Remarks/Arguments

Claims 7-10 and 15 are canceled, claim 24 is amended and claims 40-45 are newly added. Claims 1-6, 11-14 and 16-45 are pending upon entry of this amendment.

Support for amendments

Changes to the claims are supported throughout the application as originally filed. Specific support is provided for each amendment for the convenience of the examiner.

Claim 24 is amended to provide that the second well lacks a compound that modulates the beating cycle is provided as a control well. Support may be found at paragraph [0016], which provides, "[A] second well lacking compound or having a control compound is provided as a control well[.]"

Claim 40 is newly added, depends from claim 24 and adds that the step of monitoring impedance using millisecond time resolution comprises obtaining at least two consecutive impedance measurements at less than 300 milliseconds apart. Support may be found in paragraph [0015], which provides, "In some embodiments, two consecutive impedance measurements for each and every well of the system are performed less than 300 milliseconds apart."

Claim 41 is newly added, depends from claim 40 and adds that the at least two consecutive impedance measurements are less than 100 milliseconds apart. Support may be found in paragraph [0015], which provides, "In further embodiments, at least two consecutive impedance measurements for each and every well of the system are performed less than 100 milliseconds apart."

Claim 42 is newly added, depends from claim 41 and adds that the at least two consecutive impedance measurements are less than 40 milliseconds apart. Support may be found in paragraph [0015], which provides, "In further embodiments, at least two

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consecutive impedance measurements for each and every well of the system are performed less than 40 milliseconds apart."

Claim 43 is newly added, depends from claim 42, and adds that the at least two consecutive impedance measurements are less than 10 milliseconds apart. Support may be found in paragraph [0015], which provides, "In further embodiments, at least two consecutive impedance measurements for each and every well of the system are less than 10 milliseconds apart."

Claim 44 is newly added, depends from claim 24 and adds that the step of resolving the beating cycles comprises determining a rise in impedance and a decay in impedance, and extrapolating a peek from each rise and decay. Support for the amendment may be found at paragraph [0018], which provides, "The step of determining a plurality of beating cycle peaks may include determining a rise in impedance and a decay in impedance and extrapolating a peak from each rise and decay."

Clam 45 is newly added, depends from claim 24 and adds that the step of resolving cell beating cycles comprises determining beating cycle peaks by identifying impedance data points at which the first order derivative to time is zero or about zero. Support may be found at paragraph [0018]:

> Various methods can be used for determining beating cycle peaks. For example, time dependent impedance values or cell index values for a well are analyzed by deriving their first order derivatives and second order derivatives using numerical methods. The beating cycle peaks are those data points where the first order derivatives of impedance values or cell index values are zero or close to zero in its absolute value.

Election of Group III (claims 24-29) with Traverse

The claims have been examined for restriction purposes only. The examiner has identified the following six groups of inventions and requires the election of a single group:

Group I: Claims 1-15 drawn to a system, classified in class 435, subclass 288.3;

Group II: Claims 17-23 drawn to a method for assessing and quantifying excitable cells;

Group III: Claims 24-29, drawn to a method for determining whether a compound modulates a beating cycle;

Group IV: Claims 30-34, drawn to a method of characterizing rhythmic beating, classified in class 435, subclass 174;

Group V: Claims 35-37, drawn to a method of assessing genetic manipulation, classified in subclass 506, subclass 7; and

Group VI: Claims 38-39, drawn to a method comprising determining a characteristic such as cell attachment and cell growth, classified in class 324, subclass 600.

Applicants elect Group III, which includes claims 24-29 and new claims 40-45 for examination. This election is made with traverse. For completeness, the examiner identifies claim 16 as a linking claim and thus indicates upon its allowance, Groups II-VI can be rejoined.

The examiner argues the inventions are independent and distinct and that search and examination of all claims would be a burden on the office.

Applicants respectfully request Group I be added to Group III for examination. Group I is an apparatus and Group III provides a use of the apparatus. The examiner argues that the apparatus of Group I could be used to practice a materially different process, such as for detecting a particular molecule, such as a protein or antibody, in solution.

While the examiner suggests that the apparatus of Group I could be used to detect a molecule, claim 1 specifically provides that the system is for monitoring excitable cells and the device is for monitoring cell-substrate impedance. Cell substrate impedence detects changes in the interface between cell and substrate/electrodes. Thus, the device does not directly detect changes in solution but instead the cell-substrate interface. For completeness, adding a molecule to a cell can ultimately change cell-substrate impedance and thus the device can be used to detect changes in the cell-substrate interface caused by the addition of compounds into the surrounding medium. In fact, this technical approach is conducted in Group III. A compound suspected of modulating a beating cycle of an excitable cell is added to the cell and a change in beating through millisecond time resolution is detected.

Further, whether or not Group I is rejoined it will still be examined since the method of Group III has been elected and central to the method is the use of the system (apparatus) set forth in claim 1. That is, the method of claim 24 requires the system of claim 1 to permit millisecond time resolution of beating cycles. Since the examination of claim 24 requires the examination of claim 1, added search and examination burden of Group I would be minimal. Thus, principles of efficiency would support the rejoining of Group I.

In regards to Group II, Applicants acknowledge that claims 17-23 do not require the addition of a suspected modulator of a beating cycle; however, the invention set forth in Group III emphasizes further improvements for resolving a beating cycle in millisecond

time resolution in new claims 40-43, which are similar to those provided in Group II. Accordingly, the subject matter set forth in Group II will also be examined during examination of Group III. As such, the added search and examination burden of Group II would be minimal. Thus, principles of efficiency would support the rejoining of Group II.

In regards to Group IV, Applicants acknowledge that claims 30-34 do not require the addition of a suspected modulator of a beating cycle; however, when examining how beating cycles are compared in Group III, the examiner will also look to methods of comparison including comparing amplitude or frequency similar to those set forth in claims 26 and 27. As such, the search and examination burden of Group IV would be minimal. Thus, principles of efficiency would support the rejoining of Group IV.

In regards to Group V, Applicants acknowledge that claims 35-37 do not require the addition of a suspected modulator of a beating cycle and that Group III does not require a genetically manipulated cell; however, both use the impedance system of claim 1, both resolve beating cycles and both compare beating cycles. Thus, the Groups share numerous features. As such, the search and examination burden of Group V would be minimal. Thus, principles of efficiency would support the rejoining of Group V.

In regards to Group VI, Applicants acknowledge that claims 38-39 do not require the addition of a suspected modulator of a beating cycle and that Group III does not require determining a characteristic of cell attachment, cell growth or cell viability; however, these characteristics are determined through impedance measurement since they involve changes in the cell-substrate interface. Thus, the claim elements will require similar search and examination. As such, the search and examination burden of Group VI would be minimal. Accordingly, principles of efficiency would support the rejoining of Group VI.

Conclusion

Applicants respectfully submit the claims are in condition for allowance.

Respectfully submitted,

2/27/2012
Date

Raymond Wagenknecht
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5677 Oberlin Dr. Ste 204
San Diego, CA 92121
(858) 587-2510 x 201

Patent
Attorney Docket No: ACE-134U1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	)	
	)	
Wang et al	)	Examiner: Shannon Janssen
	)	
Application Number: 12/435,569	)	
	)	Group Art Unit: 1636
Filed: May 5, 2009	)	
	)	
For: LABEL-FREE MONITORING OF EXCITATION-CONTRACTION COUPLING AND EXCITABLE CELLS USING IMPEDANCE BASED SYSTEMS WITH MILLISECOND TIME RESOLUTION	)	

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Request for Extension of Time

Sir:

Applicant is responding to the Office Action mailed September 27, 2011. A shortened statutory period for reply is set to expire 1 month from the mailing date. Applicants respectfully request a four month extension of time to file a reply and provide herewith the fee under 37 CFR §1.17(a)(4).

Respectfully submitted,

2/27/2012

Date

Raymond Wagenknecht
Reg. No. 50948

Biotech Beach Law Group PC
5677 Oberlin Dr. Ste 204
San Diego, CA 92121
(858) 587-2510 x 201

Electronic Patent Application Fee Transmittal

Application Number:	12435569
Filing Date:	05-May-2009
Title of Invention:	Label-Free Monitoring of Excitation-Contraction Coupling and Excitable Cells Using Impedance Based Systems with Millisecond Time Resolution
First Named Inventor/Applicant Name:	Xiaobo Wang
Filer:	Raymond C. Wagenknecht
Attorney Docket Number:	ACE-134U1

Filed as Small Entity

Utility under 35 USC 111(a) Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Claims in excess of 20	2202	1	30	30
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension - 4 months with $0 paid	2254	1	990	990
Miscellaneous:				
			Total in USD ($)	**1020**

Electronic Acknowledgement Receipt

EFS ID:	12169571
Application Number:	12435569
International Application Number:	
Confirmation Number:	3664
Title of Invention:	Label-Free Monitoring of Excitation-Contraction Coupling and Excitable Cells Using Impedance Based Systems with Millisecond Time Resolution
First Named Inventor/Applicant Name:	Xiaobo Wang
Customer Number:	59538
Filer:	Raymond C. Wagenknecht
Filer Authorized By:	
Attorney Docket Number:	ACE-134U1
Receipt Date:	27-FEB-2012
Filing Date:	05-MAY-2009
Time Stamp:	17:12:34
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Credit Card
Payment was successfully received in RAM	$1020
RAM confirmation Number	4948
Deposit Account	504352
Authorized User	WAGENKNECHT,RAYMOND

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Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		Filed-Response-OA1.pdf	1169155 13be6cc47e6ed16f11fd9d8ad820701d028ac044	yes	16

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Response to Election / Restriction Filed	1	1
	Claims	2	9
	Applicant Arguments/Remarks Made in an Amendment	10	15
	Extension of Time	16	16

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Information:

2	Fee Worksheet (SB06)	fee-info.pdf	32028 e64f699de717e4f6bd22bc121dc8609eec4e9c2d	no	2

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New International Application Filed with the USPTO as a Receiving Office

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PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 12/435,569	Filing Date 05/05/2009	☐ To be Mailed

APPLICATION AS FILED – PART I

	(Column 1)	(Column 2)	SMALL ENTITY ☒		OR	OTHER THAN SMALL ENTITY	
FOR	NUMBER FILED	NUMBER EXTRA	RATE ($)	FEE ($)		RATE ($)	FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A			N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A			N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A			N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*	X $ =		OR	X $ =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*	X $ =			X $ =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $250 ($125 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).						
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))							
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL			TOTAL	

APPLICATION AS AMENDED – PART II

AMENDMENT	02/27/2012	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE ($)	ADDITIONAL FEE ($)	OR	OTHER THAN SMALL ENTITY RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	* 40	Minus	** 39	= 1	X $30 =	30	OR	X $ =	
	Independent (37 CFR 1.16(h))	* 1	Minus	***3	= 0	X $125 =	0	OR	X $ =	
	☐ Application Size Fee (37 CFR 1.16(s))									
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE	30	OR	TOTAL ADD'L FEE	

AMENDMENT		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)		RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	*	Minus	**	=	X $ =		OR	X $ =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	X $ =		OR	X $ =	
	☐ Application Size Fee (37 CFR 1.16(s))									
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

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Legal Instrument Examiner:
/MARY EVANS/

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Not for submission under 37 CFR 1.99)

Application Number	12435569
Filing Date	2009-05-05
First Named Inventor	Xiaobo Wang
Art Unit	1636
Examiner Name	Shannon Janssen
Attorney Docket Number	ACE-134U1

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Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1	3743581		1973-07-03	Cady et al	
	2	3890201		1975-06-17	Cady et al	
	3	4072578		1978-02-07	Cady et al	
	4	4920047		1990-04-24	Giaever et al	
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	10	5725563		1998-03-10	Klotz	
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	20	6235520	B1	2001-05-22	Malin et al	
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	30	6630359	B1	2003-10-07	Caillat et al	

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	Attorney Docket Number	ACE-134U1

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	First Named Inventor	Xiaobo Wang
	Art Unit	1636
	Examiner Name	Shannon Janssen
	Attorney Docket Number	ACE-134U1

	42	8026080	B2	2011-09-27	Wang et al	
	43	8041515	B2	2011-10-18	Wang et al	
	44					

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	1	20060057771	A1	2006-03-21	Kovacs et al	
	2	20080190783	A1	2008-08-14	Hyland	
	3	20030116447	A1	2003-06-26	Surridge et al	
	4	20030157587	A1	2003-08-21	Gomez et al	
	5	20030166015	A1	2003-09-04	Zarowitz et al	
	6	20040091397	A1	2004-05-13	Picard	

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	7	20040146849	A1	2004-07-29	Huang et al	
	8	20110039294	A1	2011-02-17	Wang et al	
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	1	1 040 345	EP	B1	2006-03-22	Sugihara et al		☐

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	1	BAUMAN ET AL., Microelectronic sensor system for microphysical application on living cells, Sensors and Actuators,1999:77-89	☐
	2	BERENS ET AL., The role of extracelluar matrix in human astrocytoma migration and proliferation studied in a microliter scale assay, Clin. Exp. Metastasis, 1994; 12(6):405-415	☐
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	Attorney Docket Number	ACE-134U1

	4	BURNETT ET AL., Fluorescent imaginng of electrically stimulated cells, Journal of Biomolecular Screening 2003; 8 (6):660-667	☐
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	Art Unit	1636
	Examiner Name	Shannon Janssen
	Attorney Docket Number	ACE-134U1

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (**Not for submission under 37 CFR 1.99)**		
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(54) **CELL POTENTIAL MEASURING ELECTRODE AND MEASURING APPARATUS USING THE SAME**

ELEKTRODE UND VORRICHTUNG ZUR MESSUNG VON ZELLPOTENTIALEN

ELECTRODE DE MESURE DE POTENTIEL DE CELLULE ET APPAREIL DE MESURE UTILISANT CETTE ELECTRODE

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EP 1 040 345 B1

Description

Field of the Invention

[0001] This invention relates to a low impedance cell potential measuring electrode assembly typically having a number of microelectrodes on an insulating substrate and having a wall enclosing the region including the microelectrodes. The device is capable of measuring electrophysiological activities of a monitored sample using the microelectrodes while cultivating those cells or tissues in the region of the microelectrodes. The invention utilizes independent reference electrodes to lower the impedance of the overall system and to therefore lower the noise often inherent in the measured data. Optimally the microelectrodes are enclosed by a physical wall used for controlling the atmosphere around the monitored sample.

Background of the Invention

[0002] Cell potential measuring apparatus have been developed to measure the activity or electrical potential generated by activity of nerve cells, other cells, or tissues (for example, Japanese Kokai 8-62209) without inserting glass electrodes or the like into the cells.

[0003] Measurement of cell potential by inserting a glass electrode or the like into the cell may damage that cell. Long term measurement of cell potential is quite difficult. It is further difficult to measure plural positions simultaneously; there is a limit to the number of electrodes one can place in a measurement electrode array and it is similarly difficult to adequately determine the position of the sample over measurement electrodes. In contrast, use of a cell potential measuring electrode having plural microelectrodes on a substrate (having a wall for enclosing a region including the microelectrodes), allows cultivation of the cells within the region enclosed by the wall and the simultaneous measurement of the potential of plural positions without damaging those cells.

[0004] These cell potential measuring devices measure cell potential against a reference. One such way is discussed with regard to Kokai 8-62209 (family member of EP-A-0 689 051). When 64 microelectrodes are arranged in eight columns and eight rows, theoretically, by using one microelectrode as the reference potential (that is, as a common reference electrode connected to the potential of the culture medium) the cell potential of the other 63 positions can be measured simultaneously by using the remaining 63 microelectrodes.

[0005] However, when measuring very low level or micro-potentials such as cell potentials, noise is a problem. Noise level varies significantly depending on the selection of the type and location of the reference electrode. As mentioned above, when using one microelectrode as a reference electrode, simultaneous measurement of potential at 63 positions by using the remaining 63 microelectrodes is impossible because of the high noise level. When the reference electrodes and measuring electrodes correspond one-by-one to each other, the potential may be measured at a very low noise level state; But if 64 microelectrodes are used, for example, corresponding to 32 reference electrodes and 32 measuring electrodes, only 32 positions can be measured simultaneously.

[0006] In theory, though, one must limit the number of reference electrodes in order to simultaneously measure the potential at as many positions as possible.

[0007] As shown in Fig. 14 of Kokai 8-62209, eight microelectrodes in one row are used as reference electrodes and seven measuring electrodes each are correlated to each of the reference electrodes, so that the potential can be measured simultaneously at 7 X 8 = 56 positions. If 56 microelectrodes are used as measuring electrodes, i.e., by using eight microelectrodes in one row as reference electrodes, the loss of measuring sites is about 12% as compared with the case of using all 64 or 63 pieces as measuring electrodes. However, even when seven measuring electrodes are used with one reference electrode, the noise is still quite large. It is quite difficult to detect a small change in cell potential from the noise.

[0008] Moreover, as shown in Fig. 14 of Kokai 8-62209, when placing a segment S of cell or tissue on the plural microelectrodes, the segment S should not be placed on the row of microelectrodes used as reference electrodes. Such a placement requires skill and is difficult because the segment S must be held by tweezers and moved while observing the segment through a microscope. It is extremely difficult to place the segment S so that the eight microelectrodes in one row are completely exposed, while the remaining 56 microelectrodes be completely covered with the segment. If the segment S is placed to completely expose the eight microelectrodes in one row, usually some of the remaining 56 are exposed, and hence the number of positions for simultaneous measurement is decreased. Sensors and Actuators B, vol. B24, no 1/03, part 1, March 1995, p 300-303, discloses an array of working electrodes with corresponding reference electrodes positioned next to the working electrodes.

SUMMARY OF THE INVENTION

[0009] The invention is intended to solve such problems. This invention provides a cell potential measuring electrode less susceptible to noise and is yet capable of simultaneously measuring the potential at many positions by effectively utilizing all of the available microelectrodes if the positioning is not very precise when placing the segment of cell or tissue to be measured.

[0010] The cell potential measuring electrode of the invention preferably includes plural microelectrodes on an insulating substrate, a conductive pattern for connecting the microelectrodes to some region out of the microelectrode area, electric contacts connected to the end of

the conductive pattern, an insulating film covering the surface of the conductive pattern, and a wall enclosing the region including the microelectrodes on the surface of the insulating film. The inventive reference electrodes have a comparatively lower impedance than the impedance of the measuring microelectrodes. They are respectively placed at plural positions in the region enclosed by the wall and often at a specific distance from the microelectrodes. The electrical contacts are further usually connected between the conductive pattern for wiring of each reference electrode and the end of the conductive pattern. The surface of the conductive pattern for wiring of the reference electrodes is typically covered with an insulating film.

[0011] According to this invention, since exclusive reference electrodes are provided at plural positions distant from the region of plural measurement microelectrodes, it is easy to place the segment of cell sample to cover all microelectrodes while not contacting with the reference electrodes. The reference electrode would typically have, for example, a larger area than a measurement microelectrode and hence is smaller in impedance. Therefore the noise level is small even if connected commonly to plural reference potentials for measuring positions. Therefore, common reference electrodes can be used with multiple measurement microelectrodes. Moreover, since each one of the plural reference electrodes is responsible for multiple measurement microelectrodes, the cell potentials may be easily measured simultaneously using all of microelectrodes.

[0012] Preferably, the plural reference electrodes are placed at nearly equal distances from the plural microelectrode region and at intervals of nearly equal angle. By "intervals of nearly equal angle", we mean that when the plural microelectrode region is viewed from above, the plural reference electrodes extend away from that region in equi-angular rays. More preferably, the plural microelectrodes are placed in a rectangular matrix, and four of the reference electrodes are provided on an extension of diagonals of the region holding that rectangular matrix. In such a symmetrical placement, the noise level to each microelectrode is averaged.

[0013] As a specific example, the microelectrodes are situated in a matrix arrangement in a rectangle having sides of, e.g., 0.8 to 2.2 mm (in the case of 300 μm microelectrode pitch)or 0.8 to 3.3 mm (in the case of 450 μm microelectrode pitch). Four reference electrodes are situated at four corners of a rectangle of 5 to 15 mm on one side. More preferably, 64 microelectrodes are disposed in eight rows and eight columns at central pitches of about 100 to 450 μm, preferably 100 to 300 μm.

[0014] In order to set the impedance of the reference electrodes to be sufficiently smaller than the impedance of the microelectrodes, the area of the reference electrodes is preferably 4 to 25 times (particularly preferably 16 times) the area of the microelectrodes. As a specific example, the area of each of the microelectrodes is preferably between about 4×10^2 and 4×10^4 μm^2 and the area of each of the reference electrodes is preferably between about 64×10^2 and 64×10^4 μm^2.

[0015] Preferably the microelectrodes and the reference electrodes are formed from the same material to both simplify the manufacturing process and obtain a cost benefit. Preferably, the microelectrodes and the reference electrodes are formed of layers of nickel plating, gold plating, and platinum black on an indium-tin oxide (ITO) film. After platinization, the impedance of the reference electrodes is preferably between 2 and 3 kilohms.

[0016] The insulating substrate (for example, a glass substrate) may be nearly square. Plural electric contacts may be connected to the end of the conductive pattern and preferably are placed on the four sides of the insulating substrate. As a result, layout of wiring patterns of multiple microelectrodes and reference electrodes is easy. Because the pitches of electric contacts may be made to be relatively large, electric connection through the electric contacts with external units is also easy.

[0017] The microelectrode region is usually very small. When observing the sample through a microscope, it is hard to distinguish position and both vertical and lateral directions. It is desirable to place indexing micro-marks near the microelectrode region to allow visual recognition through the microscope variously of direction, axes, and position.

[0018] The most preferred cell potential measuring apparatus of this invention is made up of a cell placement device having cell potential measuring electrodes, contact sites for contacting with an electric contact, and an electrode holder for fixing the insulating substrate by sandwiching from above and beneath. In a variation of the invention, a signal processor may be placed near the microelectrode matrix or region. The cell potential measuring electrodes may be connected electrically to the cell placement assembly device to allow processing of the voltage signals generated by the sample and measured between each such microelectrode and the reference electrodes. The cell potential measuring assembly usually includes a region enclosed by a wall for cultivating sample cells or tissues. It also preferably includes an optical device for magnifying and observing optically the cells or tissues cultivated in the region enclosed by the wall. This cell potential measuring apparatus preferably further comprises an image memory device for storing the magnified image obtained by the optical device.

BRIEF DESCRIPTION OF THE DRAWINGS

[0019]

Fig. 1 is a block diagram showing an entire structure of a cell potential measuring apparatus according to the invention.

Fig. 2 is an exploded view of a cell placement device including the cell potential measuring electrode of the invention.

Fig. 3 is a partial plan view showing an example of microelectrodes in the central part of the cell potential measuring apparatus and a conductive pattern for its wiring.

Fig. 4 is a plan view showing an entire structure of a cell potential measuring electrode.

Fig. 5 is a schematic diagram of a section of a cell potential measuring electrode.

Fig. 6 is a plan view and a side sectional view showing a state of fixing the cell potential measuring electrode by sandwiching with upper and lower holders.

Fig. 7 is a perspective view of the cell potential measuring electrode and upper and lower holders in Fig. 6.

Fig. 8 is a side view of contact metal fittings provided in the upper holder.

Fig. 9 is a waveform diagram showing noise level in the case of 50-micron square size of reference electrodes provided in the cell potential measuring electrode.

Fig. 10 is a waveform diagram showing noise level in the case of 200-micron square size of reference electrodes provided in the cell potential measuring electrode.

Fig. 11 is a diagram showing the central seven measurement sites within the measuring region of the cell potential measuring electrode.

Fig. 12 is a block diagram showing an example of measuring method of cell potential by using a conventional cell potential measuring electrode.

DESCRIPTION OF THE INVENTION

[0020] Fig. 1 shows a typical example of an entire cell potential measuring apparatus using a cell potential measuring electrode and reference electrode made according to the invention. This cell potential measuring apparatus comprises an integrated cell placement device 1 containing the cell potential measuring electrode of the invention, an optical observation device 20 including an inverted microscope 21 for optically measuring the sample or cells placed in the cell placement device 1, a computer 30 for giving a stimulus signal to the cells and for processing the output signal from the cells, and a cell culture system 40 for maintaining a culture atmosphere about the sample.

[0021] In addition to the inverted microscope 21 upon which the cell placement device 1 is set, the optical observation device 20 may also comprise an SIT camera 22 for the microscope 21, a high definition display 23, and an image memory device 24. The high definition display 23 may be also used as the display for the computer 30.

[0022] The computer 30 usually is a personal computer (PC) in which measurement software is installed. The computer 30 and cell placement device 1 are connected through an I/O board for measurement. The I/O board includes an A/D converter 31 and a D/A converter 32. The A/D converter 31 is usually for measuring and converting the resulting potentials; the D/A converter 32 is for stimulus signals to the sample. For example, the A/D converter 31 may have 16 bits, 64 channels, and the D/A converter 32 has 16 bits, 8 channels.

[0023] The measurement software installed in the computer 30 may include software for setting conditions for giving a stimulus signal, forming the stimulus signal, and for recording the obtained detection signal. By using such measurement software, the computer 30 may comprise means for giving a stimulus signal to the cells and means for processing the signal detected from the cells. The computer 30 may also control the optical observation device (SIT camera and image memory device) and the cell culture system.

[0024] An outline of the functionality of the desirable measurement software is described below screen-by-screen.

[0025] On a parameter setting screen, complicated stimulus conditions may be set by drawing a stimulus waveform on the screen by using a keyboard or a mouse. If a setting of recording condition is 64 input channels and a sampling rate of 10 kHz, the computer can handle consecutive recording for several hours. Moreover, electrodes to apply a stimulus signal and electrodes to pick up the detection signal from the cells can be designated by pointing out the microscope images displayed on the screen by the mouse or pen. The temperature, pH, and other conditions of the cell culture system 40 are desirably set from the keyboard.

[0026] On a recording screen, the spontaneous activity potential or induced potential detected from the cells may be displayed in real time. In addition, the recorded spontaneous activity, potential or induced potential can be displayed by overlaying on the microscope image of the cell. When measuring the induced potential, the entire recorded waveform is displayed. When measuring the spontaneous activity potential, by the spike detection function using window discriminator or waveform discriminator, the recorded waveform is displayed only when generation of spontaneous activity is detected. Together with the display of recorded waveform, measurement parameters (e.g., stimulus condition, recording conditions, temperature, pH, etc.) may also be displayed in real time. An alarm function is also provided for warning when the temperature or pH goes out of the allowable range.

[0027] Concerning data analysis or processing, Fourier Function Transform (FFT) analysis, coherence analysis, and correlation analysis are also desirable. Useable

functions may include single spike separation function using waveform discrimination, temporal profile display function, topography display function, and current source density analysis function. These analysis results may be displayed by overlaying on the microscope images stored in the image memory device.

[0028] When a stimulus signal is issued from the computer 30, this stimulus signal is sent to the cell placement device through the D/A converter 32 and an isolator 33. The cell placement device 1 includes a cell potential measuring electrode which may be formed, as described later, of 64 microelectrodes on a glass substrate in a matrix form and having an enclosing wall for maintaining the sample (e.g., segments of cells or tissues) in contact with the microelectrodes and their culture fluid. The stimulus signal sent to the cell placement device 1 is applied to arbitrary electrodes out of the 64 microelectrodes and then to the sample or samples.

[0029] The induced, evoked, or spontaneous potential occurring between each microelectrode and reference potential (which is at the potential of the culture fluid) is passed through a 64 channel high sensitivity amplifier 34 and the A/D converter 31 into the computer 30. The amplification factor of the amplifier 34 may be, e.g., about 80-100 dB, for example, in a frequency band of about 0.1 to 10 kHz, or to 20Hz. However, when measuring the potential induced by a stimulus signal, by using a low-cut filter, the frequency band is 100Hz to 10kHz. Spontaneous potentials are usually in the range of 100Hz to 20Hz.

[0030] The cell culture system 40 usually includes a temperature controller 41, a culture fluid circulation device 42, and a feeder 43 for supplying, e.g., a mixed gas of air and carbon dioxide. The cell culture system 40 may instead be made up of a commercial microincubator, a temperature controller, and CO_2 cylinder. The microincubator can be used to control in a temperature range of 0 to 50°C by means of a Peltier element and is applicable to the liquid feed rate of 3.0 ml/min or less and gas flow rate of 1.0 liter/min or less. Or, a microincubator incorporating a temperature controller may be used.

[0031] The structure of the cell placement device 1 (shown in Figure 1) is explained in more detail with regard to the exploded view found in Fig. 2. The preferred cell placement device 1 may be made up of a cell potential measuring electrode (also called an integrated multiple electrode or microelectrode assembly) 2 having a cylindrical wall 6 provided on a transparent glass substrate and having plural microelectrodes in its inside region, holders **3**, 4 divided in two sections for fixing the cell potential measuring electrode 2 by sandwiching from above and beneath, and a printed wiring board 5 for fixing the holders.

[0032] Figure 3 shows the details of the glass substrate. The size of the glass substrate for constituting the cell potential measuring electrode (integrated multiple electrode) 2 may be 1.1 mm in thickness and about 50 mm square. In the central part of the glass substrate, 64 microelectrodes 11 are formed in a matrix form of 8 x 8. The microelectrodes are insulated from each other and from the reference electrodes. A conductive pattern 12 for wiring is connected to each microelectrode 11. The microelectrode 11 may be about 50 microns square and the distance between centers of adjacent electrodes is about 150 microns. The depicted 64 microelectrodes 11 are therefore shown in a matrix form of 8 x 8, one side of the formed rectangular region is about 1.1 mm.

[0033] Although the Description of the Invention contains many specific references to particular sizes and areas, the invention is not so limited; they are only provided for the sake of guidance and are not critical to the invention unless so stated.

[0034] Also, as shown in Fig. 4, reference electrodes 10 are formed at four positions on lines extended from diagonals of the rectangular region in the central part of the glass substrate in which the microelectrodes are disposed. The reference electrodes are insulated from each other and from the microelectrodes. These reference electrodes 10 are also connected to the electric contacts 7 situated on four sides of the glass substrate by the conductive pattern 12 for wiring same as the microelectrodes 11. The reference electrodes 10 are formed in the same process as the microelectrodes 11, as mentioned below, but the size is generally significantly larger than that of the microelectrodes 11 being, e.g., a rectangle of about 200 microns in one side. Therefore, as compared with one of the microelectrodes 11 of about 50 microns square, the rectangular area is larger, preferably about 16 times larger, and, by this portion, the impedance of the reference electrodes 10 is smaller than the impedance of the microelectrodes 11.

[0035] The positions of the reference electrodes 10 are preferably on lines extending from the diagonals of the rectangular region in the central part of the glass substrate in which the microelectrodes 11 are disposed. The reference electrodes 10 in this variation are located about 6 mm from the center of the rectangular region. Said another way, they are placed at four corners of a square of about 8.5 mm in one side.

[0036] Moreover, as shown in Fig. 4, on each of the four sides of the glass substrate may be found 17 electric contacts 7. These electric contacts 7 are attached (one-by-one) to each of the 64 microelectrodes 11 and four reference electrodes 10 through the conductive pattern 12. The pitch of the 17 electric contacts is desirably spaced to the pitch of 1.27mm of the universal connector. The manufacturing process of this integrated multiple electrode 2 is explained below by referring to the sectional view in Fig. 5. The depiction in Fig. 5, for the ease of explanation, is not to scale.

[0037] On the surface of a glass substrate 13, an ITO (indium tin oxide) film in a thickness of 150 nm is applied, and a conductive pattern 12 is formed by photo resist and etching. A negative photosensitive polyimide film of about 1.4 microns in thickness is applied thereon, and an insulating film 14 is formed. On portions of the microelectrodes 11 (or alternatively reference electrodes 10)

and electric contacts 7, the ITO film is exposed, and nickel plating 15 of 500 nm in thickness and gold plating 16 of 50 nm in thickness are then applied.

[0038] A polystyrene or glass cylindrical member 6 corresponding to a wall of about 22 mm in inside diameter, about 25 mm in outside diameter, and 8 mm in height may then be placed on the central part of the glass substrate by using a Silicone adhesive (see Fig. 2 and Fig. 4). A highly preferred adhesive is an RTV (Room Temperature Vulcanization) silicone rubber, particularly those which use an acid cure system. These produce a low level of toxicity because of the acetic acid produced during the cure step. Two useful varieties include KE42T (Shin-Etsu Silicone) and Silastic Medical Adhesive Silicone Type A (Dow Corning). A cylindrical wall member 6 is shown, but the wall may be oval to allow enhanced access to the sample. The wall member 6 is affixed in the center of the glass substrate, that is, in a state aligned with the central part of the rectangular region in which the 64 microelectrodes are disposed. In the region enclosed by this cylindrical member 6, the cells or tissues are cultivated. This cylindrical member 6 is filled, e.g., with an aqueous solution of 1 wt.% of chloroplatinic acid, 0.01 wt.% of lead acetate and 0.0025 wt.% of hydrochloric acid, and by passing a current of 20 mA/cm^2 for a minute, platinum blacks 11a (or alternatively, reference electrode platinum black 10a) are precipitated on the surfaces of the microelectrodes 11 and reference electrodes 10.

[0039] The region within the cylindrical member 6 is occasionally referred to as the "measuring region" which includes the area including both the microelectrodes I 1 and reference electrodes 10. It is further within the scope of the invention that the reference electrodes be placed on the inner surface of the cylindrical member 6.

[0040] At one corner of the integrated multiple electrode 2, an indexing or arrow mark 17 showing the direction is provided. This arrow mark 17 can be formed in the same manufacturing process as the microelectrodes 11 and reference electrodes 10. However, the surface is coated with gold plating only, and platinum black is not formed. The length and width of the arrow mark 17 are both about 5 mm. Moreover, near one corner of the rectangular region of disposing the microelectrodes 11, a small indexing mark, e.g., a micro-mark 18 similar to the arrow mark is provided. This micro-mark 18 is not visible by the naked eye, but a same pattern as the arrow mark 17 is recognized in a magnified view by an optical observation device of the measuring apparatus, so that the direction, position, axes, etc. may be identified. Like the arrow mark 17, the micro-mark 18 can be also formed in the same manufacturing process as the microelectrodes 11 and reference electrodes 10.

[0041] In Fig. 2, the integrated multiple electrode 2 is sandwiched between holders 3, 4. An electrical connection is made in the same way. The holders 3, 4 are typically polymeric. The step portion is used to hold the edge of the integrated multiple electrode 2 and the rectangular openings are formed in the central part. The upper holder 3 is provided with a pair of fasteners 8 and 17 pieces x 4 pairs of contact metal areas 9. A top view of the holders 3, 4 sandwiching and fixing the integrated multiple electrode 2 is shown in Fig. 6(A), its side view (section B-B) in Fig. 6(B), and its perspective back view in Fig. 7. As clear from these diagrams, the fastener 8 is supported by and rotates about shaft pins 8a on two confronting sides of the upper holder 3. As shown in Figure 7, grooves 4a are formed in two confronting sides of the back side of the lower holder 4. Protrusions 8b of the fastener 8 are fitted in grooves 4a and the upper and lower holders 3, 4 are fixed firmly in a state of sandwiching the integrated multiple electrode 2.

[0042] A total of 68 contact metal fittings 9 provided on the upper holder 3 to correspond to the electric contacts 7 of the integrated multiple electrode 2 may be formed by processing elastic and conductive metal plates such as a Be/Cu spring alloy, plated with Ni and Au. The metal fittings 9 have a sectional shape as shown in Fig. 8. That is, it consists of a pin 9a, its base part 9b, and a movable contact part 9d extending from the base part 9b through a curved part 9c. In such structure, the movable contact part 9d can be elastically dislocated from the base part 9b. In the upper holder 3, holes for inserting the pin 9a of the contact metal fitting 9, and grooves for fitting the base part 9b are formed in 68 (17 x 4) positions.

[0043] As shown in Fig. 2 and Fig.6(B), with the contact metal fitting 9 inserted and fixed in the hole and groove, pin 9a projects from the upper holder 3. Contact metal fittings 9 are of two types which differ in the length of the base part 9b. The two sized fittings 9 are alternately disposed, 16 pins 9a projecting from the upper holder 3 are arranged in two zigzag rows. As mentioned later, these pins 9a are connected to the connectors mounted on the printed wiring board 5 for connection with the outside.

[0044] Movable contact part 9d of the contact metal fitting 9 projects from the lower side of the upper holder 3, when in contact metal fitting 9 is inserted and affixed in the hole and groove of the upper bolder 3. With the holders 3, 4 fixed on both sides of the integrated multiple electrode 2, the movable contact part 9d of each contact metal fitting 9 contacts with the electric contact 7 of the integrated multiple electrode 2, and a specified contact pressure is given to the contact area by elastic deformation of the curved part 9c. In this way, the electric contacts 7 for connecting to the microelectrodes 11 and reference electrodes 10 of the integrated multiple electrode 2 through the conductive pattern 12 are electrically connected at a lowercontact resistance (30 milliohms or less) as compared with that of the contact metal fittings 9.

[0045] As mentioned above, the holders 3, 4 firmly fixing the integrated multiple electrode 2 in a state of electric contact with the integrated multiple electrode 2 are electrically connected and affixed to the printed wiring board 5 as shown in Fig. 2. The electric connection from the microelectrodes 11 and reference electrode 10 of the integrated multiple electrode 2 to the conductive pattern

12, electric contacts 7 and contact metal fittings 9 is further connected to the cell potential measuring apparatus mentioned above through the printed wiring board 5. Handling of the integrated multiple electrode on the measuring apparatus is facilitated by use of the printed wiring board 5.

[0046] Also as shown in Figure 2, printed circuit board 5 may be made up of, e.g., a glass epoxy two-sided substrate. Connectors 5a are provided at the back side of four positions on the circumference of the circular opening formed in the center of printed circuit board 5. Since 16 pins 9a projecting in two zigzag rows from the four positions on the surface of the upper holder 3 are inserted into the individual corresponding connectors 5a, the assembly of the integrated multiple electrode 2 and holders 3, 4 is fixed to the printed wiring board 5 and is connected electrically.

[0047] At both edges 5b of the printed wiring board 5, electric contacts of 2.54 mm pitch for both edge connectors may be found. These electric contacts and central connectors 5a are connected in the conductive pattern 5c. The inside row of the both connectors 5a is wired by the surface pattern, and the outside row by the back side pattern, respectively, and 34 each on both surface and back sides of both edges 5b, that is, a total of 68 electric contacts are formed. To make the mechanical fixing secure, the upper holder 3 may be affixed to the printed wiring board 5 by fastening with screws.

[0048] The reference electrodes 10 of the integrated multiple electrode 2 are described by reference to Fig. 4. The reference electrodes 10 are usually immersed in the culture fluid as the reference potential for measuring the potential occurring in each microelectrode. Therefore, each microelectrode 11 is connected to an input of the amplifier 34 (Fig. 1), and the reference electrodes 10 are connected to the reference voltage terminals of each amplifier. The 64-channel amplifier is divided into four groups of 16 channels each, and each one of the four reference electrodes is commonly connected to the reference voltage terminal of one group for 16 channels.

[0049] First, as is clear from Fig. 4, it is preferred to position the four reference electrodes 10 on extensions of diagonals of the central rectangular region containing the microelectrodes 11. In general, this is a matter of convenience for pattern wiring. Moreover, in order to place the segments of cells or tissues easily so as to cover all of 64 microelectrodes and not to cover the four reference electrodes, the distance between the central rectangular region disposing the microelectrodes 11 and the reference electrodes 10 should be as large as reasonably possible. Moreover, by placing the four reference electrodes 10 at equal distances from the center of the rectangular region, the noise level occurring in each microelectrode is substantially uniform. Although the positions of the reference electrodes are closely specified above, the numerical values are not intended to be absolute, but are only intended to be guidelines.

[0050] The size of the reference electrodes 10 may be 4-64, preferably about 16, times the area of a microelectrode as mentioned above. As a result, the impedance is balanced between the measuring potential input side of the amplifier and the reference potential input side and the noise level is minimized. For instance, by forming the microelectrodes and reference electrodes in the same noted process and by setting the area of the reference electrode 16 times that of the microelectrode, the impedance of 16 microelectrodes and the impedance of one reference electrode responsible are nearly equal.

Example

[0051] This Example shows the difference in noise level between a system including an integrated multiple electrode such as that discussed above and reference electrodes of 50 microns square and 200 microns square. Fig. 9 and Fig. 10 show those comparative noise levels.

[0052] We fabricated integrated multiple electrodes (such as shown in Fig. 4) having, respectively, reference electrodes of 50 microns square and reference electrodes of 200 microns square. The integrated multiple electrodes each had cylindrical members 6. The same culture medium as normally used in tissue culturing was placed inside the cylindrical members 6. To limit the resulting signal to the noise, no cell or tissue sample was placed on the microelectrodes. As shown in Fig. 11, of the 64 microelectrodes, the central seven sites (channels I to 5, 7, 8) were measured.

[0053] Fig. 9 shows the noise waveform of the reference electrodes in 50 microns square, and Fig. 10, 200 microns square. In each diagram, the voltage on the axis of ordinates is 0.02 mV/div, and the time on the axis of abscissas is 5.0 ms/div. As clear from comparison between Fig. 9 and Fig. 10, the noise level is clearly smaller when the reference electrodes are 200 microns square (Fig. 10) as compared to reference electrodes with 50 microns square (Fig. 9). Incidentally, as described in relation to the prior art, by using one of the 64 microelectrodes as the reference electrode to be responsible for 16 microelectrodes, the noise level was as large as in Fig. 9.

[0054] As described herein, according to the cell potential measuring electrode and apparatus of the invention, the noise effect is small, and if positioning when setting the segments of cells or tissues to be measured is not very precise, all microelectrodes are effectively utilized, and potentials at multiple points can be measured simultaneously.

Claims

1. A cell potential measuring electrode assembly (2) suitable for measuring electrical potential in a neural sample comprising:

   a) a plurality of measurement microelectrodes

(11) insulated from each other and located on an insulating substrate (13) and forming a measurement microelectrode region,
b) a plurality of reference electrodes (10) isolated from each other and located on said insulating substrate outside of said measurement microelectrode region, each of said plurality of reference electrodes having an impedance smaller than said each of said measurement microelectrodes (11) when measured in an electrolyte covering said measuring region at 1 kHz, 50 mv.

2. The cell potential measuring electrode assembly (2) of claim 1 wherein the area of each of the plurality of reference electrodes (10) is larger than the area of each of the plurality of measurement microelectrodes (11).

3. The cell potential measuring electrode assembly (2) of claim 2 wherein the area of each of the plurality of reference electrodes (10) is 4 to 25 times the area of each of plurality of measurement microelectrodes (11).

4. The cell potential measuring electrode assembly (2) of claim 2 wherein the area of said each of plurality of measurement microelectrodes (11) is 4×10^2 to 4×10^4 μm^2 and the area of each of the plurality of reference electrodes (10) is 64×10^2 to 64×10^4 μm^2.

5. The cell potential measuring electrode assembly (2) of claim 1 wherein each of the plurality of measurement microelectrodes (11) are situated in an array in said measuring region.

6. The cell potential measuring electrode assembly (2) of claim 5 wherein 64 microelectrodes (11) are disposed in eight rows and eight lines at central pitches of 100 to 450 microns.

7. The cell potential measuring electrode assembly (2) of claim 1 wherein each of the plurality of measurement microelectrodes (11) and each of the plurality of reference electrodes (10) are connectable to a position outside of the measuring region.

8. The cell potential measuring electrode assembly (2) of claim 1 wherein the measuring region is surrounded by a wall (6).

9. The cell potential measuring electrode assembly (2) of claim 8 wherein the wall (6) is circular.

10. The cell potential measuring electrode assembly (2) of claim 8 wherein the wall (6) is oval.

11. The cell potential measuring electrode assembly (2) of claim 1, further comprising:

a conductive pattern (12) for wiring of said microelectrodes (11) and said reference electrodes (10);
an electric contact (7) connected to the end of said conductive pattern;
an insulating film (14) covering the surface of said conductive pattern; and
a wall (6) enclosing an area including said measurement microelectrode region and said reference electrodes for use in measurement of electrophysiological activities while cultivating cells or tissues in a region enclosed by said wall.

12. The cell potential measuring electrode assembly (2) of claim II, wherein said plural reference electrodes (10) are disposed at nearly equal distance from the region of disposition of said plural microelectrodes (11) and at intervals of nearly equal angle.

13. The cell potential measuring electrode assembly (2) of claim 12, wherein said plural microelectrodes (11) are disposed in a matrix in a rectangular region, and four of said reference electrodes (10) are provided on an extension of diagonals of said rectangular region.

14. The cell potential measuring electrode assembly (2) of claim 13, wherein said microelectrodes (11) are 0.8 to 3.3 mm in one side of a rectangular region in matrix arrangement, and said four reference electrodes (10) are disposed at four corners of a rectangular form of 5 to 15 mm in one side.

15. The cell potential measuring electrode assembly (2) of claim 14, wherein 64 microelectrodes (11) are disposed in eight rows and eight lines at central pitches of 100 to 450 microns.

16. The cell potential measuring electrode assembly (2) of claim 11, wherein the area of said reference electrodes (10) is 4 to 25 times the area of said microelectrodes (11).

17. The cell potential measuring electrode assembly (2) of claim 16, wherein the area of said reference electrodes (10) is 16 times the area of said microelectrodes (11).

18. The cell potential measuring electrode assembly (2) of claim 11, wherein the area of said microelectrodes (11) is 4×10^2 to 4×10^4 μm^2 and the area of said reference electrodes (10) is 64×10^2 to 64×10^4 μm^2.

19. The cell potential measuring electrode assembly (2) of claim 11, wherein said microelectrodes (11) and

said reference electrodes (10) are formed of a same material.

20. The cell potential measuring electrode assembly (2) of claim 19, wherein said microelectrodes (11) and said reference electrodes (10) are formed by layering nickel plating, gold plating, and platinum black on an indium-tin oxide film.

21. The cell potential measuring electrode assembly (2) of claim 11, wherein said insulating substrate (13) is nearly square, and plural electric contacts (7) connected to the end of said conductive pattern (12) are distributed and disposed in four sides of said insulating substrate.

22. The cell potential measuring electrode assembly (2) of claim 11, wherein indexing micro-marks (17) for visually recognizing direction when magnified are provided near the regions of disposition of said microelectrodes (11).

23. A cell potential measuring apparatus comprising:

a cell placement device (1) having a cell potential measuring electrode assembly (2) as defined in any one of claims 11 to 22, and a contact metal (9) for contacting with its electric contact (7), and including an electrode holder (3, 4) for fixing said insulating substrate (13) by sandwiching from above and beneath,
a signal processor (30) connected electrically to said cell placement device (1) for processing voltage signals generated between each microelectrode (11) and reference electrode (10) of said cell potential measuring electrode (2) by the activity of cells or tissues cultivated in a region enclosed by a wall (6), and
an optical device (21) for magnifying and observing optically the cells or tissues cultivated in the region enclosed by said wall (6).

24. A cell potential measuring apparatus of claim 23, further comprising an image memory device (22) for storing the magnified image obtained by aid optical device (21).

Patentansprüche

1. Elektrodenanordnung zur Messung von Zellpotentialen (2), die zur Messung eines elektrischen Potentials in einer neuralen Probe geeignet ist, mit:

a) einer Vielzahl von Meßmikroelektroden (11), die gegeneinander isoliert und auf einem isolierenden Substrat (13) angeordnet sind und einen Meßmikroelektrodenbereich bilden,

b) einer Vielzahl von Referenzelektroden (10), die gegeneinander isoliert und auf dem isolierenden Substrat außerhalb des Meßmikroelektrodenbereichs angeordnet sind, wobei jede aus der Vielzahl von Referenzelektroden eine Impedanz hat, die kleiner ist als jede der Meßmikroelektroden (11), wenn in einem Elektrolyt, der den Messungsbereich umfaßt, mit 1 kHz, 50 mV gemessen wird.

2. Elektrodenanordnung zur Messung von Zellpotentialen (2) nach Anspruch 1, wobei die Fläche jeder aus der Vielzahl von Referenzelektroden (10) größer ist als die Fläche jeder aus der Vielzahl von Meßmikroelektroden (11).

3. Elektrodenanordnung zur Messung von Zellpotentialen (2) nach Anspruch 2, wobei die Fläche jeder aus der Vielzahl von Referenzelektroden (10) das 4- bis 25fache der Fläche jeder aus der Vielzahl von Meßmikroelektroden (11) ist.

4. Elektrodenanordnung zur Messung von Zellpotentialen (2) nach Anspruch 2, wobei die Fläche jeder aus der Vielzahl von Meßmikroelektroden (11) 4×10^2 bis 4×10^4 μm^2 ist und die Fläche jeder aus der Vielzahl der Referenzelektroden (10) 64×10^2 bis 64×10^4 μm^2 ist.

5. Elektrodenanordnung zur Messung von Zellpotentialen (2) nach Anspruch 1, wobei jede aus der Vielzahl von Meßmikroelektroden (11) in einer Matrix in dem Messungsbereich angeordnet ist.

6. Elektrodenanordnung zur Messung von Zellpotentialen (2) nach Anspruch 5, wobei 64 Mikroelektroden (11) in acht Reihen und acht Zeilen mit mittigen Rastermaßen von 100 bis 450 μm angeordnet sind.

7. Elektrodenanordnung zur Messung von Zellpotentialen (2) nach Anspruch 1, wobei jede aus der Vielzahl von Meßmikroelektroden (11) und jede aus der Vielzahl von Referenzelektroden (10) mit einer Position außerhalb des Messungsbereichs verbindbar sind.

8. Elektrodenanordnung zur Messung von Zellpotentialen (2) nach Anspruch 1, wobei der Messungsbereich von einer Wand (6) umgeben ist.

9. Elektrodenanordnung zur Messung von Zellpotentialen (2) nach Anspruch 8, wobei die Wand (6) kreisförmig ist.

10. Elektrodenanordnung zur Messung von Zellpotentialen (2) nach Anspruch 8, wobei die Wand (6) oval ist.

11. Elektrodenanordnung zur Messung von Zellpotentialen (2) nach Anspruch 1, ferner mit:

einer Leiterstruktur (12) zum Verdrahten der Mikroelektroden (11) und der Referenzelektroden (10);
einem elektrischen Kontakt (7), der mit dem Ende der Leiterstruktur verbunden ist;
einem isolierenden Film (14), der die Oberfläche der Leiterstruktur überdeckt; und
einer Wand (6), die eine Fläche mit dem Meßmikroelektrodenbereich und den Referenzelektroden einschließt, zur Verwendung beim Messen von elektrophysiologischen Aktivitäten, während Zellen oder Gewebe in einem von der Wand eingeschlossenen Bereich kultiviert werden.

12. Elektrodenanordnung zur Messung von Zellpotentialen (2) nach Anspruch 11, wobei die mehreren Referenzelektroden (10) in nahezu gleichem Abstand vom Anordnungsbereich der mehreren Mikroelektroden (11) und in Intervallen von nahezu gleichem Winkel angeordnet sind.

13. Elektrodenanordnung zur Messung von Zellpotentialen (2) nach Anspruch 12, wobei die mehreren Mikroelektroden (11) in einer Matrix in einem rechteckigen Bereich angeordnet sind und vier der Referenzelektroden (10) auf einer Verlängerung von Diagonalen des rechteckigen Bereichs angeordnet sind.

14. Elektrodenanordnung zur Messung von Zellpotentialen (2) nach Anspruch 13, wobei die Mikroelektroden (11) 0,8 bis 3,3 mm an einer Seite eines rechteckigen Bereichs in Matrixanordnung sind und die vier Referenzelektroden (10) an vier Ekken einer rechteckigen Form von 5 bis 15 mm an einer Seite angeordnet sind.

15. Elektrodenanordnung zur Messung von Zellpotentialen (2) nach Anspruch 14, wobei 64 Mikroelektroden (11) in acht Reihen und acht Linien mit mittigen Rastermaßen von 100 bis 450 μm angeordnet sind.

16. Elektrodenanordnung zur Messung von Zellpotentialen (2) nach Anspruch 11, wobei die Fläche der Referenzelektroden (10) das 4- bis 25fache der Fläche der Mikroelektroden (11) ist.

17. Elektrodenanordnung zur Messung von Zellpotentialen (2) nach Anspruch 16, wobei die Fläche der Referenzelektroden (10) das 16fache der Fläche der Mikroelektroden (11) ist.

18. Elektrodenanordnung zur Messung von Zellpotentialen (2) nach Anspruch 11, wobei die Fläche der Mikroelektroden (11) 4×10^2 bis 4×10^4 μm^2 ist und die Fläche der Referenzelektroden (10) 64×10^2 bis 64×10^4 μm^2 ist.

19. Elektrodenanordnung zur Messung von Zellpotentialen (2) nach Anspruch 11, wobei die Mikroelektroden (11) und die Referenzelektroden (10) aus dem gleichen Material bestehen.

20. Elektrodenanordnung zur Messung von Zellpotentialen (2) nach Anspruch 19, wobei die Mikroelektroden (11) und die Referenzelektroden (10) durch Aufbringen von Nickelplattierung, Goldplattierung und Platinschwarz auf einem Indium-Zinnoxidfilm ausgebildet sind.

21. Elektrodenanordnung zur Messung von Zellpotentialen (2) nach Anspruch 11, wobei das isolierende Substrat (13) nahezu quadratisch ist und mehrere elektrische Kontakte (7), die mit dem Ende der Leiterstruktur (12) verbunden sind, an vier Seiten des isolierenden Substrats verteilt und angeordnet sind.

22. Elektrodenanordnung zur Messung von Zellpotentialen (2) nach Anspruch 11, wobei Hinweismikromarkierungen (17) zur optischen Erkennung der Richtung bei Vergrößerung nahe den Anordnungsbereichen der Mikroelektroden (11) vorgesehen sind.

23. Eine Vorrichtung zur Messung von Zellpotentialen mit:

einer Zellplazierungsvorrichtung (1) mit einer Elektrodenanordnung zur Messung von Zellpotentialen (2) nach einem der Ansprüche 11 bis 22 und einem Kontaktmetall (9) zur Kontaktherstellung mit ihrem elektrischen Kontakt (7) und mit einem Elektrodenhalter (3, 4) zum Fixieren des isolierenden Substrats (13) durch sandwichartiges Anordnen von oben und unten,
einem Signalprozessor (30), der mit der Zellplazierungsvorrichtung (1) elektrisch verbunden ist, zur Verarbeitung von Spannungssignalen, die zwischen jeder Mikroelektrode (11) und Referenzelektrode (10) der Zellpotentialmeßelektrode (2) durch die Aktivität von Zellen oder Geweben erzeugt werden, die in einem von einer Wand (6) eingeschlossenen Bereich kultiviert werden, und
einer optischen Vorrichtung (21) zum optischen Vergrößern und Beobachten der Zellen oder Gewebe, die in dem von der Wand (6) eingeschlossenen Bereich kultiviert werden.

24. Vorrichtung zur Messung von Zellpotentialen nach Anspruch 23, ferner mit einer Bildspeichervorrichtung (22) zum Speichern des durch die optische Vor-

richtung (21) gewonnenen, vergrößerten Bildes.

Revendications

1. Ensemble d'électrode de mesure de potentiel de cellule (2) adéquat pour la mesure le potentiel électrique dans un échantillon neural comprenant :

   a) une pluralité de microélectrodes de mesure (11) isolées les unes des autres et placées sur un substrat d'isolation (13) et formant une région de microélectrode de mesure,
   b) une pluralité d'électrodes de référence (10) isolées les unes des autres et placées sur ledit substrat d'isolation à l'extérieur de ladite région de microélectrode de mesure, chacune de ladite pluralité d'électrodes de référence ayant une impédance inférieure à ladite chacune desdites microélectrodes de mesure (11) lorsqu'on la mesure dans un électrolyte couvrant ladite région de mesure à 1 kHz, 50 mv.

2. Ensemble d'électrode de mesure de potentiel de cellule (2) selon la revendication 1 dans lequel l'aire de chacune de la pluralité d'électrodes de référence (10) est supérieure à l'aire de chacune de la pluralité des microélectrodes de mesure (11).

3. Ensemble d'électrode de mesure de potentiel de cellule (2) selon la revendication 2 dans lequel l'aire de chacune de la pluralité d'électrodes de référence (10) est 4 à 25 fois l'aire de chacune de la pluralité des microélectrodes de mesure (11).

4. Ensemble d'électrode de mesure de potentiel de cellule (2) selon la revendication 2 dans lequel l'aire de ladite chacune de la pluralité des électrodes de mesure (11) est de 4×10^2 à 4×10^4 μm^2 et l'aire de chacune de la pluralité des électrodes de référence (10) est de 64×10^2 à 64×10^4 μm^2.

5. Ensemble d'électrode de mesure de potentiel de cellule (2) selon la revendication 1 dans lequel chacune de la pluralité des microélectrodes de mesure (11) est située dans une matrice dans ladite région de mesure.

6. Ensemble d'électrode de mesure de potentiel de cellule (2) selon la revendication 5 dans lequel 64 microélectrodes (11) sont disposées dans huit rangées et huit lignes à des pas centraux de 100 à 450 microns.

7. Ensemble d'électrode de mesure de potentiel de cellule (2) selon la revendication 1 dans lequel chacune de la pluralité des microélectrodes de mesure (11) et chacune de la pluralité des électrodes de référence (10) sont connectables à une position à l'extérieur de la région de mesure.

8. Ensemble d'électrode de mesure de potentiel de cellule (2) selon la revendication 1 dans lequel la région de mesure est entourée par une paroi (6).

9. Ensemble d'électrode de mesure de potentiel de cellule (2) selon la revendication 8 dans lequel la paroi (6) est circulaire.

10. Ensemble d'électrode de mesure de potentiel de cellule (2) selon la revendication 8 dans lequel la paroi (6) est ovale.

11. Ensemble d'électrode de mesure de potentiel de cellule (2) selon la revendication 1, comprenant de plus :

    une impression conductrice (12) pour le câblage desdites microélectrodes (11) et desdites électrodes de référence (10) ;
    un contact électrique (7) connecté à l'extrémité de ladite impression conductrice ;
    un film d'isolation (14) couvrant la surface de ladite impression conductrice; et
    une paroi_ (6) enfermant une aire comprenant ladite région de microélectrode de mesure et lesdites électrodes de référence pour une utilisation dans la mesure des activités électrophysiologiques pendant la culture de cellules ou de tissus dans une région enfermée au moyen de ladite paroi.

12. Ensemble d'électrode de mesure de potentiel de cellule (2) selon la revendication 11, dans lequel ladite pluralité d'électrodes de référence (10) est disposée à presque une distance égale de la région de disposition desdites microélectrodes plurales (11) et à des intervalles de presque un angle égal.

13. Ensemble d'électrode de mesure de potentiel de cellule (2) selon la revendication 12, dans lequel lesdites microélectrodes plurales (11) sont disposées dans une matrice dans une région rectangulaire, et quatre desdites électrodes de référence (10) sont fournies sur une extension de diagonales de ladite région rectangulaire.

14. Ensemble d'électrode de mesure de potentiel de cellule (2) selon la revendication 13, dans lequel lesdites microélectrodes (11) sont de 0,8 à 3,3 mm dans un côté d'une région rectangulaire dans un arrangement de matrice, et lesdites quatre électrodes de référence (10) sont disposées à quatre coins d'une forme rectangulaire de 5 à 15 mm dans un côté.

15. Ensemble d'électrode de mesure de potentiel de cel-

lule (2) selon la revendication 14, dans lequel 64 microélectrodes (11) sont disposées dans huit rangées et huit lignes à des pas centraux de 100 à 450 microns.

16. Ensemble d'électrode de mesure de potentiel de cellule (2) selon la revendication 11, dans lequel l'aire desdites électrodes de référence (10) est de 4 à 25 fois l'aire desdites microélectrodes (11).

17. Ensemble d'électrode de mesure de potentiel de cellule (2) selon la revendication 16, dans lequel l'aire desdites électrodes de référence (10) est 16 fois l'aire desdites microélectrodes (11).

18. Ensemble d'électrode de mesure de potentiel de cellule (2) selon la revendication 11, dans lequel l'aire desdites microélectrodes (11) est de 4×10^2 à 4×10^4 μm^2 et l'aire desdites électrodes de référence (10) est de 64×10^2 à 64×10^4 μm^2.

19. Ensemble d'électrode de mesure de potentiel de cellule (2) selon la revendication 11, dans lequel lesdites microélectrodes (11) et lesdites électrodes de référence (10) sont formées d'un même matériau.

20. Ensemble d'électrode de mesure de potentiel de cellule (2) selon la revendication 19, dans lequel lesdites microélectrodes (11) et lesdites électrodes de référence (10) sont formées au moyen d'un placage de couche de nickel, de placage d'or, et de platine noir sur un film d'oxyde d'étain indium.

21. Ensemble d'électrode de mesure de potentiel de cellule (2) selon la revendication 11, dans lequel ledit substrat d'isolation (13) est presque carré, et les contacts électriques pluraux (7) connectés à l'extrémité de ladite impression conductrice (12) sont distribués et disposés dans quatre côtés dudit substrat d'isolation.

22. Ensemble d'électrode de mesure de potentiel de cellule (2) selon la revendication 11, dans lequel l'indexation de micro marques (17) pour reconnaître visuellement la direction lorsqu'un agrandissement est fourni près des régions de disposition desdites microélectrodes (11).

23. Appareil de mesure de potentiel de cellule comprenant :

un dispositif de placement de cellule (11) ayant un ensemble d'électrode de mesure de potentiel de cellule (2) tel que défini dans l'une quelconque des revendications 11 à 22, et un métal de contact (9) pour être en contact avec son contact électrique (7), et comprenant un support d'électrode (3, 4) pour la fixation dudit substrat d'isolation (13) en couche intermédiaire depuis le dessus et le dessous,
un processeur de signal (30) connecté électriquement audit dispositif de placement de cellule (1) pour traiter les signaux de tension générés entre chaque microélectrode (11) et l'électrode de référence (10) de ladite électrode de mesure de potentiel de cellule (2) au moyen de l'activité des cellules ou des tissus cultivés dans une région enfermée au moyen d'une paroi (6), et
un dispositif optique (21) pour agrandir et observer de façon optique les cellules ou les tissus cultivés dans la région enfermée au moyen de ladite paroi (6).

24. Appareil mesurant un potentiel de cellule selon la revendication 23, comprenant de plus un dispositif de mémoire d'image (22) pour stocker l'image agrandie obtenue au moyen du dispositif optique d'aide (21).

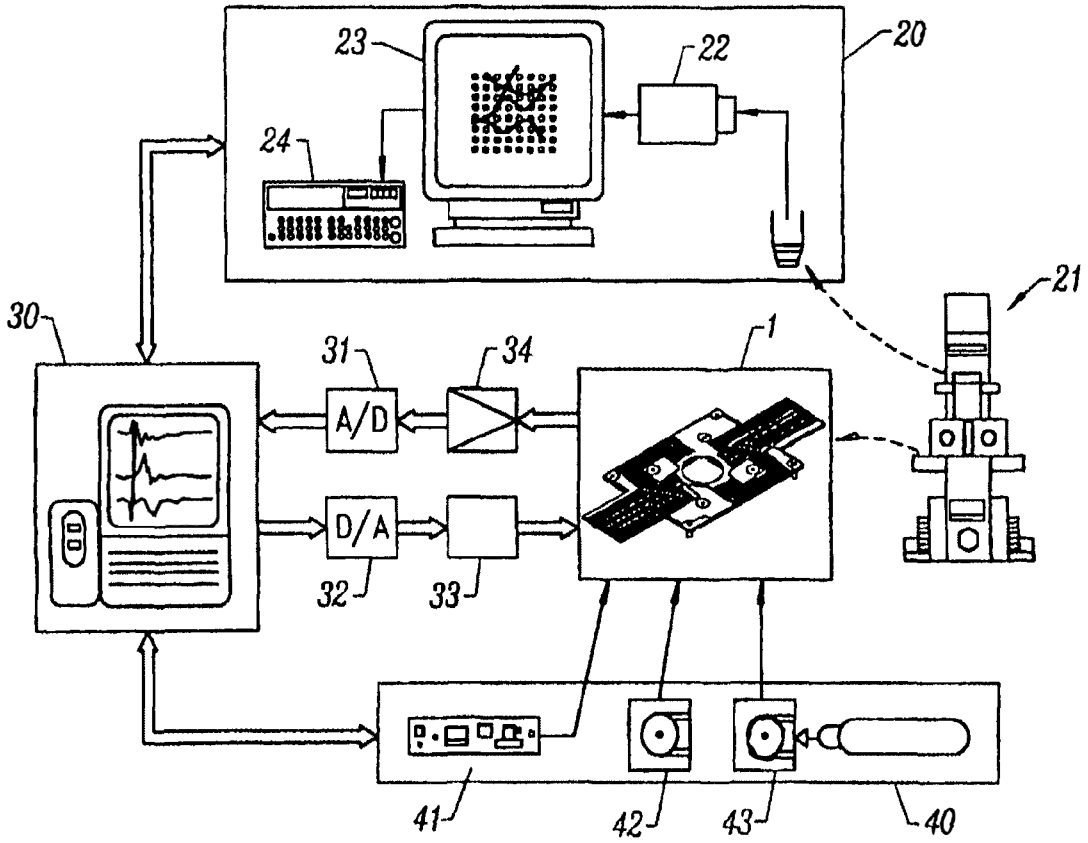


FIG. 1

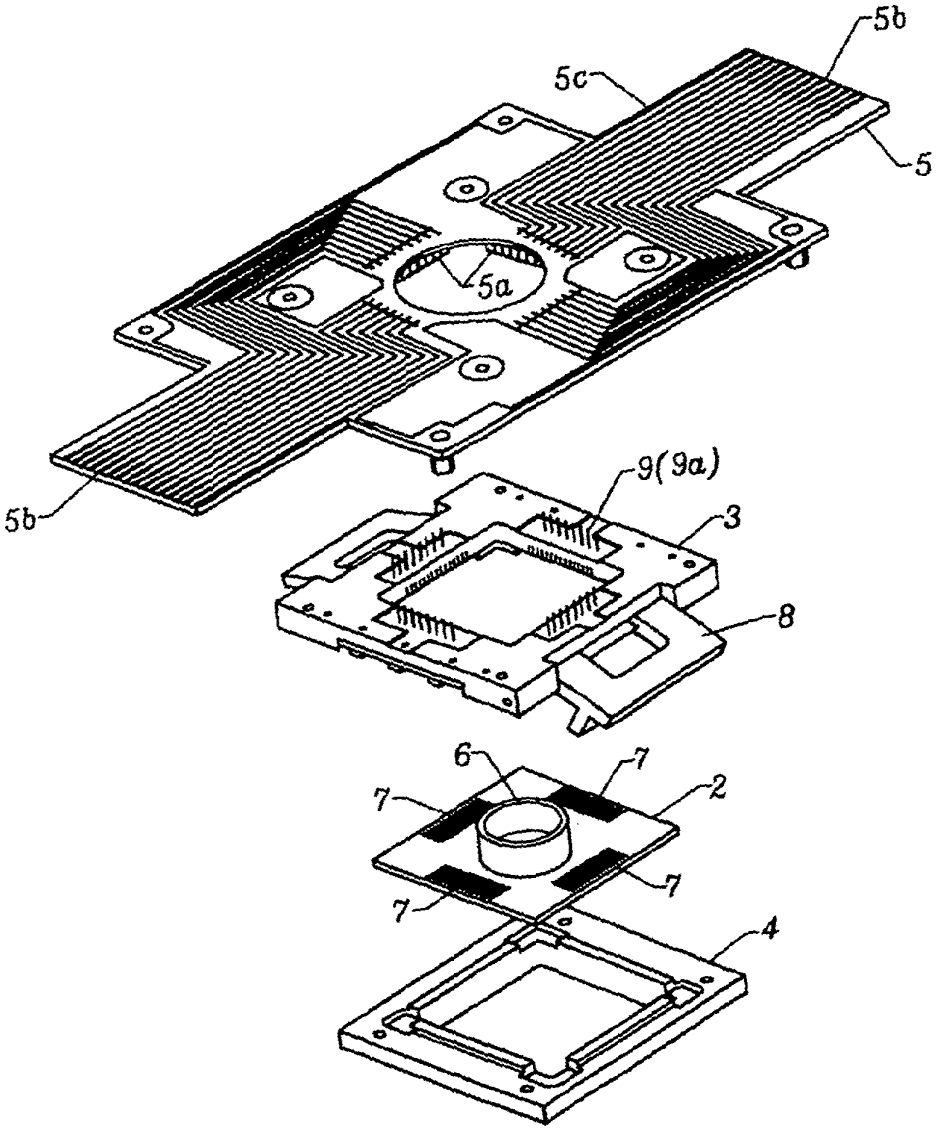


FIG. 2

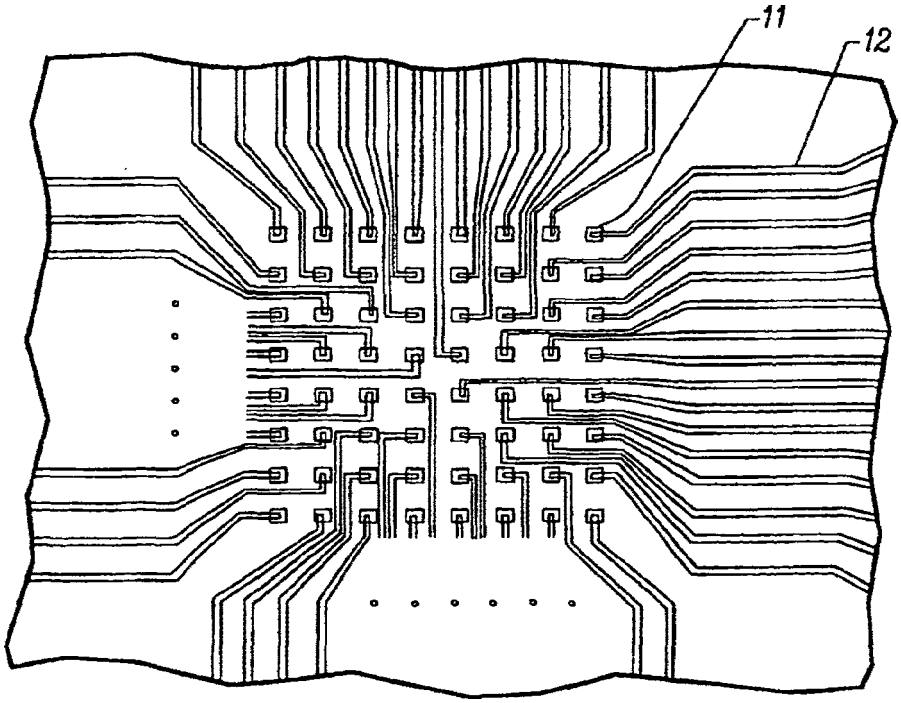


FIG. 3

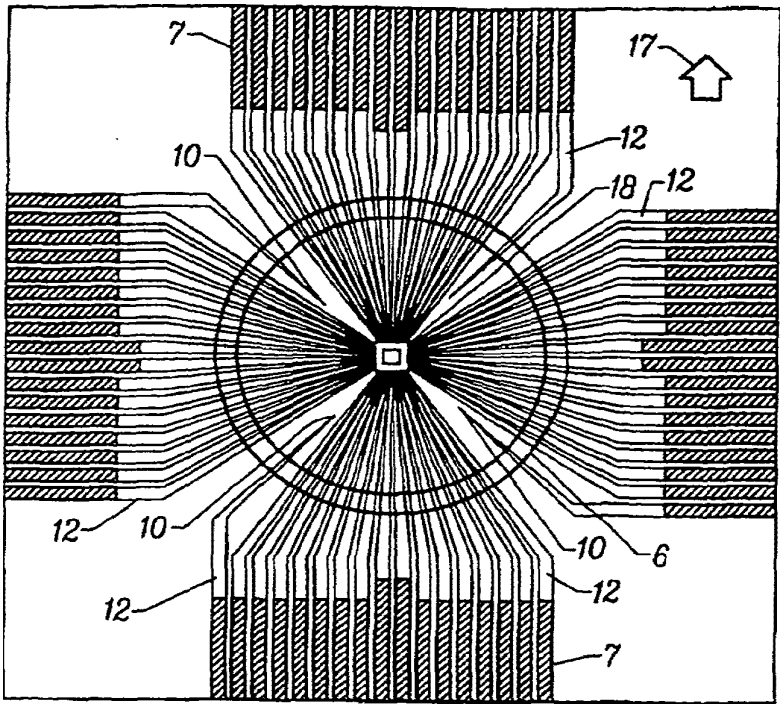


FIG. 4

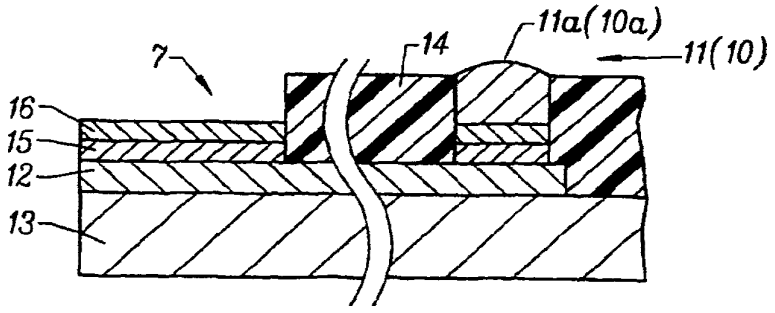


FIG. 5

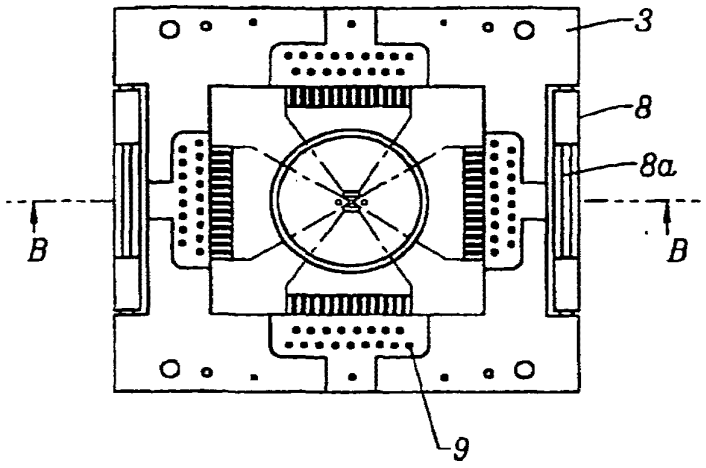


FIG. 6A

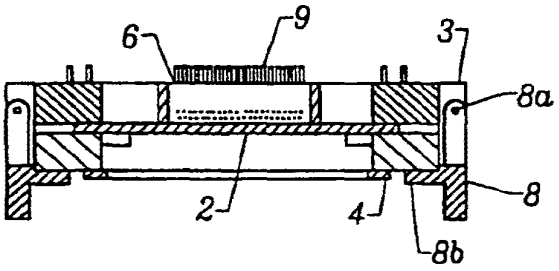


FIG. 6B

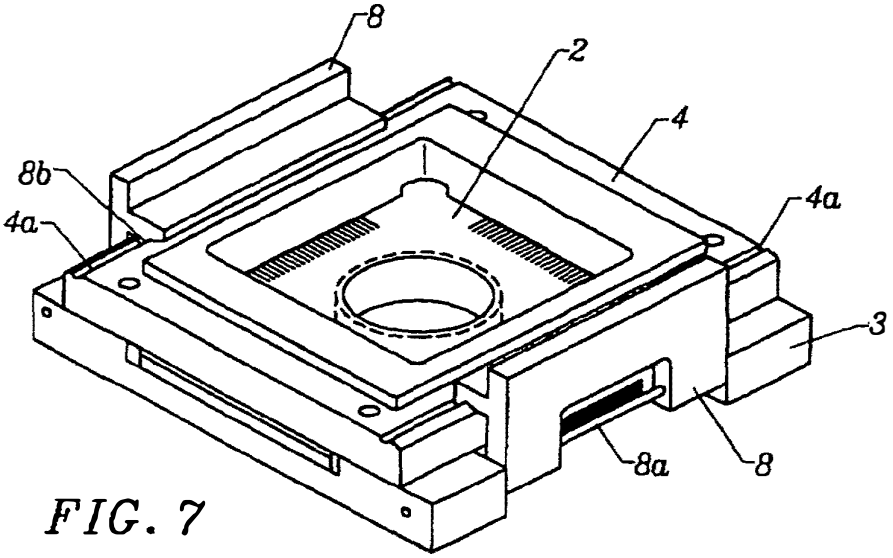


FIG. 7

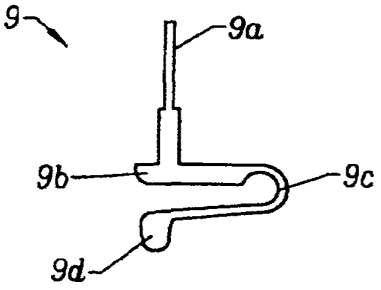


FIG. 8

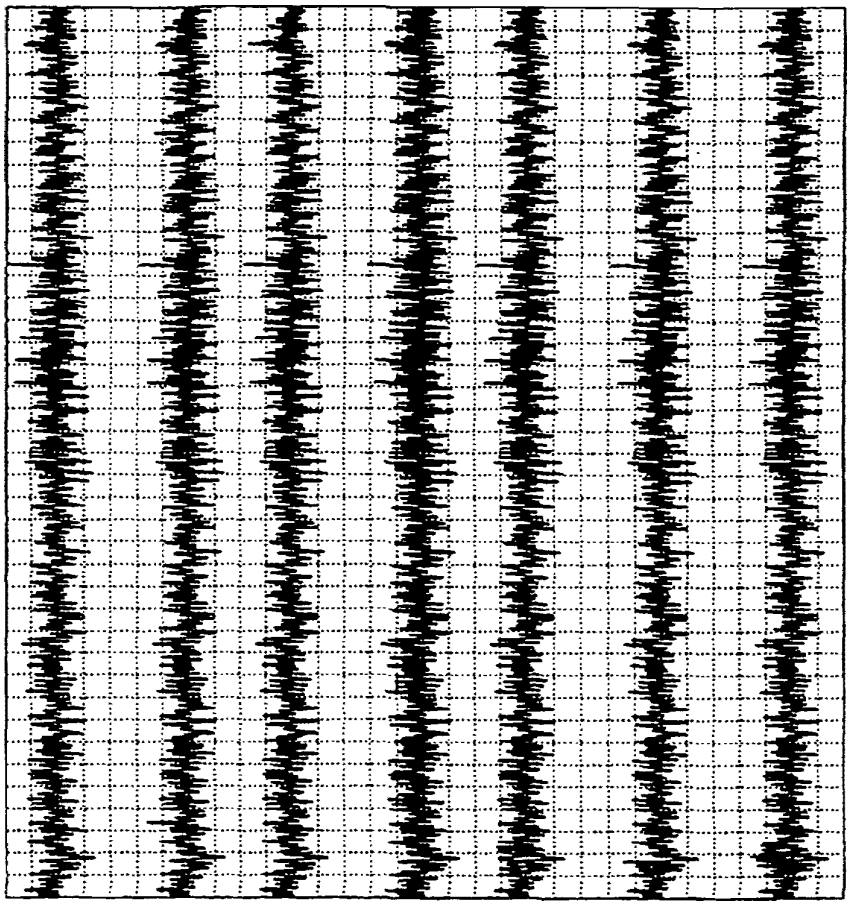


FIG. 9

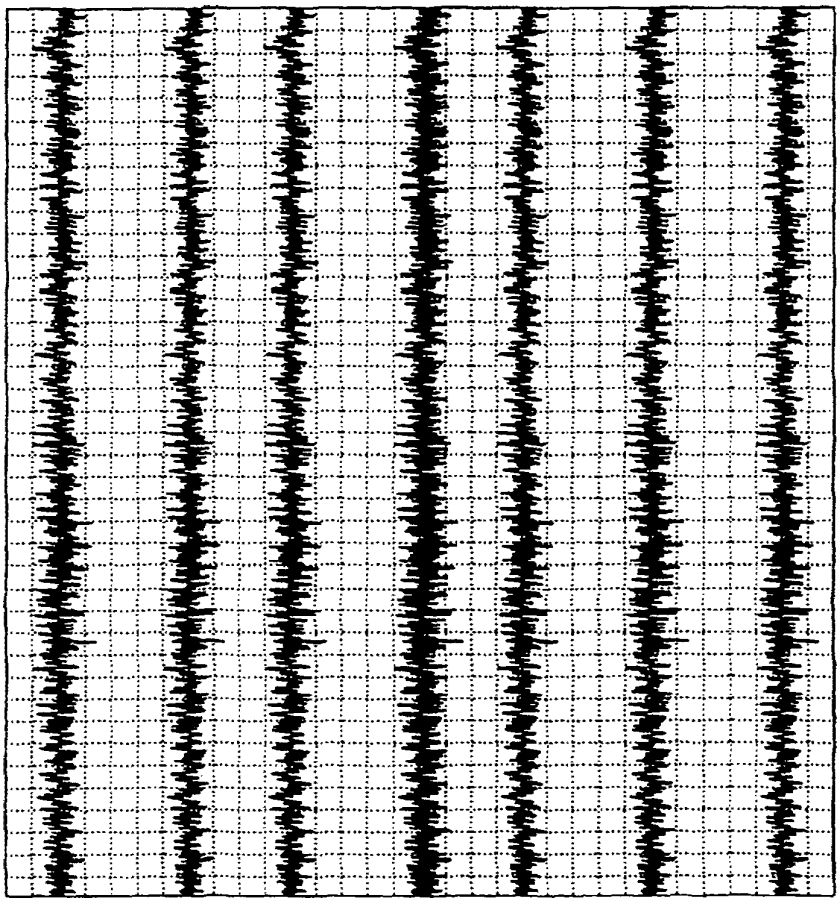


FIG. 10

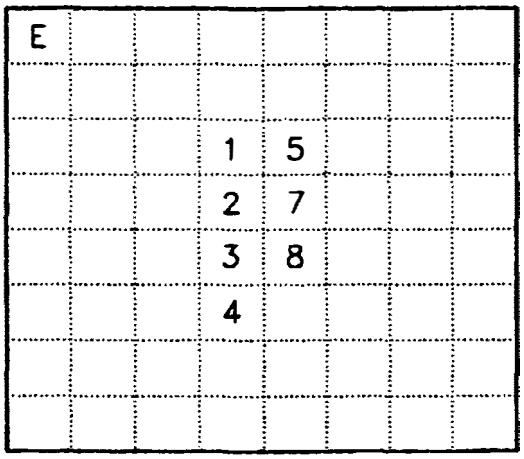


FIG. 11

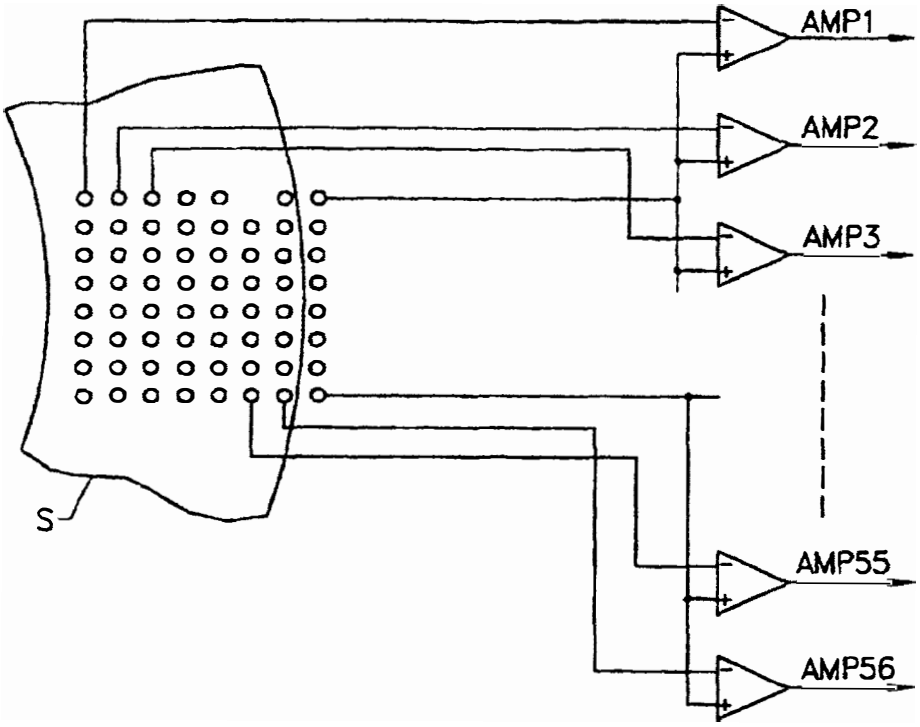


FIG. 12

Electronic Acknowledgement Receipt

EFS ID:	12643151
Application Number:	12435569
International Application Number:	
Confirmation Number:	3664
Title of Invention:	Label-Free Monitoring of Excitation-Contraction Coupling and Excitable Cells Using Impedance Based Systems with Millisecond Time Resolution
First Named Inventor/Applicant Name:	Xiaobo Wang
Customer Number:	59538
Filer:	Raymond C. Wagenknecht
Filer Authorized By:	
Attorney Docket Number:	ACE-134U1
Receipt Date:	26-APR-2012
Filing Date:	05-MAY-2009
Time Stamp:	20:53:08
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Transmittal Letter	IDS1.pdf	71584 0f2c4395c46470fc0ad8c9d048627f19bcb684c9	no	1

Warnings:	
Information:	

2	Information Disclosure Statement (IDS) Form (SB08)	updated_IDS.pdf	615744 04a585c6b064d0957c2e37226edc29dfe3d5a30	no	11
Warnings:					
Information:					
A U.S. Patent Number Citation is required in the Information Disclosure Statement (IDS) form. You may remove the form to add the required data in order to correct the Informational Message or if you chose not to, the image of the form will be processed and be made available within the Image File Wrapper (IFW) system. However, no data will be extracted from this form. Any additional data such as Foreign Patent Documents or Non Patent Literature will be manually reviewed and keyed into USPTO systems.					
3	Non Patent Literature	Baumann.pdf	1015443 8ae42b6c39283d22d5fc9b6342ba217fc0c358ae	no	16
Warnings:					
Information:					
4	Non Patent Literature	Berens.pdf	865676 292f6413c242aa8e1c465337e4fc653fcd5f0f2e	no	12
Warnings:					
Information:					
5	Non Patent Literature	Bieberich.pdf	819138 1701cc32d7b555870b894eaaf4688a281c98d8dc	no	10
Warnings:					
Information:					
6	Non Patent Literature	Burnett.pdf	714850 723cb63086c36f8c52d14c995a3483abc2132c26	no	9
Warnings:					
Information:					
7	Non Patent Literature	Cady.pdf	451901 627ee5a779db6f4ae3fa271427211e16703c0403a	no	8
Warnings:					
Information:					
8	Non Patent Literature	Ehret2.pdf	883145 6ada18585f063a4193f192c925b1515aca57330b	no	14
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9	Non Patent Literature	Ehret.pdf	790653 ec9819661c6ce07beffbbab503c5142bcfe3ff08	no	6
Warnings:					
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10	Non Patent Literature	Giaever2.pdf	405678 7010d3434e295d8a3f01cb4d97803cc67ab238a8	no	5
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11	Non Patent Literature	Giaever.pdf	300836 0b8fb2b930b18bc73e592749d8f84623e8710e18	no	4
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12	Non Patent Literature	Henning.pdf	861000 0e6bb7f7485efe2ac08364810f8921782fa07f38	no	12
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13	Non Patent Literature	Hidalgo.pdf	1579484 39f878c46368bfda520581e0b04080cd11a22dbc	no	15
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14	Non Patent Literature	Hug.pdf	850258 1f0206cb5ac09abd8cc13f64c60e6138a47f7fe5	no	10
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15	Non Patent Literature	Kleinman.pdf	764490 1ef7c66c6bc6442648bd08aff07db8e510b8cc02	no	7
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16	Non Patent Literature	Kowolenko.pdf	493238 14754448011b69de5091014add3b29319d687d5a	no	8
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17	Non Patent Literature	Lo.pdf	510071 3585ae5b2e0297edafe6ee31081df38396ce9e38	no	8
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18	Non Patent Literature	Luong.pdf	552719 ca49c94474bee8e6a1f09ead1393e8a039b5248e	no	5
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Information:					

19	Non Patent Literature	Neher.pdf	110275 fcf48c10e30fd656269a4f3808c696abf157795d	no	1
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20	Non Patent Literature	Ong.pdf	772062 03607d404c461827827ff10eb2b76ff5b797dc07	no	14
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21	Non Patent Literature	Pancrazio.pdf	580257 949b6eb4155d7a5369c1d606923eb8e5b9516c94	no	7
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22	Non Patent Literature	Slaughter.pdf	834695 585c3d0ce9b191f4a6c9bf71d492314aff2a0904	no	8
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23	Non Patent Literature	Stenger.pdf	641268 fe553c2f4865c56f927a5fd1b25d11d9bf463f9e	no	6
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24	Non Patent Literature	Wang.pdf	805123 9e407534fdb9a075a6afb090161e44aa9291fbbe	no	12
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25	Non Patent Literature	Wegener2.pdf	850778 14e926e36f2e20776e9cb70155ae5e0fdbf34716	no	9
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26	Non Patent Literature	Wolf.pdf	797085 e4134b6b89aaa828033939f502970db5e77105f3	no	10
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27	Non Patent Literature	Xiao2.pdf	326618 4c160467cd5d9f92b4253b011135d54af5a3dad	no	4
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28	Non Patent Literature	Xiao3.pdf	540917 6744ebcc9186b1c6558cf1ac9f8a13b922a47fa4	no	7
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Information:					
29	Non Patent Literature	Xiao.pdf	552586 ae170f7577d6848df60545d7c5887ad52371a10e	no	6
Warnings:					
Information:					
30	Foreign Reference	EP1040345B1-Sugihara.pdf	416975 732e3803183fae76f17b5cbef8b208552364a133	no	22
Warnings:					
Information:					
		Total Files Size (in bytes):	19774547		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Patent
Attorney Docket No: ACE-134U1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

WANG et al.

Application Number: 12/435,569

Filed: May 5, 2009

For: LABEL-FREE MONITORING OF EXCITATION-CONTRACTION COUPLING AND EXCITABLE CELLS USING IMPEDANCE BASED SYSTEMS WITH MILLISECOND TIME RESOLUTION

Examiner: Shannon Janssen
Group Art Unit: 1636

Commissioner for Patents
P. O. Box 1450
Alexandria, VA 22313-1450

Information Disclosure Statement (IDS-1)

Sir:

Applicant submits this information disclosure statement before mailing of the first action on the merits for the above identified application. Accordingly, no fee is deemed necessary. Applicant provides herewith a listing of references on the attached PT0-SB08. Copies of Non-Patent Literature and foreign patent documents are provided herewith.

Respectfully submitted,

4/26/2012
Date

Raymond Wagenknecht
Reg. No. 50,948

Biotech Beach Law Group PC
5677 Oberlin Drive, Ste 204
San Diego, CA 92121
(858) 587-2510 x 201

UNITED STATES PATENT AND TRADEMARK OFFICE

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
12/435,569	05/05/2009	Xiaobo Wang	ACE-134U1	3664

59538 7590 06/15/2012

BIOTECH BEACH LAW GROUP , PC
5677 OBERLIN DRIVE, SUITE 204
SAN DIEGO, CA 92121

EXAMINER	
JANSSEN, SHANNON L	
ART UNIT	PAPER NUMBER
1653	

MAIL DATE	DELIVERY MODE
06/15/2012	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	12/435,569	WANG ET AL.	
	Examiner	Art Unit	
	SHANNON JANSSEN	1653	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *27 February 2012*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

5)☒ Claim(s) *1-6,11-14 and 16-45* is/are pending in the application.

5a) Of the above claim(s) *1-6,11-14 and 30-39* is/are withdrawn from consideration.

6)☐ Claim(s) _____ is/are allowed.

7)☒ Claim(s) *16-29 and 40-45* is/are rejected.

8)☒ Claim(s) *16,17,24,44 and 45* is/are objected to.

9)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

12)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *4/26/12*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Claims 1-6, 11-14 and 16-45 are currently pending. Claims 1-6, 11-14 and 30-39 have been withdrawn and claims 16-29 and 40-45 are currently under consideration.

Election/Restrictions

Applicant's election with traverse of Group III, claims 24-29 and newly added claims 40-45 in the reply filed on February 27, 2012 is acknowledged. The traversal is on the ground(s) that the system/apparatus of claim 1 is required for Group III and therefore would not constitute a search burden, that the subject matter of Group II would be examined as part of Group III, and that Groups IV-VI share numerous features and there would not be a search burden. This is found persuasive, in part, and Group II has been rejoined to Group III. This is not found persuasive in regards to Groups I and IV-VI for the reasons of record. In addition, Group IV requires determining a rise in cell index, a decay in cell index, and extrapolating each peak from each rise and decay and monitoring impedance over seconds, minutes, or hours, which is not required by Groups III and V-VI. Further, although applicants assert that the apparatus is used for monitoring excitable cells, this does not exclude other methods of using the apparatus, such as for use as a biosensor for a specific antibody or protein.

Claims 1-6, 11-14 and 30-39 are withdrawn from further consideration pursuant to 37 CFR 1.142(b), as being drawn to a nonelected Inventions, there being no allowable generic or linking claim. Applicant timely traversed the restriction (election) requirement in the reply filed on February 27, 2012. However, claim 16 links inventions III-VI and upon indication of allowability, the restriction requirement between the linked inventions shall be withdrawn and

any claims depending from or otherwise requiring all the limitations of the allowable linking claim will be rejoined.

Priority

The present application claims priority based on U.S. Provisional Application Nos. 61/191,684, filed on September 11, 2008 and 61/126,533, filed on May 5, 2008.

Information Disclosure Statement

The information disclosure statement (IDS) submitted on April 26, 2012 is being considered by the examiner.

Claim Objections

Claims 16 and 24 are objected to for containing non-elected subject matter (i.e.: claim 1). Applicant is required to cancel the claim or rewrite the claim in independent form. Applicant may add in structural requirements that the method of claim 1 would impart.

Claim 17 is objected to because of the following informalities: claim 17 recites "wherein said excitable cells are cells undergo" in line 2 which appears to be missing a restrictive clause (e.g.: such as "that," between "cells" and "undergo") and appears to be a typographical error. Appropriate correction is required.

Claim 44 is objected to because of the following informalities: "peek" in line 3 appears to be a typographical error. Appropriate correction is required.

Claim 45 recites the limitation "resolving cell beating cycles" in lines 1-2. There is insufficient antecedent basis for this limitation in the claim.

Invention as Claimed

One embodiment of the present invention is drawn to a method comprising a) providing the system; b) adding excitable cells to said one or more wells; c) monitoring impedance of said one or more wells using millisecond time resolution; and d) resolving individual cycles of said excitable cells.

Another embodiment of the present invention is drawn to a method of determining whether a compound modulates a beating cycle of an excitable cell, comprising: a) providing the system according to claim 1, wherein said system comprises at least two wells; b) adding excitable cells to said at least two wells; c) monitoring impedance of said at least two wells using millisecond time resolution; d) adding a compound suspected of modulation of the beating cycle to a first of said at least two wells to provide a test well, wherein a second well lacking compound is provided as a control well; e) resolving the beating cycles of both test well and control well; and d) comparing said beating cycles between said test well and control well, wherein a difference in beating cycles indicates said compound modulates the beating cycle of the excitable cell.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claim 16 is rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 16 recites a method for assessing and quantifying excitable cells in vitro. However, the positive method steps of instant claim 16 do not recite a step of quantifying excitable cells, which results in a nexus issue. This issue renders the claim unclear as to whether there is or is not a step of quantifying the cells. Claims 17-23 fail to clarify this and are similarly rejected.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claim 16 is rejected under 35 U.S.C. 102(b) as being anticipated by Cantor (U.S. Patent No. 6,852,525, granted February 8, 2005).

Regarding present **claim 16**, Cantor teaches a method comprising a) providing a system comprising microelectrodes on a substrate, such as a glass substrate (i.e.: nonconductive), for monitoring impedance of cells and further comprising a plurality of cloning cylinders bonded to the substrate to define wells for holding the cells (see col 3, lns 29-53, col 4, lns 4-15, col 9, lns 35-37, Figs. 7 and 9, for example) and a signal monitoring and processing means such as a computer (see col 5, lns 60+, col 6, lns 1-12); b) adding excitable cells, such as neuronal cells, to said one or more wells; c) monitoring impedance of said one or more wells using millisecond time resolution (since it monitors the opening and closing of ion channels which occurs in less than a millisecond; see col 2, lns 39+); and d) resolving individual cycles (e.g.: of ion channels opening, closing, and the cell returning to a resting state) of said excitable cells (see entire

document, particularly col 2, lns 39+, col 3, lns 14-27, col 4, lns 26-46, col 5, lns 1-35, col 8, lns 14-44). Cantor further states:

> "For example, voltage-gated Na^+ channels (among other ion channels) help make nerve cells electrically excitable and enable them to conduct action potentials. When the membrane of a cell with many Na^+ channels is partially depolarized by a momentary stimulus, some of the channels promptly open, allowing Na+ ions to enter the cell. The influx of positive charge depolarizes the membrane further, thereby opening more channels, which admit more Na^+, causing still further depolarization. This process continues in a self-amplifying fashion until the membrane potential has shifted from its resting value of about -70 mV all the way to the Na^+ equilibrium potential of about +50 mV. At that point, where the net electrochemical driving force for the flow of Na^+ is zero, the cell would come to a new resting state with all its Na^+ channels permanently open, if the open channel conformation were stable. The cell is saved from such a permanent electrical tetanus by the automatic inactivation of the Na^+ channels, which now gradually close and stay closed until the membrane potential has returned to its initial negative resting value. The whole cycle, from initial stimulus to return to the original resting state, takes a few milliseconds or less." See col 2, lns 46+.

> "Examining the membrane potential relative to time, an action potential exhibits various characteristics or parameters, including action potential rate (if cells spontaneously depolarize), action potential amplitude, and action potential shape, among others. Action potential rate refers to the frequency with which a cell produces an action potential (rapid depolarization). Action potential amplitude refers to the height of the peak depolarization that occurs in the course of the action potential. Action potential shape refers to the time course of the depolarization and repolarization." See col 3, lns 14-24.

> "The present invention permits detection and monitoring of membrane, chemical, and electrical characteristics of a cell. As noted at the outset, these characteristics include cell impedance (cell membrane capacitance and conductance), action potential parameters, cell membrane capacitance, cell membrane conductance, neuronal action potential, cellular metabolic products (hormones, neurotransmitters, waste products, etc.), and cell/substrate seal resistance. These electrical characteristics, in turn, correlate well with the metabolic state of the cell. The present invention, thus, provides an apparatus and method for monitoring cells and for monitoring the impact that an analyte has upon the metabolism of a cell. It is to be appreciated that such analytes include pharmaceutical agents, drugs, environmental factors, toxins, chemical agents, biological agents, viruses, and cellular adhesion promoters." See Col 4, lns 25-45.

Therefore, the teachings of Cantor anticipate the presently claimed invention.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The factual inquiries set forth in *Graham* **v.** *John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 16-29 and 40-45 are rejected under 35 U.S.C. 103(a) as being unpatentable over Xu et al. (U.S. Patent Application Publication No. 2005/0153425, published July 14, 2005), Banach et al. (Development of electrical activity in cardiac myocyte aggregates derived from mouse embryonic stem cells, Am. J. Physiol. Heart Circ. Physiol., 2003, Vol 284, pp H2114-H2123) as evidenced by Hescheler et al. (Determination of electrical properties of ES cell-

derived cardiomyocytes using MEAs, 2004, Journal of Electrocardiology, Vol 37, pp 110-116), Cantor (U.S. Patent No. 6,852,525, granted February 8, 2005), and Schecter (U.S. Patent Application Publication No. 2005/0182447, published August 18, 2005).

Regarding present **claims 16 and 24, Xu** et al. teach a method for performing real-time cell-based assays investigating the effects of one or more compound on cells comprising providing a system for monitoring cell-substrate impedance comprising a nonconducting substrate, two or more electrode arrays fabricated on the substrate, where each of the two or more electrode arrays comprises two electrode structures, and at least two connection pads, each of which is located on an edge of the substrate, at least one multiple-well device monitoring cell-substrate impedance, in which at least two of the multiple wells each comprise an electrode array at the bottom of the well which are independently addressable, an impedance analyzer, a device station capable of engaging the one or more multiple-well devices and capable of selecting and electrically connecting electrode arrays within any of the multiple wells in to the impedance analyzer, and a software program to control the device station and perform data acquisition and data analysis on impedance values measured by the impedance analyzer, depositing cells on at least one of the two or more arrays of the device, adding one or more compounds to the one or more wells, providing at least one control well lacking compound, and monitoring cell-substrate impedance at regular or irregular time intervals on one or more arrays of the device to ascertain if the compound modulates the cell (see [0018-0020, 0023, 0024, 0029, 0031, 0032, 0036, 0130, 0131, 0134, 0244, 0246], for example).

Regarding present **claim 29**, Xu et al. provides methods for calculating a Cell Index for quantifying and comparing cell-substrate impedance (see [0021, 0140+], for example).

Regarding present **claim 45**, Xu et al. teach setting the first order derivatives to zero (see [0209], for example).

While Xu et al. teach a method for performing real-time cell-based assays investigating the effects of one or more compound on cells, Xu et al. do not teach excitable cells which undergo excitation-contraction coupling or cardiomyocytes as recited in instant **claims 16-18, 24 and 25**, resolving individual cycles or beating cycles as recited in instant **claims 16 and 24**, at least 2 consecutive impedance measurements at millisecond (ms) time resolution as recited in instant **claims 19-23 and 40-43**, or identifying changes in beating amplitude or beating frequency as recited in instant **claims 26-29 and 45**.

However, **Banach** et al. teach a microelectrode array for monitoring the beating cycle of cardiomyocytes and further teach monitoring electrical activity from multiple electrodes simultaneously and continuously with ms time resolution and resolving signal delays of less than 10 ms (e.g.: at least two consecutive measurements less than 10 milliseconds apart since it is continuously measured and this could be broadly and reasonably interpreted as consecutive measurements; see entire document, particularly p H2115, cols 1-2, Figs. 1, 3, and 4, for example). Banach et al. teach monitoring the interspike interval to determine the beating frequency and measuring peak amplitude (e.g.: would be regular or irregular changes; see p 2115, col 1, para 2, p H2116, col 1, para 1). Banach et al. teach measuring the effect of different compounds on beating frequency and action potential in the form of FP_{MIN} to FP_{MAX} (e.g.: amplitude; see p H2115, col 2, para 1, p H2118, col 2, para 2, p H2119, p H2120, col 1, para 1, col 2, para 4, Figs. 4, 6, and 7). Banach et al. further state:

> "In the present study, we analyzed the developmental changes of spontaneous electrical activity with repeated multielectrode recordings using substrate-integrated

> microelectrode arrays (MEAs). This technique allows not only the long-term culture of the preparation but also the analysis of the spatial electrophysiological differences within the preparation. In the present study, we analyzed different field potential (FP) parameters to characterize the development of the spontaneous electrical activity of multicellular aggregates of ESCs and their electrophysiological properties. Furthermore, the use of an ES cell line deficient in the expression of connexin43 ($Cx43^{-/-}$), the major cardiac gap junction protein, enabled us to distinguish developmental changes of the active, voltage-dependent membrane properties from changing passive components like the intercellular resistance." See p H2114, col 2, Para 1.

In addition, Hescheler, which cites to the microelectrode array system taught by Banach et al. (see p 113, Fig. 3) and, regarding the arrays, state:

> "It may provide a new experimental model to study the impact of cell-cell-interactions and growth factors as well as of extracellular matrix proteins. In addition, the cellular system described, may be useful as an in vitro assay for pharmacological investigations of chronotropic drugs and as a cellular system for studying cardiotoxic effects during embryogenesis in vitro." See p 115, cols 1-2, bridging para.

Further, **Cantor** teaches a method comprising a) providing a system comprising microelectrodes on a substrate, such as a glass substrate (i.e.: nonconductive), for monitoring impedance of cells and further comprising a plurality of cloning cylinders bonded to the substrate to define wells for holding the cells (see col 3, lns 29-53, col 4, lns 4-15, col 9, lns 35-37, Figs. 7 and 9, for example) and a signal monitoring and processing means such as a computer (see col 5, lns 60+, col 6, lns 1-12); b) adding excitable cells, such as neuronal cells, to said one or more wells; c) monitoring impedance of said one or more wells using millisecond time resolution (since it monitors the opening and closing of ion channels which occurs in less than a millisecond; see col 2, lns 39+; and d) resolving individual cycles (e.g.: of ion channels opening, closing, and the cell returning to a resting state) of said excitable cells (e.g.: measuring impedance peaks in millisecond time resolution; see entire document, particularly col 2, lns 39+, col 3, lns 14-27, col 4, lns 26-46, col 5, lns 1-35, col 8, lns 14-44). Cantor further states:

> "For example, voltage-gated Na^+ channels (among other ion channels) help make nerve cells electrically excitable and enable them to conduct action potentials. When the membrane of a cell with many Na^+ channels is partially depolarized by a momentary stimulus, some of the channels promptly open, allowing Na+ ions to enter the cell. The influx of positive charge depolarizes the membrane further, thereby opening more channels, which admit more Na^+, causing still further depolarization. This process continues in a self-amplifying fashion until the membrane potential has shifted from its resting value of about -70 mV all the way to the Na^+ equilibrium potential of about +50 mV. At that point, where the net electrochemical driving force for the flow of Na^+ is zero, the cell would come to a new resting state with all its Na^+ channels permanently open, if the open channel conformation were stable. The cell is saved from such a permanent electrical tetanus by the automatic inactivation of the Na^+ channels, which now gradually close and stay closed until the membrane potential has returned to its initial negative resting value. The whole cycle, from initial stimulus to return to the original resting state, takes a few milliseconds or less." See col 2, lns 46+.

> "Examining the membrane potential relative to time, an action potential exhibits various characteristics or parameters, including action potential rate (if cells spontaneously depolarize), action potential amplitude, and action potential shape, among others. Action potential rate refers to the frequency with which a cell produces an action potential (rapid depolarization). Action potential amplitude refers to the height of the peak depolarization that occurs in the course of the action potential. Action potential shape refers to the time course of the depolarization and repolarization." See col 3, lns 14-24.

> "The present invention permits detection and monitoring of membrane, chemical, and electrical characteristics of a cell. As noted at the outset, these characteristics include cell impedance (cell membrane capacitance and conductance), action potential parameters, cell membrane capacitance, cell membrane conductance, neuronal action potential, cellular metabolic products (hormones, neurotransmitters, waste products, etc.), and cell/substrate seal resistance. These electrical characteristics, in turn, correlate well with the metabolic state of the cell. The present invention, thus, provides an apparatus and method for monitoring cells and for monitoring the impact that an analyte has upon the metabolism of a cell. It is to be appreciated that such analytes include pharmaceutical agents, drugs, environmental factors, toxins, chemical agents, biological agents, viruses, and cellular adhesion promoters." See Col 4, lns 25-45.

While Xu et al., Banach et al., and Cantor teach a method for performing real-time cell-based assays investigating the effects of one or more compound on cells and measuring beating frequency and amplitude, Xu et al., Banach et al., and Cantor do not *specifically* teach determining a rise in impedance and a decay in impedance and extrapolating a peak from each

rise and decay as recited in instant **claim 44** or identifying impedance data points at which the first order derivative to time is zero or about zero as recited in instant **claim 45**.

However, **Schecter** teaches a method of monitoring beating cycle of the heart using impedance measurements and further teach calculating the derivative of impedance to time at 0 and the peak and delays in time to peak incidence and changes in the impedance waveform (e.g. time to onset of positive dZ/dt, time to peak Z, time to peak dZ/dt, which can be broadly and reasonably interpreted as rise in impedance and decay in impedance and determining a peak) and/or reductions in the quantity of the integral of Z or changes in the morphology of Z(t)dt (see entire document, particularly [0048, 0049, 0060, 0077, 0086-0090, 0100-0101, 0118, 0136], Figs. 5, 6, 8-10, 12). Schecter further state:

> "Derivation of impedance waveforms using implanted lead systems is well known in the art and has been described in the literature. Data acquisition can be accomplished, for example, by delivering pulses of 200 uA, 30 us pulse width at a frequency of 128 Hz applied to two electrodes positioned along one vector (electrode pairs) and measuring the resulting voltage between electrodes located along the same vector. These pulses will not depolarize myocardium, cause only limited battery drain and have a frequency with an acceptable signal to noise ratio. The resultant time dependent impedance, Z(t) peaks when there is maximal systolic ventricular wall thickness and minimal intracardiac blood volume." See [0048].
>
> "The time dependent impedance signals or waveforms derived in this fashion relate to intrinsic myocardial properties. If signals are acquired between specific electrode pairs, the regional myocardial properties can be derived. The integrity of these waveforms may be subject to significant noise (poor signal to noise ratio) and inferior signal quality may result. This is especially true if data sampling occurs in a vector where there is impairment in myocardial contractile properties. Derivation of specific characteristics of these waveforms may suffice, even though overall signal quality is poor. Measurement of peak impedance and first and second order derivatives of impedance waveforms will relate to myocardial contractility. Assessment of the time required for a waveform to reach peak impedance will relate to myocardial synchrony if comparisons can be made to waveforms derived in alternate regions (e.g. right and left ventricular vectors)." See [0049].

Therefore it would have been obvious to one of skill in the art at the time of the instant invention to measure the beating cycle of excitable cells such as cardiomyocytes at millisecond time resolution, to record multiple measurements over time in millisecond time resolution, to characterize the beating cycle of the cardiomyocytes and identify changes in beating amplitude and frequency by using a cell index, extrapolating a peak, and/or identifying data points where the first order derivative to time is zero.

One would have been motivated to characterize the beating cycle and to examine various parameters of the beating cycle, such as beating frequency and amplitude, or the beating frequency and amplitude relative to the number of cells (e.g.: cell index), in order to characterize the culture of beating cardiomyocytes *in vitro* as taught by Banach et al. (e.g.: for use as a model; see p H2114, col 2, para 1, for example) and to utilize the culture to characterize cardiotoxic effects of drugs as taught by Hescheler et al. (see above). In light of the fact that measuring the various peaks and rise and decay of a cardiac cycle will detail the condition of the heart, or in this case, the culture representing the heart, it would have been obvious to one of ordinary skill in the art to measure the peaks comprising the rise and decay of the cardiac cycle to gain a better determining of the state of the heart. Further, due to the inherent property of signals and peaks, it is well known that for a signal that has a rise and fall (e.g.: a peak), the first derivative yields a signal that crosses zero at the maximum peak height of the original signal. One would have been motivated to utilize impedance measurements because Xu et al. teach that the impedance system can be used for real-time cell-based assays in high throughput, e.g., rather than just an end-point assay which limits the time resolution and number of time points for large library screening (see [0014, 0016, 0024, 0025, 0031, 0032], for example) and Cantor teaches the need for electrodes

which can monitor cellular changes including ion channel conductance (e.g.: which are responsible for the action potentials that cause cell beating; see above and col 2, lns 31-40).

One would have had a reasonable expectation for success because Banach et al. teach successfully establishing cultures of beating cardiomyocytes and measuring electrical properties of the cultures to establish beating cycle parameters and beating frequency, including changes in the parameters after administration of drugs, Cantor teaches that electrode arrays can be used to measure cell impedance, secretion of cellular products, monitoring of ion channels and action potentials, and monitoring of compounds which affect those parameters in millisecond time resolution (see above and cols 1-2), Xu et al. teach successfully using impedance to characterize cellular properties (see [0428], for example), and Schecter teach that it is routine and known to derive impedance waveforms (see supra).

Therefore the teachings of Xu et al., Banach et al. as evidenced by Hescheler et al., Cantor, and Schecter render the present invention *prima facie* obvious.

Double Patenting

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory obviousness-type double patenting rejection is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference

claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); and *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the conflicting application or patent either is shown to be commonly owned with this application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement.

Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

Claims 16-19, 24-29, and 44 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-15 of copending Application No. 13/109809. Although the conflicting claims are not identical, they are not patentably distinct from each other because both are directed to methods of monitoring a beating cycle of cardiomyocytes comprising monitoring impedance changes in multiple wells in millisecond time resolution, adding a test compound to the wells, and monitoring changes in beating parameters for the test compound compared to a control, wherein the beating parameters are cell index, beating rate, beating amplitude, rising time, falling time, beating period, rising slope, and falling slope.

This is a provisional obviousness-type double patenting rejection because the conflicting claims have not in fact been patented.

Claims 16-19 and 24-29 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 12-41 of copending Application No. 12/774709. Although the conflicting claims are not identical, they are not patentably distinct from each other because both are directed to methods of monitoring a beating cycle of cardiomyocytes comprising monitoring impedance changes in multiple wells in millisecond time resolution, adding a test compound to the wells, and monitoring changes in beating cycle for the test compound compared to a control and calculating a cell index.

This is a provisional obviousness-type double patenting rejection because the conflicting claims have not in fact been patented.

Future Communication

Any inquiry concerning this communication or earlier communications from the examiner should be directed to SHANNON JANSSEN whose telephone number is (571)270-1303. The examiner can normally be reached on Generally M-F 10:00AM-7:00PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Sharmila Landau can be reached on (571) 272-0614. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

Shannon L Janssen
SLJ

/Amber D. Steele/

Primary Examiner, Art Unit 1654

Notice of References Cited	Application/Control No. 12/435,569	Applicant(s)/Patent Under Reexamination WANG ET AL.	
	Examiner SHANNON JANSSEN	Art Unit 1653	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
*	A	US-2005/0153425	07-2005	Xu et al.	435/287.1
*	B	US-6,852,525	02-2005	Cantor, Hal C.	435/288.3
*	C	US-2005/0182447	08-2005	Schecter, Stuart O.	607/002
	D	US-			
	E	US-			
	F	US-			
	G	US-			
	H	US-			
	I	US-			
	J	US-			
	K	US-			
	L	US-			
	M	US-			

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	Banach et al. (Development of electrical activity in cardiac myocyte aggregates derived from mouse embryonic stem cells, Am. J. Physiol. Heart Circ. Physiol., 2003, Vol 284, pp H2114-H2123)
	V	Hescheler et al. (Determination of electrical properties of ES cell-derived cardiomyocytes using MEAs, 2004, Journal of Electrocardiology, Vol 37, pp 110-116)
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	12435569	WANG ET AL.
	Examiner	Art Unit
	SHANNON JANSSEN	1653

SEARCHED			
Class	Subclass	Date	Examiner

SEARCH NOTES		
Search Notes	Date	Examiner
Inventor Name Search - PALM, EAST	6/6/12, 6/8/12	SLJ
USPAT, USPGPUB, EPO, JPO, DERWENT- see search notes	6/8/12	SLJ
Pubmed, Scirus - search terms: impedance, resistance, excitable, cardiac, cardiomyocyte, ion channel, neuron, neuronal, neural, array, beat, beating, cycle, well, screen, assay, and various combinations	6/8/12	SLJ

INTERFERENCE SEARCH			
Class	Subclass	Date	Examiner

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12/435,569	05/05/2009 RULE	506	1653	ACE-134U1

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**** CONTINUING DATA **************************

This appln claims benefit of 61/191,684 09/11/2008
and claims benefit of 61/126,533 05/05/2008

**** FOREIGN APPLICATIONS **************************

**** IF REQUIRED, FOREIGN FILING LICENSE GRANTED **** ** SMALL ENTITY **
05/14/2009

Foreign Priority claimed	35 USC 119(a-d) conditions met	Verified and Acknowledged	Met after Allowance	STATE OR COUNTRY	SHEETS DRAWINGS	TOTAL CLAIMS	INDEPENDENT CLAIMS
☐ Yes ☑ No	☐ Yes ☑ No	/SHANNON L JANSSEN/ Examiner's Signature	☐ Initials	CA	42	39	1

ADDRESS

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UNITED STATES

TITLE

Label-Free Monitoring of Excitation-Contraction Coupling and Excitable Cells Using Impedance Based Systems with Millisecond Time Resolution

FILING FEE RECEIVED	FEES: Authority has been given in Paper	
1051	No._____ to charge/credit DEPOSIT ACCOUNT No._____ for following:	☐ All Fees ☐ 1.16 Fees (Filing) ☐ 1.17 Fees (Processing Ext. of time) ☐ 1.18 Fees (Issue) ☐ Other _____ ☐ Credit

EAST Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
S1	85	((XIAOBO) near2 (WANG)).INV.	US-PGPUB; USPAT; USOCR	SAME	ON	2012/06/08 15:54
S2	22	((YAMA) near2 (ABASSI)).INV.	US-PGPUB; USPAT; USOCR	SAME	ON	2012/06/08 15:54
S3	5	((BIAO) near2 (XI)).INV.	US-PGPUB; USPAT; USOCR	SAME	ON	2012/06/08 15:54
S4	180	((WEN) near2 (ZHANG)).INV.	US-PGPUB; USPAT; USOCR	SAME	ON	2012/06/08 15:54
S5	120	((XIAO) near2 (XU)).INV.	US-PGPUB; USPAT; USOCR	SAME	ON	2012/06/08 15:54
S6	98	acea.as.	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	SAME	ON	2012/06/08 15:55
S7	0	S1 S2 S3 S4 S5 S6	US-PGPUB; USPAT; USOCR	SAME	ON	2012/06/08 15:55
S8	355	S1 S2 S3 S4 S5 S6	US-PGPUB; USPAT; USOCR	OR	ON	2012/06/08 15:55
S9	38	S8 and impedance.clm.	US-PGPUB; USPAT; USOCR	OR	ON	2012/06/08 15:55
S10	28	S8 and impedance.clm. and cell.clm.	US-PGPUB; USPAT; USOCR	OR	ON	2012/06/08 15:56
S11	3	S10 and (excitable or cardiac or cardiomyocyte).clm.	US-PGPUB; USPAT; USOCR	OR	ON	2012/06/08 15:56
S12	8	S10 and (excitable or cardiac or cardiomyocyte)	US-PGPUB; USPAT; USOCR	OR	ON	2012/06/08 15:57
S13	8	S10 and (excitable or cardiac or cardiomyocyte or beat$3)	US-PGPUB; USPAT; USOCR	OR	ON	2012/06/08 15:57
S14	3	S10 and (excitable or cardiac or cardiomyocyte or beat$3).clm.	US-PGPUB; USPAT; USOCR	OR	ON	2012/06/08 15:58

EAST Search History (Prior Art)

S15	29565	impedance cell	US-PGPUB; USPAT; USOCR	SAME	ON	2012/06/08 15:59
S16	1983	S15 and (excitable or cardiac or cardiomyocyte)	US-PGPUB; USPAT; USOCR	SAME	ON	2012/06/08 15:59
S17	1162	S15 and (excitable or (cardiac cell) or cardiomyocyte)	US-PGPUB; USPAT; USOCR	SAME	ON	2012/06/08 16:00
S18	7645	impedance (beat$3 or contract or contraction)	US-PGPUB; USPAT; USOCR	SAME	ON	2012/06/08 16:00
S19	174	S17 and S18	US-PGPUB; USPAT; USOCR	SAME	ON	2012/06/08 16:00
S20	36	S19 and (culture or vitro)	US-PGPUB; USPAT; USOCR	SAME	ON	2012/06/08 16:01
S21	45782	impedance (beat$3 or cycle)	US-PGPUB; USPAT; USOCR	SAME	ON	2012/06/08 16:01
S22	241	S17 and S21	US-PGPUB; USPAT; USOCR	SAME	ON	2012/06/08 16:02
S23	50	S22 and (culture or vitro)	US-PGPUB; USPAT; USOCR	SAME	ON	2012/06/08 16:02
S42	345	cell impedance (cardiomyocyte or cardiac)	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	SAME	ON	2012/06/08 22:10
S43	248	S42 and @ay<="2008"	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	SAME	ON	2012/06/08 22:11
S44	150	S43 and (beat$3 or cycle)	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	SAME	ON	2012/06/08 22:11
S45	71	S43 and (beat$3 or cycle) cell	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	SAME	ON	2012/06/08 22:11

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Not for submission under 37 CFR 1.99)

Application Number	12435569
Filing Date	2009-05-05
First Named Inventor	Xiaobo Wang
Art Unit	1636
Examiner Name	Shannon Janssen
Attorney Docket Number	ACE-134U1

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	Examiner Name	Shannon Janssen
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	Filing Date	2009-05-05
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	Art Unit	1636
	Examiner Name	Shannon Janssen
	Attorney Docket Number	ACE-134U1

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Examiner Signature	/Shannon Janssen/	Date Considered	06/06/2012

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (**Not for submission under 37 CFR 1.99)**		
	Application Number	12435569
	Filing Date	2009-05-05
	First Named Inventor	Xiaobo Wang
	Art Unit	1636
	Examiner Name	Shannon Janssen
	Attorney Docket Number	ACE-134U1

CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

☐ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

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☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

☒ See attached certification statement.

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A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Raymond Wagenknecht/	Date (YYYY-MM-DD)	2012-04-26
Name/Print	Raymond Wagenknecht	Registration Number	50948

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1 hour to complete, including gathering, preparing and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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Patent
Attorney Docket No: ACE-134U1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	
WANG et al.	
Application Number: 12/435,569	Examiner: Shannon Janssen Group Art Unit: 1636
Filed: May 5, 2009	
For: LABEL-FREE MONITORING OF EXCITATION-CONTRACTION COUPLING AND EXCITABLE CELLS USING IMPEDANCE BASED SYSTEMS WITH MILLISECOND TIME RESOLUTION	

Commissioner for Patents
P. O. Box 1450
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Response to Office Action 2
(Non-Final Action)

Sir:

Applicants are responding to the Office Action mailed June 15, 2012 which provides a three month period for reply. This response is filed within six months and is therefore accompanied with a request for a three month extension of time.

Amendments to the Claims begin on page 2 of this paper.

Remarks / Arguments begin on page 12 of this paper.

Amendments to the Claims

Changes to the claims are provided in the below listing of claims for the convenience of the examiner.

Listing of Claims

1-15. (Cancelled)

16. (Currently amended) A method for assessing ~~and quantifying~~ excitable cells in vitro, comprising

a) providing ~~the system according to Claim 1~~; a system for monitoring impedance of excitable cells in vitro, comprising:

i) a device for monitoring cell-substrate impedance, comprising:

a) a nonconductive substrate,

b) one or more electrode arrays fabricated on said substrate,

c) one or more wells on said substrate, wherein each of said one or more arrays is associated with one of said one or more wells, wherein each electrode array is individually addressable, further wherein said substrate has a surface suitable for cell attachment, wherein said cell attachment on said substrate can result in a detectable change in impedance between electrodes within each electrode array,

ii) an impedance analyzer capable of impedance measurement at millisecond time resolution,

iii) electronic circuitry that can engage said device and selectively connect said two or more electrode arrays of said device to said impedance analyzer, and

(iv) a software program that controls said electronic circuitry and records and analyzes data obtained from said impedance analyzer;

b) adding excitable cells to said one or more wells;

c) monitoring cell-substrate impedance of said one or more wells using millisecond time resolution to detect changes in cell morphology or cell attachment to said substrate; and

d) resolving individual cycles of said excitable cells.

17. (Currently amended) The method according to claim 16, wherein said excitable cells are cells that undergo excitation-contraction coupling.

18. (Original) The method according to claim 17, wherein said excitable cells are cardiomyocytes.

19. (Original) The method according to claim 16, wherein the step of monitoring impedance comprises performing impedance measurements at millisecond time resolution simultaneously across all wells.

20. (Original) The method according to claim 19, wherein said impedance measurements at millisecond time resolution comprises at least two consecutive impedance measurements at less than 300 milliseconds apart.

21. (Original) The method according to claim 20, wherein said at least two consecutive impedance measurements are less than 100 milliseconds apart.

22. (Original) The method according to claim 21, wherein said at least two consecutive impedance measurements are less than 40 milliseconds apart.

23. (Original) The method according to claim 22, wherein said at least two consecutive impedance measurements are less than 10 milliseconds apart.

24. (Currently amended) A method of determining whether a compound modulates a beating cycle of an excitable cell, comprising:

a) providing ~~the system according to claim 1, wherein said system comprises at least two wells;~~ a system for monitoring impedance of excitable cells in vitro, comprising:

i) a device for monitoring cell-substrate impedance, comprising:

a) a nonconductive substrate,

b) one or more electrode arrays fabricated on said substrate,

c) at least two wells on said substrate, wherein each of said one or more arrays is associated with each of said at least two wells, wherein each electrode array is individually addressable, further wherein said substrate has a surface suitable for cell attachment, wherein said cell attachment on said substrate can result in a detectable change in impedance between electrodes within each electrode array,

ii) an impedance analyzer capable of impedance measurement at millisecond time resolution,

iii) electronic circuitry that can engage said device and selectively connect said two or more electrode arrays of said device to said impedance analyzer, and

(iv) a software program that controls said electronic circuitry and records and analyzes data obtained from said impedance analyzer;

b) adding excitable cells to said at least two wells;

c) monitoring cell-substrate impedance of said at least two wells using millisecond time resolution to detect changes in cell morphology or cell attachment to said substrate;

d) adding a compound suspected of modulation of the beating cycle to a first of said at least two wells to provide a test well, wherein a second well lacking a compound that modulates the beating cycle is provided as a control well;

e) resolving the beating cycles of both test well and control well; and

d) comparing said beating cycles between said test well and control well, wherein a difference in beating cycles indicates said compound modulates the beating cycle of the excitable cell.

25. (Original) The method according to claim 24, wherein the excitable cells are cardiomyocytes or embryonic stem cell derived cardiomyocytes.

26. (Original) The method according to claim 24, wherein said beating cycles are compared to identify changes in beating amplitude.

27. (Original) The method according to claim 24, wherein said beating cycles are compared to identify changes in beating frequency.

28. (Original) The method according to claim 27, wherein said changes in beating frequency are regular changes or irregular changes.

29. (Original) The method according to claim 24, further comprising determining a cell index for each of said test well and control well, wherein the step of comparing said beating cycles comprises comparing said cell indexes between test well and control well.

30. (Withdrawn-Currently amended) A method of characterizing rhythmic beating of a cardiomyocyte comprising:

a) providing ~~the system according to claim 1;~~ a system for monitoring impedance of excitable cells in vitro, comprising:

i) a device for monitoring cell-substrate impedance, comprising:

a) a nonconductive substrate,

b) one or more electrode arrays fabricated on said substrate,

c) one or more wells on said substrate, wherein each of said one or more arrays is associated with each of said one or more wells, wherein each electrode array is individually addressable, further wherein said substrate has a surface suitable for cell attachment, wherein said cell attachment on said substrate can result in a detectable change in impedance between electrodes within each electrode array,

ii) an impedance analyzer capable of impedance measurement at millisecond time resolution,

iii) electronic circuitry that can engage said device and selectively connect said two or more electrode arrays of said device to said impedance analyzer, and

(iv) a software program that controls said electronic circuitry and records and analyzes data obtained from said impedance analyzer;

b) adding excitable cells to said one or more wells;

c) monitoring cell-substrate impedance of said one or more wells in millisecond time resolution to detect changes in cell morphology or cell attachment to said substrate; and

d) determining a plurality of beating cycle peaks in a time unit; and

e) comparing amplitude or frequency of said peaks over said time unit.

31. (Withdrawn) The method according to claim 30, wherein the step of determining a plurality of beating cycle peaks comprises determining a rise in impedance and a decay in impedance and extrapolating a peak from each rise and decay.

32. (Withdrawn) The method according to claim 30, further comprising determining a cell index for each of said one or more wells, wherein the step of determining a plurality of beating cycle peaks comprises for each cycle determining a rise in cell index, a decay in cell index and extrapolating each peak from each rise and decay.

33. (Withdrawn) The method according to claim 30, wherein beating cycle peaks are determined by identifying impedance data points at which the first order derivative to time is zero or close to zero.

34. (Withdrawn) The method according to claim 30, further comprising monitoring impedance of said one or more wells over seconds, minutes or hours, optionally wherein said monitoring of impedance in millisecond time resolution is performed within said monitoring over seconds, minutes or hours.

35. (Withdrawn-Currently amended) A method of assessing genetic manipulation of an embryonic stem cell, comprising:

a) providing ~~the system according to claim 1, wherein said device comprises at least two wells;~~ a system for monitoring impedance of excitable cells in vitro, comprising:

i) a device for monitoring cell-substrate impedance, comprising:

a) a nonconductive substrate,

b) one or more electrode arrays fabricated on said substrate,

c) at least two wells on said substrate, wherein each of said one or more arrays is associated with each of said at least two wells, wherein each electrode array is individually addressable, further wherein said substrate has a surface suitable for cell attachment, wherein said cell attachment on said substrate can result in a detectable change in impedance between electrodes within each electrode array,

ii) an impedance analyzer capable of impedance measurement at millisecond time resolution,

iii) electronic circuitry that can engage said device and selectively connect said two or more electrode arrays of said device to said impedance analyzer, and

(iv) a software program that controls said electronic circuitry and records and analyzes data obtained from said impedance analyzer;

b) mutating embryonic stem cells to comprise a genetic mutation;

c) adding said mutated embryonic stem cells to a first well of said at least two wells to form a test well and control embryonic stem cells to a second of said at least two wells to form a control well;

c) monitoring cell-substrate impedance of said at least two wells in millisecond time resolution to detect changes in cell morphology or cell attachment to said substrate; and

e) resolving the beating cycles of both test well and control well, if any; and

d) comparing said beating cycles between said test well and control well, wherein a difference in beating cycles indicates said genetic mutation modulates embryonic stem cell differentiation.

36. (Withdrawn) The method according to claim 35, wherein said embryonic stem cell differentiation is differentiation into a cardiomyocyte.

37. (Withdrawn) The method according to claim 35, wherein said genetic mutation is selected from the group consisting of a genetic knock out, a knock down and an inserted transgene.

38. (Withdrawn-Currently amended) A method for assessing and quantifying excitable cells in vitro, comprising

a) providing ~~the system according to claim 1;~~ a system for monitoring impedance of excitable cells in vitro, comprising:

i) a device for monitoring cell-substrate impedance, comprising:

a) a nonconductive substrate,

b) one or more electrode arrays fabricated on said substrate,

c) one or more wells on said substrate, wherein each of said one or more arrays is associated with one of said one or more

wells, wherein each electrode array is individually addressable, further wherein said substrate has a surface suitable for cell attachment, wherein said cell attachment on said substrate can result in a detectable change in impedance between electrodes within each electrode array,

ii) an impedance analyzer capable of impedance measurement at millisecond time resolution,

iii) electronic circuitry that can engage said device and selectively connect said two or more electrode arrays of said device to said impedance analyzer, and

(iv) a software program that controls said electronic circuitry and records and analyzes data obtained from said impedance analyzer;

b) adding excitable cells to said one or more wells;

c) monitoring cell-substrate impedance of said one or more wells over a first time period, which is characterized as a longer time period comprising seconds, minutes or hours;

d) monitoring cell-substrate impedance of said one or more wells over a second time period in millisecond time resolution to detect changes in cell morphology or attachment;

e) determining a characteristic selected from the group consisting of cell attachment, cell growth and cell viability from monitoring over said first time period; and

f) resolving individual cycles of said excitable cells from monitoring over said second time period.

39. (Withdrawn) The method according to claim 38, wherein monitoring impedance over said second time period is performed at regular or irregular time intervals within monitoring impedance over said first time period.

40. (Previously presented) The method according to claim 24, wherein the step of monitoring impedance using millisecond time resolution comprises obtaining at least two consecutive impedance measurements at less than 300 milliseconds apart.

41. (Previously presented) The method according to claim 40, wherein said at least two consecutive impedance measurements are less than 100 milliseconds apart.

42. (Previously presented) The method according to claim 41, wherein said at least two consecutive impedance measurements are less than 40 milliseconds apart

43. (Previously presented) The method according to claim 42, wherein said at least two consecutive impedance measurements are less than 10 milliseconds apart.

44. (Previously presented) The method according to claim 24, wherein the step of resolving the beating cycles comprises determining a rise in impedance and a decay in impedance, and extrapolating a peek from each rise and decay.

45. (Currently amended) The method according to claim 24, wherein the step of resolving the ~~cell~~ beating cycles comprises determining beating cycle peaks by identifying impedance data points at which the first order derivative to time is zero or about zero.

Remarks / Arguments

Claims 16, 17, 24, 30, 35, 38, 44 and 45 are amended and claims 1-6 and 11-14 are canceled. Claims 16-45 remain pending with claims 30-39 remaining withdrawn.

I.
Support for Amendments

Claims 16, 24, 30, 35 and 38 are amended to include the limitations of claim 1 and thus are rewritten in independent form. These claims are also amended to provide the step of monitoring cell-substrate impedance to detect changes in cell morphology or cell attachment to the substrate. Support for the amendment may be found in claim 1, which originally presented a system for monitoring impedance of excitable cells in vitro including a device for monitoring cell-substrate impedance. Further support can be found in paragraph [00173], "Such regular or periodic changes in cell morphology and cell adhesion/attachment are then reflected in the changes in cell-electrode or cell-substrate impedances." Still further support may be found in paragraph [00137]:

> Because the impedance-based system can sensitively and precisely detect transient changes in morphology and adhesive capacity of the cells, it can be used to monitor cardiac function in vitro. Further, the development of millisecond time resolution permits impedance monitoring of the cardiomyocyte beating cycle as well as fluctuations, even minor alterations, in response to external stimuli.

Claim 17 is amended to correct a grammatical error.

Claim 44 is amended to correct a typographical error.

Claim 45 is amended to correct antecedent basis.

II.
Amendment to Withdrawn claims

Claims 30, 35 and 38 are amended consistent with the amendment to claim 16 and thus upon allowance of claim 16, Applicants respectfully request the rejoining of claims 30, 35 and 38.

III.
Response to Claim Objections

Claims 16 and 24 are objected to for containing nonelected subject matter. Specifically, claims 16 and 24 depend from claim 1, which was subject to a restriction requirement. Claims 16 and 24 are rewritten in independent form including the limitations of claim 1. For completeness, claim 1 is cancelled herein.

Claim 17 is objected to as having a grammatical error in the use of the term "undergo". The grammatical error is corrected to "that undergo."

Claim 44 is objected to as having a typographical error, namely "peek". The typographical error in "peak" is corrected.

Claim 45 is objected to as lacking insufficient antecedent basis for "resolving cell beating cycles." Claim 45 is amended to recite, "resolving the beating cycles."

In view of the above remarks, Applicants respectfully request the objections be withdrawn.

IV.
Response to Claim Rejections Under 35 U.S.C. §112

Claim 16 is rejected under 35 U.S.C. §112, second paragraph as being indefinite. Specifically, the examiner is confused as what elements support the term "quantifying" in the preamble. To avoid confusion the preamble is amended to delete the term "quantifying."

V.

Response to Claim Rejections Under 35 U.S.C. 102

(Anticipation)

Claim 16 is rejected under 35 U.S.C. §102(b) as being anticipated by US 6,852,525 issued to Cantor. Specifically, Cantor is cited as teaching a method including providing a glass substrate with a plurality of cloning cylinders with microelectrodes and a computer for signal monitoring and processing; adding neuronal cells to the one or more wells, monitoring the opening and closing of ion channels (which occurs in less than a millisecond) and resolving the opening and closing cycle of the neurons.

Anticipation requires a single prior art reference disclose each and every element of the claim under consideration." *W.L. Gore & Assocs. V. Garlock, Inc.*, 220 USPQ 303, 313 (Fed. Cir.1983). However, it is not enough that the reference discloses all of the claimed elements in isolation. Rather, as stated by the Federal Circuit, the prior art reference must disclose each and every element of the claimed invention "arranged as in the claim." *Lindemann Maschinenfabrik GMBH v. American Hoist & Derrick Co.*, 221 USPQ 481, 485 (Fed. Cir.1984). Further, the prior art must be such that a person of ordinary skill in the art would consider there to be no difference between the claimed invention and the referenced disclosure. *In re Gurley*, 31 USPQ2d 1130, 1132 (Fed Cir. 1994). Applicants respectfully request reconsideration of the rejection in view of the following remarks.

Claim 16 is amended to clarify that the method includes monitoring cell-substrate impedance of the one or more wells using millisecond time resolution to detect changes in cell morphology or cell attachment to the substrate. Cantor does not teach this element nor this technical approach.

Cantor provides a mammalian cell culture chamber having a cover for allowing for transfer of gasses and a sensor array. Col. 3, ll.29-33. Cantor's system provides an approach for screening and assaying broad classes of materials for their impact on cellular metabolism. Col. 5, ll. 1-3. In regards to the sensor array, Cantor utilizes a small microelectrode/ large reference electrode approach where measurement occurs solely at

the small microelectrode. Col. 5, ll. 4-36. Cantor alleges that the small microelectrodes can be used to monitor the impedance of the individual cell, action potential parameters of the individual cell, the cell membrane capacitance, the cell membrane conductance, and the cell/substrate seal resistance, and cell membrane potential. Col. 5, ll. 13-22. Specifically, Cantor utilizes the cell culture chamber to monitor changes in membrane potential in response to the opening of ion channels. Col. 10, ll.22-37. The opening and closing of voltage-gated Na^+ channels takes only a few milliseconds. Col. 2, ll. 46-67.

In view of the above, while Cantor proposes a system for measuring changes in cell membrane potential characterized by ion channels opening and closing, the approach does not teach monitoring cell-substrate impedance of one or more wells using millisecond time resolution to detect changes in cell morphology or cell attachment to the substrate. That is, while Cantor measures cell membrane potential, which is an electrical property, the present invention monitors cell-substrate impedance, which involves the interaction between cell and substrate. Accordingly, whether or not Cantor can resolve cycles of opening and closing of ion channels, Cantor does not utilize the cell-substrate impedance approach as claimed and thus does not teach each element "as arranged in the claims."

As such, Applicants respectfully request the rejection be withdrawn and the claim allowed.

VI.

Response to Claim Rejections Under 35 U.S.C. §§103 (Obviousness)

Claims 16-29 and 40-45 are rejected under 35 U.S.C. §103(a) as being obvious over US 2005/0153425 published by Xu et al. (Xu), Am. J. Phyisol. Heart Circ. Physiol. 2003:H2114-23 by Banach et al. (Banach), as evidenced by Journal of Electrocardiology 37:110-16 published by Hescheler et al. (Hescheler), US 6,852,525 issued to Cantor, and US 2005/0182447 published by Schecter.

Specifically, Xu is cited as teaching methods for performing real-time cell-based assays investigating the effects of one or more compounds using an impedance-based system. The examiner indicates that Xu does not teach the measurement of excitable cells

that undergo excitation-contraction coupling or cardiomyocytes, resolving individual cycles or beating cycles at millisecond time resolution or identifying changes in beating amplitude or beating frequency. However, Banach is cited as teaching a microelectrode array for monitoring the beating cycle of cardiomyocytes and monitoring electrical activity from multiple electrodes simultaneously and continuously with millisecond time resolution and resolving signal delays of less than 10 milliseconds. Hescheler is cited as incorporating the microelectrode array of Banach and adding that the system may provide a new experimental model to study the impact of cell-cell interactions, growth factors, extracellular matrix proteins pharmacological investigations or chronic drugs and cardiotoxic effects during embryogenesis in vitro. Cantor is reintroduced as providing a system that monitors neuronal cells, such as through ion channel measurements. Schecter is cited as teaching a method of monitoring the beating cycle of a heart using impedance measurements and calculating the derivative of impedance to time at 0 and the peak and delays in time to peak incidence and changes in the impedance waveform. The examiner concludes that it would have been obvious to one skilled in the art to measure the beating cycle of excitable cells, such as cardiomyocytes at millisecond time resolution, to record multiple measurements over time in millisecond time resolution, to characterize the beating cycle of the cardiomyocytes and identify changes in beating amplitude and frequency by using a cell index, extrapolating a peak, and/or data points where the first order derivative to time is zero.

A proper obviousness rejection requires consideration of the factual inquiries provided in *Graham v. John Deere Co.*, 38 U.S. 1, 148 USPQ 459 (1966), including: 1) determining the scope and contents of the prior art; 2) ascertaining the differences between the prior art and the claims at issue; 3) resolving the level of ordinary skill in the pertinent art; and 4) considering the objective evidence present in the application indicating obviousness or nonobviousness. Although *Graham v. John Deere* requires that certain factual inquiries be conducted to support a determination of the issue of obviousness, the actual determination of the issue requires an elevation in light of the findings in those inquiries as to the obviousness *of the claimed invention as a whole*, not merely the differences between the claimed invention and the prior art. *Lear Siegeler,*

Inc. v. Aeroquip Corp., 221 USPQ 1025, 1033 (Fed. Cir. 1984). Applicants respectfully request reconsideration of the rejections in view of the following remarks

Claim 16 provides a method, which includes providing a device for monitoring cell-substrate impedance, monitoring cell-substrate impedance of the wells using millisecond time resolution to detect changes in cell morphology or cell attachment to the substrate; and resolving individual cycles of the excitable cells. Claim 24 provides a method, which includes monitoring cell-substrate impedance of at least two wells using millisecond time resolution to detect changes in cell morphology or cell attachment to the substrate; resolving the beating cycles of both test well and control well; and comparing the beating cycles between the test well and control well. These methods reveal the development of an approach of resolving beating cycles of excitable cells through the use of millisecond time resolution of cell-substrate impedance monitoring.

It is acknowledged that Xu provides an impedance-based system for monitoring cell-substrate impedance; however, as indicated by the examiner the methods in Xu do not achieve millisecond time resolution of impedance-based measurement nor teach or suggest the use of such an approach to resolve the beating of cycle of excitable cells. That is, while Xu does monitor changes that occur over longer periods of time, such as cytotoxicity, Xu does not teach or suggest to one skilled in the art that further advances in impedance-based monitoring to achieve millisecond time resolution could be achieved or such an achievement would be useful in a method to resolve the individual cycle or beating cycle of excitable cells. The addition of Banach, Hescheler, Cantor and Schecter does not correct the deficiency of this absence.

Banach analyzed the developmental changes of spontaneous electrical activity of multicellular aggregates of embryonic stem cells (ESCs) by measuring field potential parameters using substrate integrated microelectrode arrays (MEAs). Pg. H2114, right col., Para 1. That is, Banach analyzed changes in electrical activity of the ESC aggregates by measuring the membrane potential of the cells. Thus, while Banach is cited as resolving signal delays of less than 10 milliseconds, the technology is directed towards measurement of membrane potential, which provides a different technical approach compared to that of claims 16 and 24. That is, Banach does not teach the development of cell-substrate impedance measurements at millisecond time resolution.

Further, Banach does not teach or suggest to one skilled in the art that further advances in impedance-based monitoring to achieve millisecond time resolution would be useful in a method to resolve the individual cycle or the beating cycle of excitable cells. As such, Banach would not apply to the invention as set forth in the claims and would not contribute to its obviousness.

Hescheler provides an overview of the use of extracellular recording of embryonic stem cell-derived cardiomyocytes using microelectrode arrays (MEAs). Abstract. As with Banach, Hescheler confirms at page 112, right col. that the measurement used in the extracellular recording approach involves measuring the membrane potential:

> Although the signal recording with an extracellular electrode reflects local changes of the membrane potential, currents generated in the electrically connected neighboring tissue influence it.

Since the object of Hescheler is to provide an overview of a technical approach that records changes in cell membrane potential, the approach would not apply to a method that involves the resolution of individual cycles or beating cycles of excitable cells through measuring cell-substrate impedance. That is, measuring cell membrane potential provides a different technical approach than measuring cell-substrate impedance. As such, Hescheler would not apply to the invention as set forth in the claims and would not contribute to its obviousness.

Cantor is discussed above. Since Cantor measures cell membrane potential, Cantor does not teach the achievement of cell-substrate impedance measurements at millisecond time resolution nor does Cantor teach or suggest to one skilled in the art that further advances in impedance-based monitoring to achieve millisecond time resolution would be useful in a method to resolve the individual cycle or beating cycle of excitable cells. That is, Cantor uses a different technical approach, which would not apply to the present invention and would not contribute to its obviousness.

Schecter provides a method of optimizing acquisition of impedance signals between electrodes present on implanted lead systems, and automatically determining

which electrodes or electrode combinations acquire impedance waveforms that have the best signal to noise ratio and characterize data most representative of dysynchronous electromechanical events. Abstract. Schecter's technical approach is to utilize impedance waveforms and corresponding impedance data to direct the programming of a cardiac resynchronization therapy (CRT). Para [0046]. That is, the impedance data is from an implanted pacemaker or defribillator. Abstract. Since Schecter utilizes impedance signals from an patient's organ and not from a cell-substrate impedance device as claimed Schecter would not apply to the invention set forth in claims 16 or 24. That is, Schecter does not teach the technical achievement of cell-substrate impedance measurements at millisecond time resolution nor does Schecter teach or suggest to one skilled in the art that further advances in impedance-based monitoring to achieve millisecond time resolution would be useful in a method to resolve the individual cycle or the beating cycle of excitable cells. Thus, Schector would not apply to the invention set forth in claims 16 or 24.

Accordingly, Applicants respectfully request the rejections be withdrawn.

VII.

Response to Provisional Double Patenting Rejections

Claims 16-19, 24-29 and 44 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting over claims 1-15 of copending Application No. 13/109,809. Provided herewith Applicants provide a terminal disclaimer

Claims 16-19 and 24-29 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting over claims 12-41 of copending Application No. 12/774,709. Provided herewith Applicants provide a terminal disclaimer.

Accordingly, Applicants respectfully request the rejections be withdrawn.

VIII.

Conclusion

In view of the above remarks, Applicants respectfully submit the claims are in condition for allowance.

Respectfully submitted,

12/18/2012

Date

Raymond Wagenknecht
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Biotech Beach Law Group PC
5677 Oberlin Dr. Ste 204
San Diego, CA 92121

Patent
Attorney Docket No: ACE-134U1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	)	
	)	
WANG et al.	)	
	)	Examiner: Shannon Janssen
Application Number: 12/435,569	)	Group Art Unit: 1636
	)	
Filed: May 5, 2009	)	
	)	
For: LABEL-FREE MONITORING OF EXCITATION-CONTRACTION COUPLING AND EXCITABLE CELLS USING IMPEDANCE BASED SYSTEMS WITH MILLISECOND TIME RESOLUTION	)	

Commissioner for Patents
P. O. Box 1450
Alexandria, VA 22313-1450

Extension of Time Request

Sir:

Applicants respectfully request a three month extension of time to file a reply to the Office Action mailed June 15, 2012. Provided herewith please find the fee deemed necessary under 37 CFR 1.17(a)(3).

Respectfully submitted,

12/13/2012

Date

Raymond Wagenknecht
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TERMINAL DISCLAIMER TO OBVIATE A PROVISIONAL DOUBLE PATENTING REJECTION OVER A PENDING "REFERENCE" APPLICATION	Docket Number (Optional) ACE-134U1

In re Application of: Xiaobo Wang et al

Application No.: 12/435,569

Filed: May 5, 2009

For: LABEL-FREE MONITORING OF EXCITATION-CONTRACTION COUPLING AND EXCITABLE CELLS USING IMPEDANCE BASED SYSTEMS WTIH MILLISECOND TIME RESOLUTION

The owner*, ACEA BIOSCIENCES, INC, of 100 percent interest in the instant application hereby disclaims, except as provided below, the terminal part of the statutory term of any patent granted on the instant application which would extend beyond the expiration date of the full statutory term of any patent granted on pending **reference** Application Number 12/774,709, filed May 5, 2010, as the term of any patent granted on said **reference** application may be shortened by any terminal disclaimer filed prior to the grant of any patent on the pending **reference** application. The owner hereby agrees that any patent so granted on the instant application shall be enforceable only for and during such period that it and any patent granted on the **reference** application are commonly owned. This agreement runs with any patent granted on the instant application and is binding upon the grantee, its successors or assigns.

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2. ☑ The undersigned is an attorney or agent of record. Reg. No. 50948



Signature — 12/13/2012 Date

Raymond Wagenknecht
Typed or printed name

858-587-2510 x 201
Telephone Number

☑ Terminal disclaimer fee under 37 CFR 1.20(d) is included.

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Electronic Patent Application Fee Transmittal

Application Number:	12435569
Filing Date:	05-May-2009
Title of Invention:	Label-Free Monitoring of Excitation-Contraction Coupling and Excitable Cells Using Impedance Based Systems with Millisecond Time Resolution
First Named Inventor/Applicant Name:	Xiaobo Wang
Filer:	Raymond C. Wagenknecht/Diana Meisenholder
Attorney Docket Number:	ACE-134U1

Filed as Small Entity

Utility under 35 USC 111(a) Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Independent claims in excess of 3	2201	2	125	250
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension - 3 months with $0 paid	2253	1	645	645
Miscellaneous:				
Statutory or terminal disclaimer	2814	2	80	160
		Total in USD ($)		**1055**

Electronic Acknowledgement Receipt

EFS ID:	14466600
Application Number:	12435569
International Application Number:	
Confirmation Number:	3664
Title of Invention:	Label-Free Monitoring of Excitation-Contraction Coupling and Excitable Cells Using Impedance Based Systems with Millisecond Time Resolution
First Named Inventor/Applicant Name:	Xiaobo Wang
Customer Number:	59538
Filer:	Raymond C. Wagenknecht/Diana Meisenholder
Filer Authorized By:	Raymond C. Wagenknecht
Attorney Docket Number:	ACE-134U1
Receipt Date:	13-DEC-2012
Filing Date:	05-MAY-2009
Time Stamp:	19:22:33
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
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Payment was successfully received in RAM	$1055
RAM confirmation Number	6986
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Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		ACE-134U1FiledResponseOA2.pdf	7496993 d69dc2b8cc1bf381a85ab7d4a5bc06a9955fac7a	yes	20

Multipart Description/PDF files in .zip description

Document Description	Start	End
Amendment/Req. Reconsideration-After Non-Final Reject	1	1
Claims	2	11
Applicant Arguments/Remarks Made in an Amendment	12	20

Warnings:

Information:

2		ACE-134U1RequestTimeExtension.pdf	1513357 a0d388beb8d286fed1ccc51b05e4b7c5e9b44078	yes	3

Multipart Description/PDF files in .zip description

Document Description	Start	End
Extension of Time	1	1
Terminal Disclaimer Filed	2	2
Terminal Disclaimer Filed	3	3

Warnings:

Information:

3	Fee Worksheet (SB06)	fee-info.pdf	33764 aea3c103cec256b9bd604cd767a6dff498a72bad	no	2

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New International Application Filed with the USPTO as a Receiving Office

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PTO/SB/25 (08-11)
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TERMINAL DISCLAIMER TO OBVIATE A PROVISIONAL DOUBLE PATENTING REJECTION OVER A PENDING "REFERENCE" APPLICATION	Docket Number (Optional) ACE-134U1

In re Application of: Xiaobo Wang et al

Application No.: 12/435,569

Filed: May 5, 2009

For: LABEL-FREE MONITORING OF EXCITATION-CONTRACTION COUPLING AND EXCITABLE CELLS USING IMPEDANCE BASED SYSTEMS WTIH MILLISECOND TIME RESOLUTION

The owner*, ACEA BIOSCIENCES, INC, of 100 percent interest in the instant application hereby disclaims, except as provided below, the terminal part of the statutory term of any patent granted on the instant application which would extend beyond the expiration date of the full statutory term of any patent granted on pending **reference** Application Number 13/109,809, filed May 17, 2011, as the term of any patent granted on said **reference** application may be shortened by any terminal disclaimer filed prior to the grant of any patent on the pending **reference** application. The owner hereby agrees that any patent so granted on the instant application shall be enforceable only for and during such period that it and any patent granted on the **reference** application are commonly owned. This agreement runs with any patent granted on the instant application and is binding upon the grantee, its successors or assigns.

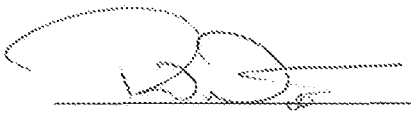
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Check either box 1 or 2 below, if appropriate.

1. ☐ For submissions on behalf of a business/organization (e.g., corporation, partnership, university, government agency, etc.), the undersigned is empowered to act on behalf of the business/organization.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

2. ☑ The undersigned is an attorney or agent of record. Reg. No. 50948



Signature — 12/13/2012 — Date

Raymond Wagenknecht
Typed or printed name

858-587-2510 x 201
Telephone Number

☑ Terminal disclaimer fee under 37 CFR 1.20(d) is included.

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

*Statement under 37 CFR 3.73(b) is required if terminal disclaimer is signed by the assignee (owner).
Form PTO/SB/96 may be used for making this statement. See MPEP § 324.

This collection of information is required by 37 CFR 1.321. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 12/435,569	Filing Date 05/05/2009	☐ To be Mailed

APPLICATION AS FILED – PART I

	(Column 1)	(Column 2)	SMALL ENTITY ☒		OR	OTHER THAN SMALL ENTITY	
FOR	NUMBER FILED	NUMBER EXTRA	RATE ($)	FEE ($)		RATE ($)	FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A			N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A			N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A			N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*	X $ =		OR	X $ =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*	X $ =			X $ =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $250 ($125 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).						
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))							
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL			TOTAL	

APPLICATION AS AMENDED – PART II

		(Column 1)		(Column 2)	(Column 3)	SMALL ENTITY		OR	OTHER THAN SMALL ENTITY	
	12/13/2012	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)		RATE ($)	ADDITIONAL FEE ($)
AMENDMENT	Total (37 CFR 1.16(i))	* 30	Minus	** 39	= 0	X $31 =	0	OR	X $ =	
	Independent (37 CFR 1.16(h))	* 5	Minus	***3	= 2	X $125 =	250	OR	X $ =	
	☐ Application Size Fee (37 CFR 1.16(s))									
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE	**250**	OR	TOTAL ADD'L FEE	

		(Column 1)		(Column 2)	(Column 3)					
		CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)		RATE ($)	ADDITIONAL FEE ($)
AMENDMENT	Total (37 CFR 1.16(i))	*	Minus	**	=	X $ =		OR	X $ =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	X $ =		OR	X $ =	
	☐ Application Size Fee (37 CFR 1.16(s))									
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.

** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".

*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".

The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

Legal Instrument Examiner:
/LAJUAN HICKSON/

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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Application Number	Application/Control No.	Applicant(s)/Patent under Reexamination	
	12/435,569	WANG ET AL.	

Document Code - DISQ	Internal Document – DO NOT MAIL

TERMINAL DISCLAIMER	☒ APPROVED	☐ DISAPPROVED
Date Filed : 12/13/12	This patent is subject to a Terminal Disclaimer	

Approved/Disapproved by:

Felicia D. Roberts - 2 TDs approved with this filing date:

1. 13/109,809
2. 12/774,709

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
12/435,569	05/05/2009	Xiaobo Wang	ACE-134U1	3664

59538 7590 11/04/2013

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EXAMINER	
JANSSEN, SHANNON L	
ART UNIT	PAPER NUMBER
1653	

MAIL DATE	DELIVERY MODE
11/04/2013	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	12/435,569	WANG ET AL.	
	Examiner	Art Unit	
	SHANNON JANSSEN	1653	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *13 December 2012*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

5)☒ Claim(s) *16-45* is/are pending in the application.

5a) Of the above claim(s) *30-39* is/are withdrawn from consideration.

6)☐ Claim(s) _____ is/are allowed.

7)☒ Claim(s) *16-29 and 40-45* is/are rejected.

8)☒ Claim(s) *44* is/are objected to.

9)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

12)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Claims 16-45 are currently pending. Claims 16, 17, 24, 30, 35, 38, and 45 have been amended and claims 1-6 and 11-14 have been cancelled as filed on December 13, 2012. Claims 30-39 have been withdrawn and claims 16-29 and 40-45 are currently under consideration.

Election/Restrictions

Applicants elected Group III, claims 24-29 and newly added claims 40-45 with traverse in the reply filed on February 27, 2012.

Claims 1-6, 11-14 and 30-39 are withdrawn from further consideration pursuant to 37 CFR 1.142(b), as being drawn to a nonelected Inventions, there being no allowable generic or linking claim. However, claim 16 links inventions III-VI and upon indication of allowability, the restriction requirement between the linked inventions shall be withdrawn and any claims depending from or otherwise requiring all the limitations of the allowable linking claim will be rejoined.

Priority

The present application claims priority based on U.S. Provisional Application Nos. 61/191,684, filed on September 11, 2008 and 61/126,533, filed on May 5, 2008.

Withdrawn Objection(s)/Rejection(s)

Any objection or rejection presented in the Office Action mailed June 15, 2012 and not reiterated below is hereby withdrawn.

Maintained Objection

Claim Objections

Claim 44 is objected to because of the following informalities: "peek" in line 3 appears to be a typographical error. Appropriate correction is required.

Response to Arguments

Applicant's arguments filed December 13, 2012 have been fully considered but they are not persuasive for the following reasons. Applicants' arguments are presented in Italics.

Applicants assert that the claim has been amended to correct the typographical error (Response, p 13).

However, the claim has not been amended. Therefore the objection is maintained.

Invention as Claimed

One embodiment of the present invention is drawn to a method comprising a) providing a system for monitoring impedance of excitable cells in vitro comprising: i) a device for monitoring cell-substrate impedance comprising: a) a nonconductive substrate, b) one or more electrode arrays fabricated on said substrate, c) one or more wells on said substrate, wherein each of said one or more arrays is associated with one of said one or more wells, wherein each electrode array is individually addressable, further wherein said substrate has a surface suitable for cell attachment, wherein said cell attachment on said substrate can result in a detectable change in impedance between electrodes within each electrode array, ii) an impedance analyzer capable of impedance measurement at millisecond time resolution, iii) electronic circuitry that can engage said device and selectively connect said two or more electrode arrays of said device

to said impedance analyzer, and iv) a software program that controls said electronic circuitry and records and analyzes data obtained from said impedance analyzer; b) adding excitable cells to said one or more wells; c) monitoring cell-substrate impedance of said one or more wells using millisecond time resolution to detect changes in cell morphology or cell attachment to said substrate; and d) resolving individual cycles of said excitable cells.

Another embodiment of the present invention is drawn to a method of determining whether a compound modulates a beating cycle of an excitable cell, comprising: a) providing a system for monitoring impedance of excitable cells in vitro comprising: i) a device for monitoring cell-substrate impedance comprising: a) a nonconductive substrate, b) one or more electrode arrays fabricated on said substrate, c) at least two wells on said substrate, wherein each of said one or more arrays is associated with each of said at least two wells, wherein each electrode array is individually addressable, further wherein said substrate has a surface suitable for cell attachment, wherein said cell attachment on said substrate can result in a detectable change in impedance between electrodes within each electrode array, ii) an impedance analyzer capable of impedance measurement at millisecond time resolution, iii) electronic circuitry that can engage said device and selectively connect said two or more electrode arrays of said device to said impedance analyzer, and iv) a software program that controls said electronic circuitry and records and analyzes data obtained from said impedance analyzer; b) adding excitable cells to said at least two wells; c) monitoring cell-substrate impedance of said at least two wells using millisecond time resolution to detect changes in cell morphology or cell attachment to said substrate; d) adding a compound suspected of modulation of the beating cycle to a first of said at least two wells to provide a test well, wherein a second well lacking compound is provided as a

control well; e) resolving the beating cycles of both test well and control well; and d) comparing said beating cycles between said test well and control well, wherein a difference in beating cycles indicates said compound modulates the beating cycle of the excitable cell.

Maintained Rejections

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The factual inquiries set forth in *Graham* **v.** *John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later

invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 16-29 and 40-45 are rejected under 35 U.S.C. 103(a) as being unpatentable over Xu et al. (U.S. Patent Application Publication No. 2005/0153425, published July 14, 2005), Banach et al. (Development of electrical activity in cardiac myocyte aggregates derived from mouse embryonic stem cells, Am. J. Physiol. Heart Circ. Physiol., 2003, Vol 284, pp H2114-H2123) as evidenced by Hescheler et al. (Determination of electrical properties of ES cell-derived cardiomyocytes using MEAs, 2004, Journal of Electrocardiology, Vol 37, pp 110-116), Cantor (U.S. Patent No. 6,852,525, granted February 8, 2005), and Schecter (U.S. Patent Application Publication No. 2005/0182447, published August 18, 2005). Modifications to this rejection were necessitated by the claim amendments.

Regarding present **claims 16 and 24, Xu** et al. teach a method for performing real-time cell-based assays investigating the effects of one or more compound on cells comprising providing a system for monitoring cell-substrate impedance comprising a nonconducting substrate, two or more electrode arrays fabricated on the substrate, where each of the two or more electrode arrays comprises two electrode structures, and at least two connection pads, each of which is located on an edge of the substrate, at least one multiple-well device monitoring cell-substrate impedance, in which at least two of the multiple wells each comprise an electrode array at the bottom of the well which are independently addressable, an impedance analyzer, a device station capable of engaging the one or more multiple-well devices and capable of selecting and electrically connecting electrode arrays within any of the multiple wells in to the impedance analyzer, and a software program to control the device station and perform data acquisition and

data analysis on impedance values measured by the impedance analyzer, depositing cells on at least one of the two or more arrays of the device, adding one or more compounds to the one or more wells, providing at least one control well lacking compound, and monitoring cell-substrate impedance at regular or irregular time intervals on one or more arrays of the device to ascertain if the compound modulates the cell (see [0018-0020, 0023, 0024, 0029, 0031, 0032, 0036, 0130, 0131, 0134, 0244, 0246], for example). Xu et al. further teach wherein the system monitors cell attachment, morphology, degree of adhesion (e.g.: how well the cells area attached to the electrodes), and wherein measurements can be taken in timeframes of less than a second (see [0125, 0250, 0255, 0259], for example).

Regarding present **claim 29**, Xu et al. provides methods for calculating a Cell Index for quantifying and comparing cell-substrate impedance (see [0021, 0140+], for example).

Regarding present **claim 45**, Xu et al. teach setting the first order derivatives to zero (see [0209], for example).

While Xu et al. teach a method for performing real-time cell-based assays investigating the effects of one or more compound on cells, Xu et al. do not teach excitable cells which undergo excitation-contraction coupling or cardiomyocytes as recited in instant **claims 16-18, 24 and 25**, resolving individual cycles or beating cycles as recited in instant **claims 16 and 24**, at least 2 consecutive impedance measurements at millisecond (ms) time resolution as recited in instant **claims 19-23 and 40-43**, or identifying changes in beating amplitude or beating frequency as recited in instant **claims 26-29 and 45**.

However, **Banach** et al. teach a microelectrode array for monitoring the beating cycle of cardiomyocytes and further teach monitoring electrical activity from multiple electrodes

simultaneously and continuously with ms time resolution and resolving signal delays of less than 10 ms (e.g.: at least two consecutive measurements less than 10 milliseconds apart since it is continuously measured and this could be broadly and reasonably interpreted as consecutive measurements; see entire document, particularly p H2115, cols 1-2, Figs. 1, 3, and 4, for example). Banach et al. teach monitoring the interspike interval to determine the beating frequency and measuring peak amplitude (e.g.: would be regular or irregular changes; see p 2115, col 1, para 2, p H2116, col 1, para 1). Banach et al. teach measuring the effect of different compounds on beating frequency and action potential in the form of FP_{MIN} to FP_{MAX} (e.g.: amplitude; see p H2115, col 2, para 1, p H2118, col 2, para 2, p H2119, p H2120, col 1, para 1, col 2, para 4, Figs. 4, 6, and 7). Banach et al. further state:

> "In the present study, we analyzed the developmental changes of spontaneous electrical activity with repeated multielectrode recordings using substrate-integrated microelectrode arrays (MEAs). This technique allows not only the long-term culture of the preparation but also the analysis of the spatial electrophysiological differences within the preparation. In the present study, we analyzed different field potential (FP) parameters to characterize the development of the spontaneous electrical activity of multicellular aggregates of ESCs and their electrophysiological properties. Furthermore, the use of an ES cell line deficient in the expression of connexin43 ($Cx43^{-/-}$), the major cardiac gap junction protein, enabled us to distinguish developmental changes of the active, voltage-dependent membrane properties from changing passive components like the intercellular resistance." See p H2114, col 2, Para 1.

In addition, Hescheler, which cites to the microelectrode array system taught by Banach et al. (see p 113, Fig. 3) and, regarding the arrays, state:

> "It may provide a new experimental model to study the impact of cell-cell-interactions and growth factors as well as of extracellular matrix proteins. In addition, the cellular system described, may be useful as an in vitro assay for pharmacological investigations of chronotropic drugs and as a cellular system for studying cardiotoxic effects during embryogenesis in vitro." See p 115, cols 1-2, bridging para.

Further, **Cantor** teaches a method comprising a) providing a system comprising microelectrodes on a substrate, such as a glass substrate (i.e.: nonconductive), for monitoring impedance of cells and further comprising a plurality of cloning cylinders bonded to the substrate to define wells for holding the cells (see col 3, lns 29-53, col 4, lns 4-15, col 9, lns 35-37, Figs. 7 and 9, for example) and a signal monitoring and processing means such as a computer (see col 5, lns 60+, col 6, lns 1-12); b) adding excitable cells, such as neuronal cells, to said one or more wells; c) monitoring impedance of said one or more wells using millisecond time resolution (since it monitors the opening and closing of ion channels which occurs in less than a millisecond; see col 2, lns 39+; and d) resolving individual cycles (e.g.: of ion channels opening, closing, and the cell returning to a resting state) of said excitable cells (e.g.: measuring impedance peaks in millisecond time resolution; see entire document, particularly col 2, lns 39+, col 3, lns 14-27, col 4, lns 26-46, col 5, lns 1-35, col 8, lns 14-44). Cantor further states:

> "For example, voltage-gated Na^+ channels (among other ion channels) help make nerve cells electrically excitable and enable them to conduct action potentials. When the membrane of a cell with many Na^+ channels is partially depolarized by a momentary stimulus, some of the channels promptly open, allowing Na+ ions to enter the cell. The influx of positive charge depolarizes the membrane further, thereby opening more channels, which admit more Na^+, causing still further depolarization. This process continues in a self-amplifying fashion until the membrane potential has shifted from its resting value of about -70 mV all the way to the Na^+ equilibrium potential of about +50 mV. At that point, where the net electrochemical driving force for the flow of Na^+ is zero, the cell would come to a new resting state with all its Na^+ channels permanently open, if the open channel conformation were stable. The cell is saved from such a permanent electrical tetanus by the automatic inactivation of the Na^+ channels, which now gradually close and stay closed until the membrane potential has returned to its initial negative resting value. The whole cycle, from initial stimulus to return to the original resting state, takes a few milliseconds or less." See col 2, lns 46+.

> "Examining the membrane potential relative to time, an action potential exhibits various characteristics or parameters, including action potential rate (if cells spontaneously depolarize), action potential amplitude, and action potential shape, among others. Action potential rate refers to the frequency with which a cell produces an action

> potential (rapid depolarization). Action potential amplitude refers to the height of the peak depolarization that occurs in the course of the action potential. Action potential shape refers to the time course of the depolarization and repolarization." See col 3, lns 14-24.
>
> "The present invention permits detection and monitoring of membrane, chemical, and electrical characteristics of a cell. As noted at the outset, these characteristics include cell impedance (cell membrane capacitance and conductance), action potential parameters, cell membrane capacitance, cell membrane conductance, neuronal action potential, cellular metabolic products (hormones, neurotransmitters, waste products, etc.), and cell/substrate seal resistance. These electrical characteristics, in turn, correlate well with the metabolic state of the cell. The present invention, thus, provides an apparatus and method for monitoring cells and for monitoring the impact that an analyte has upon the metabolism of a cell. It is to be appreciated that such analytes include pharmaceutical agents, drugs, environmental factors, toxins, chemical agents, biological agents, viruses, and cellular adhesion promoters." See Col 4, lns 25-45.

While Xu et al., Banach et al., and Cantor teach a method for performing real-time cell-based assays investigating the effects of one or more compound on cells and measuring beating frequency and amplitude, Xu et al., Banach et al., and Cantor do not *specifically* teach determining a rise in impedance and a decay in impedance and extrapolating a peak from each rise and decay as recited in instant **claim 44** or identifying impedance data points at which the first order derivative to time is zero or about zero as recited in instant **claim 45**.

However, **Schecter** teaches a method of monitoring beating cycle of the heart using impedance measurements and further teach calculating the derivative of impedance to time at 0 and the peak and delays in time to peak incidence and changes in the impedance waveform (e.g. time to onset of positive dZ/dt, time to peak Z, time to peak dZ/dt, which can be broadly and reasonably interpreted as rise in impedance and decay in impedance and determining a peak) and/or reductions in the quantity of the integral of Z or changes in the morphology of Z(t)dt (see entire document, particularly [0048, 0049, 0060, 0077, 0086-0090, 0100-0101, 0118, 0136], Figs. 5, 6, 8-10, 12). Schecter further state:

> "Derivation of impedance waveforms using implanted lead systems is well known in the art and has been described in the literature. Data acquisition can be accomplished, for example, by delivering pulses of 200 uA, 30 us pulse width at a frequency of 128 Hz applied to two electrodes positioned along one vector (electrode pairs) and measuring the resulting voltage between electrodes located along the same vector. These pulses will not depolarize myocardium, cause only limited battery drain and have a frequency with an acceptable signal to noise ratio. The resultant time dependent impedance, Z(t) peaks when there is maximal systolic ventricular wall thickness and minimal intracardiac blood volume." See [0048].
>
> "The time dependent impedance signals or waveforms derived in this fashion relate to intrinsic myocardial properties. If signals are acquired between specific electrode pairs, the regional myocardial properties can be derived. The integrity of these waveforms may be subject to significant noise (poor signal to noise ratio) and inferior signal quality may result. This is especially true if data sampling occurs in a vector where there is impairment in myocardial contractile properties. Derivation of specific characteristics of these waveforms may suffice, even though overall signal quality is poor. Measurement of peak impedance and first and second order derivatives of impedance waveforms will relate to myocardial contractility. Assessment of the time required for a waveform to reach peak impedance will relate to myocardial synchrony if comparisons can be made to waveforms derived in alternate regions (e.g. right and left ventricular vectors)." See [0049].

Therefore it would have been obvious to one of skill in the art at the time of the instant invention to measure the beating cycle of excitable cells such as cardiomyocytes at millisecond time resolution, to record multiple measurements over time in millisecond time resolution, to characterize the beating cycle of the cardiomyocytes and identify changes in beating amplitude and frequency by using a cell index, extrapolating a peak, and/or identifying data points where the first order derivative to time is zero.

One would have been motivated to characterize the beating cycle and to examine various parameters of the beating cycle, such as beating frequency and amplitude, or the beating frequency and amplitude relative to the number of cells (e.g.: cell index), in order to characterize the culture of beating cardiomyocytes *in vitro* as taught by Banach et al. (e.g.: for use as a model; see p H2114, col 2, para 1, for example) and to utilize the culture to characterize cardiotoxic

effects of drugs as taught by Hescheler et al. (see above). In light of the fact that measuring the various peaks and rise and decay of a cardiac cycle will detail the condition of the heart, or in this case, the culture representing the heart, it would have been obvious to one of ordinary skill in the art to measure the peaks comprising the rise and decay of the cardiac cycle to gain a better determining of the state of the heart. Further, due to the inherent property of signals and peaks, it is well known that for a signal that has a rise and fall (e.g.: a peak), the first derivative yields a signal that crosses zero at the maximum peak height of the original signal. One would have been motivated to utilize impedance measurements because Xu et al. teach that the impedance system can be used for real-time cell-based assays in high throughput, e.g., rather than just an end-point assay which limits the time resolution and number of time points for large library screening (see [0014, 0016, 0024, 0025, 0031, 0032], for example) and Cantor teaches the need for electrodes which can monitor cellular changes including ion channel conductance (e.g.: which are responsible for the action potentials that cause cell beating; see above and col 2, lns 31-40).

One would have had a reasonable expectation for success because Banach et al. teach successfully establishing cultures of beating cardiomyocytes and measuring electrical properties of the cultures to establish beating cycle parameters and beating frequency, including changes in the parameters after administration of drugs, Cantor teaches that electrode arrays can be used to measure cell impedance, secretion of cellular products, monitoring of ion channels and action potentials, and monitoring of compounds which affect those parameters in millisecond time resolution (see above and cols 1-2), Xu et al. teach successfully using impedance to characterize cellular properties (see [0428], for example), and Schecter teach that it is routine and known to derive impedance waveforms (see supra).

Therefore the teachings of Xu et al., Banach et al. as evidenced by Hescheler et al., Cantor, and Schecter render the present invention *prima facie* obvious.

Response to Arguments

Applicant's arguments filed December 13, 2012 have been fully considered but they are not persuasive for the following reasons. Applicants' arguments are presented in Italics.

Applicants assert that Xu et al. does not teach millisecond time resolution and that the other references do not cure these deficiencies and do not teach measuring cell-substrate impedance and rather teach measuring membrane potential (Response, pp 15+).

In response, it is noted that the test for obviousness is not whether the features of a secondary reference may be bodily incorporated into the structure of the primary reference; nor is it that the claimed invention must be expressly suggested in any one or all of the references. Rather, the test is what the combined teachings of the references would have suggested to those of ordinary skill in the art. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981).

In the instant case, it is unclear how the references do not teach measuring cell-substrate impedance. It is held that measuring the impedance using the electrodes integrated into the substrate would constitute measuring the cell-substrate impedance. Further, Xu et al. teach measuring cell substrate impedance. It would have been obvious to measure the impedance in ms time resolution as taught by the other cited references in order to measure the effects of compounds on the beating cycle of cardiomyocytes (e.g.: which occurs in ms time resolution), as discussed supra. Further, Xu et al. teach wherein each measurement can take place over a narrow time frame such as "less than one second" (i.e.: less than one second would be ms; see [0125]). Given the combined teachings of all the cited references, it would have been obvious to one of

skill in the art to conduct measurements in ms time resolution in order to measure the beating cycle of cardiomyocytes. Further, the instant claims recite measuring a change in morphology. It is respectfully held that measuring the beating cycle of the cardiomyocyte inherently measures a change in morphology since the morphology of the cell changes during the beating cycle. Therefore the rejection is maintained.

Conclusion

THIS ACTION IS MADE FINAL. Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

Future Communication

Any inquiry concerning this communication or earlier communications from the examiner should be directed to SHANNON JANSSEN whose telephone number is (571)270-1303. The examiner can normally be reached on Monday-Friday 10:00AM-2:00PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Sharmila Landau can be reached on (571) 272-0614. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

Shannon L Janssen
SLJ

/SHARMILA G. LANDAU/
Supervisory Patent Examiner, Art Unit 1653

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	12435569	WANG ET AL.
	Examiner	Art Unit
	SHANNON JANSSEN	1653

CPC- SEARCHED		
Symbol	Date	Examiner

CPC COMBINATION SETS - SEARCHED		
Symbol	Date	Examiner

US CLASSIFICATION SEARCHED			
Class	Subclass	Date	Examiner

SEARCH NOTES		
Search Notes	Date	Examiner
Inventor Name Search - PALM, EAST	10/29/13	SLJ
USPAT, USPGPUB, EPO, JPO, DERWENT- updated dearch	10/23/13	SLJ
Pubmed, Scirus - updated search	10/23/13	SLJ

INTERFERENCE SEARCH			
US Class/ CPC Symbol	US Subclass / CPC Group	Date	Examiner

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Not for submission under 37 CFR 1.99)

Application Number	12435569
Filing Date	2009-05-05
First Named Inventor	Xiaobo Wang
Art Unit	1636
Examiner Name	Shannon Janssen
Attorney Docket Number	ACE-134-U1

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	12435569
	Filing Date	2009-05-05
	First Named Inventor	Xiaobo Wang
	Art Unit	1636
	Examiner Name	Shannon Janssen
	Attorney Docket Number	ACE-134-U1

	1	European Search Report for 09743426.0	☐
	2	KLOSS et al. "Microcavity array (MCA)-based biosensor chip for functional drug screening of 3D tissue models" Biosensors and Bioelectronics 23 (2008) 1473-1480.	☐
	3	QIU et al. "Real-Time Monitoring Primary Cardiomyocyte Adhesion Based on Electrochemical Impedance Spectroscopy and Electrical Cell-Substrate Impedance Sensing" Anal. Chem. 2008, 80, 990-996.	☐
	4	XING et al. "Dynamic Monitoring of Cytotoxicity on Microelectronic Sensors" Chem. Res. Toxicol., 2005, 18 (2), 154-161.	☐
	5	YU et al. "Real-Time Monitoring of Morphological Changes in Living Cells by Electronic Cell Sensor Arrays: An Approach To Study G Protein-Coupled Receptors" Anal. Chem. 2006, 78, 35-43.	☐

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EXAMINER SIGNATURE

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1] See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. [2] Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). [3] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [4] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [5] Applicant is to place a check mark here if English language translation is attached.

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (**Not for submission under 37 CFR 1.99)**		
	Application Number	12435569
	Filing Date	2009-05-05
	First Named Inventor	Xiaobo Wang
	Art Unit	1636
	Examiner Name	Shannon Janssen
	Attorney Docket Number	ACE-134-U1

CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

☒ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

☐ See attached certification statement.

☐ The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☐ A certification statement is not submitted herewith.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Raymond Wagenknecht/	Date (YYYY-MM-DD)	2013-11-25
Name/Print	Raymond Wagenknecht	Registration Number	50948

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1 hour to complete, including gathering, preparing and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these record s.

2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.

3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.

4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.

6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Electronic Acknowledgement Receipt

EFS ID:	17491725
Application Number:	12435569
International Application Number:	
Confirmation Number:	3664
Title of Invention:	Label-Free Monitoring of Excitation-Contraction Coupling and Excitable Cells Using Impedance Based Systems with Millisecond Time Resolution
First Named Inventor/Applicant Name:	Xiaobo Wang
Customer Number:	59538
Filer:	Raymond C. Wagenknecht/Diana Meisenholder
Filer Authorized By:	Raymond C. Wagenknecht
Attorney Docket Number:	ACE-134U1
Receipt Date:	25-NOV-2013
Filing Date:	05-MAY-2009
Time Stamp:	13:00:36
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Information Disclosure Statement (IDS) Form (SB08)	ACE134U1_IDS2.pdf	612630 118e69ee64e97972c6f72e3ba0456703[illegible] 94335	no	4

Warnings:	
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A U.S. Patent Number Citation or a U.S. Publication Number Citation is required in the Information Disclosure Statement (IDS) form for autoloading of data into USPTO systems. You may remove the form to add the required data in order to correct the Informational Message if you are citing U.S. References. If you chose not to include U.S. References, the image of the form will be processed and be made available within the Image File Wrapper (IFW) system. However, no data will be extracted from this form. Any additional data such as Foreign Patent Documents or Non Patent Literature will be manually reviewed and keyed into USPTO systems.

2	Non Patent Literature	European_Search_Report.pdf	212904 55d4d2a6b1c46ffeb011caeabffe77b4c47643eb	no	6
Warnings:					
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3	Non Patent Literature	Kloss_et_al.pdf	963800 c7a20cc0ab82e292501bb9f3e40bb3b9a4c11ba3	no	8
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New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

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REQUEST FOR CONTINUED EXAMINATION(RCE)TRANSMITTAL
(Submitted Only via EFS-Web)

Application Number	12435569	Filing Date	2009-05-05	Docket Number (if applicable)	ACE-134U1	Art Unit	1653
First Named Inventor	Xiaobo Wang			Examiner Name	Shannon Janssen		

This is a Request for Continued Examination (RCE) under 37 CFR 1.114 of the above-identified application.
Request for Continued Examination (RCE) practice under 37 CFR 1.114 does not apply to any utility or plant application filed prior to June 8, 1995, or to any design application. The Instruction Sheet for this form is located at WWW.USPTO.GOV

SUBMISSION REQUIRED UNDER 37 CFR 1.114

Note: If the RCE is proper, any previously filed unentered amendments and amendments enclosed with the RCE will be entered in the order in which they were filed unless applicant instructs otherwise. If applicant does not wish to have any previously filed unentered amendment(s) entered, applicant must request non-entry of such amendment(s).

☐ Previously submitted. If a final Office action is outstanding, any amendments filed after the final Office action may be considered as a submission even if this box is not checked.

- ☐ Consider the arguments in the Appeal Brief or Reply Brief previously filed on _____
- ☐ Other _____

☒ Enclosed

- ☒ Amendment/Reply
- ☐ Information Disclosure Statement (IDS)
- ☐ Affidavit(s)/ Declaration(s)
- ☐ Other _____

MISCELLANEOUS

☐ Suspension of action on the above-identified application is requested under 37 CFR 1.103(c) for a period of months _____ (Period of suspension shall not exceed 3 months; Fee under 37 CFR 1.17(i) required)

☐ Other _____

FEES

The RCE fee under 37 CFR 1.17(e) is required by 37 CFR 1.114 when the RCE is filed.

☒ The Director is hereby authorized to charge any underpayment of fees, or credit any overpayments, to Deposit Account No 504352

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT REQUIRED

☒ Patent Practitioner Signature

☐ Applicant Signature

PTO/SB/30EFS (07-09)
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Signature of Registered U.S. Patent Practitioner			
Signature	/Raymond Wagenknecht/	Date (YYYY-MM-DD)	2014-05-05
Name	Raymond Wagenknecht	Registration Number	50948

This collection of information is required by 37 CFR 1.114. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450.

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2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.

3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.

4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.

6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

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PETITION FOR EXTENSION OF TIME UNDER 37 CFR 1.136(a)

Docket Number (Optional): ACE134U1

Application Number: 12/435,569

Filed: 5 May 2009

For: LABEL-FREE MONITORING OF EXCITATION-CONTRACTION COUPLING AND EXCITABLE CELLS USING IMPEDANCE BASED SYSTEMS WITH MILLISECOND TIME RESOLUTION

Art Unit: 1653

Examiner: Shannon Janssen

This is a request under the provisions of 37 CFR 1.136(a) to extend the period for filing a reply in the above-identified application.

The requested extension and fee are as follows (check time period desired and enter the appropriate fee below):

	Fee	Small Entity Fee	Micro Entity Fee	
☐ One month (37 CFR 1.17(a)(1))	$200	$100	$50	$
☐ Two months (37 CFR 1.17(a)(2))	$600	$300	$150	$
☑ Three months (37 CFR 1.17(a)(3))	$1,400	$700	$350	$ 700
☐ Four months (37 CFR 1.17(a)(4))	$2,200	$1,100	$550	$
☐ Five months (37 CFR 1.17(a)(5))	$3,000	$1,500	$750	$

☑ Applicant asserts small entity status. See 37 CFR 1.27.

☐ Applicant certifies micro entity status. See 37 CFR 1.29.
Form PTO/SB/15A or B or equivalent must either be enclosed or have been submitted previously.

☐ A check in the amount of the fee is enclosed.

☐ Payment by credit card. Form PTO-2038 is attached.

☐ The Director has already been authorized to charge fees in this application to a Deposit Account.

☐ The Director is hereby authorized to charge any fees which may be required, or credit any overpayment, to Deposit Account Number _____.

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I am the

☐ applicant.

☑ attorney or agent of record. Registration number 50948.

☐ attorney or agent acting under 37 CFR 1.34. Registration number _____.

/Raymond Wagenknecht/ — Signature

May 5, 2014 — Date

Raymond Wagenknecht — Typed or printed name

858-587-2510 ext 201 — Telephone Number

NOTE: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications. Submit multiple forms if more than one signature is required, see below*.

☐ * Total of _____ forms are submitted.

This collection of information is required by 37 CFR 1.136(a). The information is required to obtain or retain a benefit by the public, which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 6 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Mail Stop PCT, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: WANG et al.	)	Application No.: 12/435,569
Filed: 5 May 2009	)	Examiner: Shannon Janssen
Docket No.: ACE-134U1	)	Group Art Unit: 1653
Title: LABEL-FREE MONITORING OF EXCITATION-CONTRACTION COUPLING AND EXCITABLE CELLS USING IMPEDANCE BASED SYSTEMS WITH MILLISECOND TIME RESOLUTION	)	Confirmation No.: 3664
	)	Customer No.: 59538

Commissioner for Patents
P. O. Box 1450
Alexandria, VA 22313-1450

RESPONSE TO OFFICE ACTION 3 WITH REQUEST FOR CONTINUED EXAMINATION (RCE)

Sir:

Applicants are responding to the Office Action mailed November 4, 2013 which provides a three month period for reply. This response is filed within six months and is therefore accompanied with a request for a three month extension of time.

AMENDMENTS TO THE CLAIMS begin on page 2 of this paper.

REMARKS / ARGUMENTS begin on page 13 of this paper.

AMENDMENTS TO THE CLAIMS

Changes to the claims are provided in the below listing of claims for the convenience of the examiner.

Listing of Claims

1-15. (Cancelled)

16. (Previously presented) A method for assessing excitable cells in vitro, comprising

a) providing a system for monitoring impedance of excitable cells in vitro, comprising:

i) a device for monitoring cell-substrate impedance, comprising:

a) a nonconductive substrate,

b) one or more electrode arrays fabricated on said substrate,

c) one or more wells on said substrate, wherein each of said one or more arrays is associated with one of said one or more wells, wherein each electrode array is individually addressable, further wherein said substrate has a surface suitable for cell attachment, wherein said cell attachment on said substrate can result in a detectable change in impedance between electrodes within each electrode array,

ii) an impedance analyzer capable of impedance measurement at millisecond time resolution,

iii) electronic circuitry that can engage said device and selectively connect said two or more electrode arrays of said device to said impedance analyzer, and

(iv) a software program that controls said electronic circuitry and records and analyzes data obtained from said impedance analyzer;

b) adding excitable cells to said one or more wells;

c) monitoring cell-substrate impedance of said one or more wells using millisecond time resolution to detect changes in cell morphology or cell attachment to said substrate; and

d) resolving individual cycles of said excitable cells.

17. (Previously presented) The method according to claim 16, wherein said excitable cells are cells that undergo excitation-contraction coupling.

18. (Original) The method according to claim 17, wherein said excitable cells are cardiomyocytes.

19. (Original) The method according to claim 16, wherein the step of monitoring impedance comprises performing impedance measurements at millisecond time resolution simultaneously across all wells.

20. (Original) The method according to claim 19, wherein said impedance measurements at millisecond time resolution comprises at least two consecutive impedance measurements at less than 300 milliseconds apart.

21. (Original) The method according to claim 20, wherein said at least two consecutive impedance measurements are less than 100 milliseconds apart.

22. (Original) The method according to claim 21, wherein said at least two consecutive impedance measurements are less than 40 milliseconds apart.

23. (Original) The method according to claim 22, wherein said at least two consecutive impedance measurements are less than 10 milliseconds apart.

24. (Previously presented) A method of determining whether a compound modulates a beating cycle of an excitable cell, comprising:

a) providing a system for monitoring impedance of excitable cells in vitro, comprising:

i) a device for monitoring cell-substrate impedance, comprising:

a) a nonconductive substrate,

b) one or more electrode arrays fabricated on said substrate,

c) at least two wells on said substrate, wherein each of said one or more arrays is associated with each of said at least two wells, wherein each electrode array is individually addressable, further wherein said substrate has a surface suitable for cell attachment, wherein said cell attachment on said substrate can result in a detectable change in impedance between electrodes within each electrode array,

ii) an impedance analyzer capable of impedance measurement at millisecond time resolution,

iii) electronic circuitry that can engage said device and selectively connect said two or more electrode arrays of said device to said impedance analyzer, and

(iv) a software program that controls said electronic circuitry and records and analyzes data obtained from said impedance analyzer;

b) adding excitable cells to said at least two wells;

c) monitoring cell-substrate impedance of said at least two wells using millisecond time resolution to detect changes in cell morphology or cell attachment to said substrate;

d) adding a compound suspected of modulation of the beating cycle to a first of said at least two wells to provide a test well, wherein a second well lacking a compound that modulates the beating cycle is provided as a control well;

e) resolving the beating cycles of both test well and control well; and

d) comparing said beating cycles between said test well and control well, wherein a difference in beating cycles indicates said compound modulates the beating cycle of the excitable cell.

25. (Original) The method according to claim 24, wherein the excitable cells are cardiomyocytes or embryonic stem cell derived cardiomyocytes.

26. (Original) The method according to claim 24, wherein said beating cycles are compared to identify changes in beating amplitude.

27. (Original) The method according to claim 24, wherein said beating cycles are compared to identify changes in beating frequency.

28. (Original) The method according to claim 27, wherein said changes in beating frequency are regular changes or irregular changes.

29. (Original) The method according to claim 24, further comprising determining a cell index for each of said test well and control well, wherein the step of comparing said beating cycles comprises comparing said cell indexes between test well and control well.

30. (Withdrawn) A method of characterizing rhythmic beating of a cardiomyocyte comprising:

a) providing a system for monitoring impedance of excitable cells in vitro, comprising:

i) a device for monitoring cell-substrate impedance, comprising:

a) a nonconductive substrate,

b) one or more electrode arrays fabricated on said substrate,

c) one or more wells on said substrate, wherein each of said one or more arrays is associated with each of said one or more wells, wherein each electrode array is individually addressable, further wherein said substrate has a surface suitable for cell attachment, wherein said cell attachment on said substrate can result in a detectable change in impedance between electrodes within each electrode array,

ii) an impedance analyzer capable of impedance measurement at millisecond time resolution,

iii) electronic circuitry that can engage said device and selectively connect said two or more electrode arrays of said device to said impedance analyzer, and

(iv) a software program that controls said electronic circuitry and records and analyzes data obtained from said impedance analyzer;

b) adding excitable cells to said one or more wells;

c) monitoring cell-substrate impedance of said one or more wells in millisecond time resolution to detect changes in cell morphology or cell attachment to said substrate; and

d) determining a plurality of beating cycle peaks in a time unit; and

e) comparing amplitude or frequency of said peaks over said time unit.

31. (Withdrawn) The method according to claim 30, wherein the step of determining a plurality of beating cycle peaks comprises determining a rise in impedance and a decay in impedance and extrapolating a peak from each rise and decay.

32. (Withdrawn) The method according to claim 30, further comprising determining a cell index for each of said one or more wells, wherein the step of determining a plurality of beating cycle peaks comprises for each cycle determining a rise in cell index, a decay in cell index and extrapolating each peak from each rise and decay.

33. (Withdrawn) The method according to claim 30, wherein beating cycle peaks are determined by identifying impedance data points at which the first order derivative to time is zero or close to zero.

34. (Withdrawn) The method according to claim 30, further comprising monitoring impedance of said one or more wells over seconds, minutes or hours, optionally wherein said monitoring of impedance in millisecond time resolution is performed within said monitoring over seconds, minutes or hours.

35. (Withdrawn) A method of assessing genetic manipulation of an embryonic stem cell, comprising:

a) providing a system for monitoring impedance of excitable cells in vitro, comprising:

i) a device for monitoring cell-substrate impedance, comprising:

a) a nonconductive substrate,

b) one or more electrode arrays fabricated on said substrate,

c) at least two wells on said substrate, wherein each of said one or more arrays is associated with each of said at least two wells, wherein each electrode array is individually addressable, further wherein said substrate has a surface suitable for cell attachment, wherein said cell attachment on said substrate can result in a detectable change in impedance between electrodes within each electrode array,

ii) an impedance analyzer capable of impedance measurement at millisecond time resolution,

iii) electronic circuitry that can engage said device and selectively connect said two or more electrode arrays of said device to said impedance analyzer, and

(iv) a software program that controls said electronic circuitry and records and analyzes data obtained from said impedance analyzer;

b) mutating embryonic stem cells to comprise a genetic mutation;

c) adding said mutated embryonic stem cells to a first well of said at least two wells to form a test well and control embryonic stem cells to a second of said at least two wells to form a control well;

c) monitoring cell-substrate impedance of said at least two wells in millisecond time resolution to detect changes in cell morphology or cell attachment to said substrate; and

e) resolving the beating cycles of both test well and control well, if any; and

d) comparing said beating cycles between said test well and control well, wherein a difference in beating cycles indicates said genetic mutation modulates embryonic stem cell differentiation.

36. (Withdrawn) The method according to claim 35, wherein said embryonic stem cell differentiation is differentiation into a cardiomyocyte.

37. (Withdrawn) The method according to claim 35, wherein said genetic mutation is selected from the group consisting of a genetic knock out, a knock down and an inserted transgene.

38. (Withdrawn) A method for assessing and quantifying excitable cells in vitro, comprising

a) providing a system for monitoring impedance of excitable cells in vitro, comprising:

i) a device for monitoring cell-substrate impedance, comprising:

a) a nonconductive substrate,

b) one or more electrode arrays fabricated on said substrate,

c) one or more wells on said substrate, wherein each of said one or more arrays is associated with one of said one or more wells, wherein each electrode array is individually addressable, further wherein said substrate has a surface suitable for cell attachment, wherein said cell attachment on said substrate can result in a detectable change in impedance between electrodes within each electrode array,

ii) an impedance analyzer capable of impedance measurement at millisecond time resolution,

iii) electronic circuitry that can engage said device and selectively connect said two or more electrode arrays of said device to said impedance analyzer, and

(iv) a software program that controls said electronic circuitry and records and analyzes data obtained from said impedance analyzer;

b) adding excitable cells to said one or more wells;

c) monitoring cell-substrate impedance of said one or more wells over a first time period, which is characterized as a longer time period comprising seconds, minutes or hours;

d) monitoring cell-substrate impedance of said one or more wells over a second time period in millisecond time resolution to detect changes in cell morphology or attachment;

e) determining a characteristic selected from the group consisting of cell attachment, cell growth and cell viability from monitoring over said first time period; and

f) resolving individual cycles of said excitable cells from monitoring over said second time period.

39. (Withdrawn) The method according to claim 38, wherein monitoring impedance over said second time period is performed at regular or irregular time intervals within monitoring impedance over said first time period.

40. (Previously presented) The method according to claim 24, wherein the step of monitoring impedance using millisecond time resolution comprises obtaining at least two consecutive impedance measurements at less than 300 milliseconds apart.

41. (Previously presented) The method according to claim 40, wherein said at least two consecutive impedance measurements are less than 100 milliseconds apart.

42. (Previously presented) The method according to claim 41, wherein said at least two consecutive impedance measurements are less than 40 milliseconds apart

43. (Previously presented) The method according to claim 42, wherein said at least two consecutive impedance measurements are less than 10 milliseconds apart.

44. (Currently amended) The method according to claim 24, wherein the step of resolving the beating cycles comprises determining a rise in impedance and a decay in impedance, and extrapolating a ~~peek~~ peak from each rise and decay.

45. (Previously presented) The method according to claim 24, wherein the step of resolving the beating cycles comprises determining beating cycle peaks by identifying impedance data points at which the first order derivative to time is zero or about zero.

REMARKS /ARGUMENTS

Claim 44 is amended. Claims 16-45 are pending and claims 30-39 remain withdrawn.

I.
Support for Amendments

Claim 44 is amended to correct a typographical error.

II.
Response to Claim Objections

Claim 44 is objected to as having a typographical error. Specifically, "peak" is misspelled as "peek". The typographical error is corrected.

III.
Response to Rejections under 35 USC §103

Claims 16-29 and 40-45 are rejected under 35 U.S.C. §103(a) as being obvious over US 2005/0153425 published by Xu et al. (Xu), in view of Am. J. Physiol. Heart Circ. Physiol., 2003, Vol. 284, pp. H2114-23 published by Banach et al. (Banach) as evidenced by Journal of Electrocardiology, Vol. 37, pp 110-116 published by Hescheler et al. (Hescheler), US 6,852,525 by Cantor, and US 2005/0182447 by Schecter.

Specifically, Xu is cited as teaching methods for performing real-time cell-based assays for investigating the effects of one or more compounds using an impedance-based system. Xu is also cited as teaching such a system monitors cell attachment, morphology, degree of adhesion and at time frames of less than a second.

The examiner acknowledges that Xu does not teach the measurement of excitable cells that undergo excitation-contraction coupling or cardiomyocytes, resolving

individual cycles or beating cycles at millisecond time resolution or identifying changes in beating amplitude or beating frequency.

Banach is cited as teaching a microelectrode array for monitoring the beating cycle of cardiomyocytes, monitoring electrical activity from multiple electrodes simultaneously and continuously with millisecond time resolution, and resolving signal delays of less than 10 milliseconds. Banach is also cited as teaching monitoring an interspike interval to determine a beating frequency, measuring peak amplitude, and measuring the effect of different compounds on beating frequency and action potential in the form of FP_{min} to FP_{max}.

Hescheler is cited as a further reference suggesting that the microelectrode array of Banach may provide a new experimental model to study a cellular system for studying cardiotoxic effects during embryogenesis in vitro.

Cantor is cited as teaching a method including a microelectrode system for monitoring impedance of cells, and monitoring impedance of the opening and closing of ion channels, which occurs in less than a millisecond, and resolving individual opening and closing cycles of excitable cells.

The examiner acknowledges that Xu, Banach and Cantor do not specifically teach determining a rise in impedance and a decay in impedance and extrapolating a peak from the rise and decay; however, Schecter is cited as teaching a method of monitoring a beating cycle of the heart using impedance measurements and calculating the derivative of impedance to time at 0, peak and delays in time to peak incidence, and changes in the impedance waveform and/or reductions in the quantity of the integral of Z or changes in the morphology of Z(t)(Dt).

Applicant respectfully requests reconsideration of the rejection in view of the following remarks. A proper obviousness rejection requires the cited references teach or suggest all of limitations in the claim.[1] In addition, there must also be an objective

[1] *Ex parte Wada and Murphy*, Appeal 2007-3733, Slip Opinion at 7, BPAI January 14, 2008 ("When determining whether a claim is obvious, an examiner must make "a searching comparison of the claimed invention – including all its limitations – with the teaching of the prior art." *In re Ochiai*, 71 F.3d 1565, 1572 (Fed. Cir. 1995) (emphasis added). Thus, "obviousness requires a suggestion of all limitations in a claim." *CFMT, Inc. v. Yieldup Intern. Corp.*, 349 F.3d 1333, 1342 (Fed. Cir. 2003) (citing *In re Royka*, 490 F.2d 981, 985 (C.C.P.A. 1974)"); *see also* MPEP 2143.03.

reasoning for combining the teachings in the references cited.[2] Accordingly, the mere fact that certain teachings in cited references can be combined or that modifications of the prior art were within the ability of one of ordinary skill in the art does not itself render a claimed invention obvious.[3]

It is not disputed that Xu provides a cell-substrate impedance monitoring system and methods for investigating effects of one or more compounds. However, Xu indicates that cell-substrate impedance is primarily influenced by three main parameters: the number of cells seeded on the microelectronic sensor arrays, the shape of the cell, and the strength of cell adhesion to the electrode surface. Para. [0430]. Thus, changes in cell-substrate impedance occur from differences in attachment or spreading of cells on the device. However, as indicated by the examiner the methods in Xu do not monitor cell-substrate impedance at millisecond time resolution, do not monitor excitable cells that undergo excitation contraction coupling or cardiomyocytes, and do not resolve the individual or beating cycle of excitable cells. That is, while Xu does monitor changes in cell-substrate impedance that occur over longer periods of time, such as in case of cytotoxicity (e.g. cells detaching from electrodes during death), Xu does not teach or suggest conducting measurements in millisecond time resolution or that that the individual or beating cycle of excitable cells can be resolved through cell-substrate impedance monitoring. The addition of Banach, Hescheler, Cantor and Schecter does not correct the deficiency of this absence.

Banach analyzed the developmental changes of spontaneous *electrical activity* of multicellular aggregates of embryonic stem cells (ESCs) by extracellular recording of *field potential* using substrate integrated microelectrode arrays (MEAs). Pg. H2114, right col., Para 1. That is, Banach analyzed changes in electrical activity of ESC aggregates by measuring the membrane potential across the membrane channels of the cells. By

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[2] *KSR International Co. v. Teleflex Inc. et al.* 127 S. Ct. 1727, 1741 (2007) (an obvious analysis must determine "whether there was an apparent reason to combine the known elements in the fashion claimed by the patent at issue") citing *In re Kahn*, 441 F.3d 977, 988 (Fed. Cir. 2006) ("Rejections on obviousness grounds cannot be sustained by mere conclusory statements; instead, there must be some articulated reasoning with some rational underpinning to support the legal conclusion of obviousness").

[3] MPEP 2143.01, III and IV; *see also KSR, supra* at 1740-1741; *Ex parte Levengood,* 28 U.S.P.Q.2d 1300 (BPAI 1993).

recording changes in field potential an interspike interval (ISI) was determined and associated with the beating frequency. Pg. H2115, right col.

Compared to claims 16 and 24, Banach's recording of *field potential*, to determine a beating frequency through an interspike interval (ISI) does not teach or suggest that *cell-substrate impedance* can be used to resolve the individual or beating cycle of excitable cells. That is, the skilled artisan would appreciate that recording field potential and monitoring cell-substrate impedance are significantly different measurements. Specifically, field potential refers to a current sink or source that fluctuates when positive charges enter or leave a group of cells through membrane channels. In contrast, cell-substrate impedance refers to an impedance measurement that assesses the attachment or spreading of cells over a substrate/electrode. Since recording of field potential and cell-substrate impedance monitoring are significantly different, the ability to determine an interspike interval (ISI) through recording of field potential would not teach or suggest that cell-substrate impedance monitoring could be used to resolve the individual or beating cycle of an excitable cell. In addition, whether or not extracellular recording of field potential can be accomplished in millisecond time resolution is not material as to whether one could successfully achieve cell-substrate impedance monitoring at millisecond time resolution. Again, they are different measurements.

Hescheler provides an overview of the use of extracellular recording of embryonic stem cell-derived cardiomyocytes using microelectrode arrays (MEAs). Abstract. As with Banach, Hescheler confirms at page 112, right col. that the signal in the extracellular recording approach involves recording *membrane potential*:

> Although the signal recording with an extracellular electrode reflects local changes of the *membrane potential*, currents generated in the electrically connected neighboring tissue influence it. (emphasis added)

Thus, like Banach the teaching of Hescheler is limited to technologies that record membrane potential. Such approaches do not teach or suggest that cell-substrate impedance can be used to resolve the individual or beating cycle of excitable cells, such as cardiomyoctes. In addition, Hescheler does not teach one skilled in the art how to

achieve *cell-substrate impedance* monitoring at millisecond time resolution as claimed since each is a significantly different measurement.

Cantor provides a mammalian cell culture chamber having a cover for allowing for transfer of gasses and a sensor array. Col. 3, ll.29-33. Cantor's system provides an approach for screening and assaying broad classes of materials for their impact on cellular metabolism. Col. 5, ll. 1-3. In regards to the sensor array, Cantor utilizes a small microelectrode/ large reference electrode approach where measurement occurs solely at the small microelectrode. Col. 5, ll. 4-36. Cantor suggests that the small microelectrodes can be used to monitor the impedance of the individual cell, action potential parameters of the individual cell, the cell membrane capacitance, the cell membrane conductance, and the cell/substrate seal resistance, and cell membrane potential. Col. 5, ll. 13-22. Specifically, Cantor utilizes the cell culture chamber to detect changes in *membrane potential* in response to the opening of ion channels. Col. 10, ll.22-37. The opening and closing of voltage-gated Na+ channels takes only a few milliseconds. Col. 2, ll. 46-67.

For completeness, the examiner cites Cantor as measuring impedance peaks in millisecond time resolution (citing col. 2, l.39+, col. 3, ll.14-27, col. 5, ll. 1-35, col. 8, ll. 14-44.) Office Action at pg. 9. Although Cantor does indicate that the device can measure impedance and that depolarization and repolarization of Na+ channels takes a few milliseconds or less, Cantor clarifies at Col. 3, ll. 14-23, that examining *membrane potential* is used to assess the opening and closing of these membrane channels since action potential amplitude corresponds to the height of peak depolarization and action potential shape corresponds to the time course of depolarization and repolarization. In other words, Cantor does not teach or suggest the use of *cell-substrate impedance* at millisecond time resolution to resolve the individual or beating cycle of excitable cells. Instead, like Banach and Heschler Cantor measures cell *membrane potential* relative to time. Col. 3, l. 14. Again, cell membrane potential is an electrical property regarding the passage of ions through channels in the cell membrane, whereas the present invention monitors cell-substrate impedance, which is an impedance measurement directed towards the physical interaction between cell and substrate/electrode. That is, cell-substrate impedance is not a membrane potential measurement. Therefore obtaining peaks associated with *membrane potential* does not teach or suggest resolving the individual or

beating cycle of cardiomyocytes through *cell-substrate impedance* monitoring or achieving *cell-substrate impedance* monitoring at millisecond time resolution as claimed.

The object of Schecter is to utilize impedance waveforms and corresponding impedance data to direct the programming of a cardiac resynchronization therapy (CRT). Para [0046]. That is, the impedance data is from an implanted pacemaker or defribillator. Abstract. Schecter's methods involve optimizing acquisition of impedance signals between electrodes present on implanted lead systems, automatically determining which electrodes or electrode combinations acquire impedance waveforms that have the best signal to noise ratio, and characterizing data most representative of dysynchronous electromechanical events. Abstract. Since Schecter utilizes impedance signals from a patient's implant and not from a cell-substrate impedance device as claimed Schecter would not apply to the invention set forth in claims 16 or 24.

In addition to the above distinctions, Applicants respectfully disagree with the analysis by the examiner in determining likelihood of success of combining the references in the Office Action at pg. 12 for the following reasons.

In regards to Banach, the examiner argues that Banach teaches successfully establishing cultures of beating cardiomyocytes and measuring electrical properties of the cultures to establish beating cycle parameters and beating frequency. While Banach does establish cultures of cardiomyocytes, Banach uses a different technical approach to measurement. Banach records changes in field potential, which is an electrical property resulting from changes in membrane potential. The ability to record field potential from a population of cardiomyocytes and to determine a beating frequency through interspike intervals (ISI) would not support a reasonable expectation of success of using cell-substrate impedance monitoring of a cardiomyocyte population to resolve an individual or beating cycle of a cardiomyocyte population. As discussed above, each is a significantly different measurement and thus success of one is not a predictor of success of the other. In other words, cell-substrate impedance does not measure depolarization/repolarization of cells.

In regards to Cantor, the examiner argues that Cantor teaches that electrode arrays can be used to measure cell impedance, secretion of cellular products, monitoring of ion channels and action potentials, and monitoring of compounds which affect those

parameters in millisecond time resolution. While Cantor suggests that different parameters can be measured, the only effect referencing a millisecond time period would be the depolarization/repolarization of Na+ channels. Cantor indicates this is measured by recording membrane potential. An expectation of success in recording membrane potential would not support a reasonable expectation of success of cell-substrate impedance monitoring of a cardiomyocyte population to resolve an individual or beating cycle of a cardiomyocyte population. As discussed above, each is a significantly different measurement and thus success of one is not a predictor of success of the other.

In regards to Xu, the examiner argues that Xu teaches successfully using impedance to characterize cellular properties (citing para. [0428]). It is agreed Xu was able to assess mast cell activation through the use of cell-substrate impedance monitoring as discussed in para. [0428], but this does not teach millisecond time resolution and does not teach resolving the individual or beating cycle of an excitable cell through the use of cell-substrate impedance technology.

In regards to Schecter, the examiner argues that it is routine and known in the art to derive impedance waveforms. Whether or not changes in impedance waveforms can be determined does not demonstrate millisecond time resolution of cell-substrate impedance monitoring of a cardiomyocyte population.

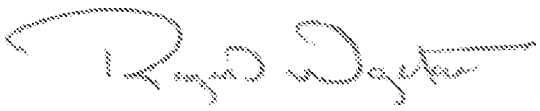
Accordingly, Applicant respectfully requests the rejection of independent claims 16, 24 and dependent claims 17-23, 25-29, and 40-45 be withdrawn.

IV.
Conclusion

Applicants believe that the application as currently presented is in a condition for allowance and such favorable action is respectfully requested. If the Examiner desires, Applicant welcomes a telephone interview to expedite prosecution and is available at the telephone number below.

Respectfully submitted,

BIOTECH BEACH LAW GROUP PC



May 5, 2014

Date

Raymond Wagenknecht
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5677 Oberlin Dr. Ste 204
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Electronic Patent Application Fee Transmittal

Application Number:	12435569
Filing Date:	05-May-2009
Title of Invention:	Label-Free Monitoring of Excitation-Contraction Coupling and Excitable Cells Using Impedance Based Systems with Millisecond Time Resolution
First Named Inventor/Applicant Name:	Xiaobo Wang
Filer:	Raymond C. Wagenknecht
Attorney Docket Number:	ACE-134U1

Filed as Small Entity

Utility under 35 USC 111(a) Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Extension - 3 months with $0 paid	2253	1	700	700

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
Request for Continued Examination	2801	1	600	600
		Total in USD ($)		**1300**

Electronic Acknowledgement Receipt

EFS ID:	18949108
Application Number:	12435569
International Application Number:	
Confirmation Number:	3664
Title of Invention:	Label-Free Monitoring of Excitation-Contraction Coupling and Excitable Cells Using Impedance Based Systems with Millisecond Time Resolution
First Named Inventor/Applicant Name:	Xiaobo Wang
Customer Number:	59538
Filer:	Raymond C. Wagenknecht
Filer Authorized By:	
Attorney Docket Number:	ACE-134U1
Receipt Date:	05-MAY-2014
Filing Date:	05-MAY-2009
Time Stamp:	20:33:09
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Credit Card
Payment was successfully received in RAM	$1300
RAM confirmation Number	7422
Deposit Account	504352
Authorized User	WAGENKNECHT, RAYMOND

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Charge any Additional Fees required under 37 C.F.R. Section 1.20 (Post Issuance fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.21 (Miscellaneous fees and charges)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Request for Continued Examination (RCE)	ACE134U1_RCE.pdf	797442 a76a03d4bda81eb08cc6d80ff9bb2c9d047bf1fc	no	3
Warnings:					
Information:					
2	Extension of Time	ACE134U1_Extension_of_Time.pdf	33139 1d4cbdf87834d377982fd766e269436d26d64cae	no	1
Warnings:					
Information:					
3		ACE-134U1_Filed_Response_OA3.pdf	161065 a346299d167d9a374f3e228c15613f7cf6d88156	yes	20

Multipart Description/PDF files in .zip description

Document Description	Start	End
Amendment Submitted/Entered with Filing of CPA/RCE	1	1
Claims	2	12
Applicant Arguments/Remarks Made in an Amendment	13	20

Warnings:					
Information:					
4	Fee Worksheet (SB06)	fee-info.pdf	32151 2ee25bbc82864e03b9608916ef83c6dff15e208f	no	2
Warnings:					
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			Total Files Size (in bytes):	1023797	

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PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 12/435,569	Filing Date 05/05/2009	☐ To be Mailed

ENTITY: ☐ LARGE ☒ SMALL ☐ MICRO

APPLICATION AS FILED – PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	RATE ($)	FEE ($)
☒ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A	**165**
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*	X $ =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*	X $ =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).			
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))				
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL	**165**

APPLICATION AS AMENDED – PART II

AMENDMENT	**05/05/2014**	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	* 30	Minus	** 39	= 0	X $40 =	0
	Independent (37 CFR 1.16(h))	* 5	Minus	***5	= 0	X $210 =	0
	☐ Application Size Fee (37 CFR 1.16(s))						
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
						TOTAL ADD'L FEE	**0**

AMENDMENT		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	*	Minus	**	=	X $ =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	X $ =	
	☐ Application Size Fee (37 CFR 1.16(s))						
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
						TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.

** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".

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(Not for submission under 37 CFR 1.99)

Application Number	12435569
Filing Date	2009-05-05
First Named Inventor	Xiaobo Wang
Art Unit	1653
Examiner Name	Shannon L. Janssen
Attorney Docket Number	ACEA134U1

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Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1	8206903		2012-06-26	Abassi et al.	

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	1	20140203818		2014-06-24	Wang et al.	
	2	20060161073		2006-07-20	Singer et al.	
	3	20090142790		2009-06-04	Fang et al.	
	4	20030211500		2003-11-13	Woosley et al.	
	5	20070042347		2007-02-22	Rosen et al.	

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	12435569
	Filing Date	2009-05-05
	First Named Inventor	Xiaobo Wang
	Art Unit	1653
	Examiner Name	Shannon L. Janssen
	Attorney Docket Number	ACEA134U1

	6	20040106095		2004-06-03	Thomson et al.	
	7	20070212423		2007-09-13	Epstein et al.	
	8	20090017465		2009-01-15	Xu et al.	

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	1	2012/043820	WO		2012-04-05	Nat'l Univ Corp Tokyo Med & Dent Univ		X
	2	2014/085727	WO		2014-06-05	ACEA Biosciences, Inc.		☐
	3	2291645	EP		2015-09-09	ACEA Biosciences, Inc.		☐
	4	2009/137440	WO		2009-11-12	ACEA Biosciences, Inc.		☐
	5	2010/129725	WO		2009-11-11	ACEA Biosciences, Inc.		☐
	6	2011/146531	WO		2011-11-24	ACEA Biosciences, Inc.		☐

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (**Not for submission under 37 CFR 1.99)**		
	Application Number	12435569
	Filing Date	2009-05-05
	First Named Inventor	Xiaobo Wang
	Art Unit	1653
	Examiner Name	Shannon L. Janssen
	Attorney Docket Number	ACEA134U1

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Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published.	T[5]
	1	BERDONDINI et al. "High-density electrode array for imaging in vitro electrophysiological activity." Biosensors and Bioelectronics, 2005, 21:167-174	
	2	CHANG et al. "Impedimetric monitoring of cell attachment on interdigitated microelectrodes." Sensors and Actuators, 2005, B 105:159-163	
	3	YANG et al. "A novel microfluidic impedance assay for monitoring endothelin-induced cardiomyocyte hypertrophy." Biosensors and Bioelectronics, 2007, 22:1688-1693	
	4	PCT/US2009/033801 International Search Report and Written Opinion mailed 09 July 2010	
	5	PCT/US2009/042787 International Search Report and Written Opinion mailed 24 June 2009	
	6	PCT/US2011/036877 International Search Report mailed 02 September 2011	
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	Application Number	12435569
	Filing Date	2009-05-05
	First Named Inventor	Xiaobo Wang
	Art Unit	1653
	Examiner Name	Shannon L. Janssen
	Attorney Docket Number	ACEA134U1

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	Application Number	12435569
	Filing Date	2009-05-05
	First Named Inventor	Xiaobo Wang
	Art Unit	1653
	Examiner Name	Shannon L. Janssen
	Attorney Docket Number	ACEA134U1

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Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

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See attached certification statement.

The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☒ A certification statement is not submitted herewith.

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A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Raymond Wagenknecht/	Date (YYYY-MM-DD)	2015-12-08
Name/Print	Raymond Wagenknecht	Registration Number	50948

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1. (JPWO2012043820) 心筋毒性検査および心筋細胞評価のための方法および装置 .

National Biblio. Data

...

Permanent Link/ Bookmark:

Machine translation: original->english

Application Number: JP2011072618 **Application Date:** 30.09.2011

Publication Number: WO2012043820 **Publication Date:** 05.04.2012

Publication Kind : A1

IPC: C12Q 1/06
C12M 1/34
C12M 1/42

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金子 智行
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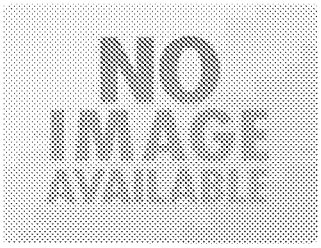
Agents: 小林 浩
藤田 尚
鈴木 康仁

Priority Data: 2010222636 30.09.2010 JP
2010234498 19.10.2010 JP
2011090536 14.04.2011 JP

Title: (JA) 心筋毒性検査および心筋細胞評価のための方法および装置

Abstract: (JA)

透明基板上に心筋細胞集団を配置し、心筋拍動細胞に与えた強制拍動刺激に対する心筋細胞の応答から心筋細胞の品質を評価する。透明基板上に心筋細胞集団を配置し、ネットワークを構成する細胞には薬物が作用するように薬物を含む液体の流れに曝す。ネットワークの隣接する心筋拍動細胞の比較から得られるゆらぎ計測から薬物による心毒性の程度を評価する。



ENGLISH TRANSLATION OF WO2012/043820

WO2012043820A1 2012-04-05 **METHOD AND DEVICE FOR MYOCARDIAL CELL EVALUATION AND MYOCARDIAL TOXICITY INSPECTION**

English Abstract:

In the present invention, a myocardial cell cluster is disposed on a transparent substrate, and the quality of the myocardial cells is evaluated from the response of the cells to a forced pulsation stimulus applied to the cardiomyocytes. The myocardial cell cluster is disposed on the transparent substrate, and is exposed to the flow of a liquid containing a drug in a manner so that the drug acts on the cells, which configure a network. The extent of cardiac toxicity resulting from the drug is evaluated from measuring the fluctuations obtained from a comparison of adjacent cardimyocytes of the network.

Standardized Assignees: NATIONAL UNIVERSITY CORPORATION TOKYO MEDICAL & DENTAL UNIVERSITY;
MITSUBISHI CHEMICAL MEDIENCE;
YASUDA KENJI;
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Attorneys: KOBAYASHI Hiroshi et al, 小林　浩, ABE, IKUBO & KATAYAMA, Fukuoka Bldg. 9th Fl., 8-7, Yaesu 2-chome, Chuo-ku, Tokyo, 1040028, 〒1040028, Japan〒1040028 東京都中央区八重洲二丁目８番７号　福岡ビル９階　阿部・井窪・片山法律事務所 Tokyo, 〒1040028, Japan

Application Number: WOJP11072618

Application/Filing Date: 2011-09-30
National Register

PCT Application: WOJP11072618 2011-09-30

English Description:

TECHNICAL FIELD

The present invention, a method and a device for inspecting and evaluating a myocardal cardiomyocyte of toxicity.

BACKGROUND ART

The change of state of the cells or, medicine or observation of the response to the bioassay processiblity. In the conventional bioassay, many cultured cells are generally used. Using this system, since a plurality of cells assays, the average of the values of the cell group 1 were observed in the characteristics of the cell.

However, actually, the cells in a cell that is tuned to cycles and rarely, in a period of each cell protein is different. Therefore, a response to the stimulation of the results of the analysis are always fluctuationally hang on problems.

That is, the cell reaction mechanism itself having universally responds to the presence of flactal, always, only an average response cannot be obtained. In order to solve these problems, tuning culture method or the like is developed, always the same stage in the use of a group of cells, the cell is always must be supplied continuously, and extend generally bioassay fault.

Furthermore, the irritation to cell (signal), a signal substance contained in the periphery of the cell soln., nutrition, and given by the amount of dissolved gas, physical contact of other cell 2 · interaction between cells, there is a kind of even one, it is difficult to judge the fluctuationally for stepwise.

· Physical contact of the cell, the problem of interaction between cells, tissue fragments bioassay is performed in such a degree of mass. However, in this case, unlike and cultured cells, cell aggregates of a constantly uniform resistance cannot be obtained. Therefore, the data obtained is dispersed, or the information during the embedding of the group.

One group of cells 1 of the cell 1 and a minimum of two units of measurement for processing model, the inventors of the present invention (patent document 1), etc. in the patent 2006-94703 as shown in, in a specific spatial arrangement of the cells are closed by partitions and a plurality of cell culture, between adjacent sections of the cells cannot pass each other in the groove is connected to the tunnel or, if necessary, or tunnel or groove in the cell culture, cell for measuring the change in the potential of the electrode assembly of the cell structure having a plurality of micro-array pattern (bioassay chip).

Furthermore, a complicated function of the heart is reflected as a method for evaluating a electrocardial acquired, nonlinear dynamics of the usually used in measuring the electrocardial analysis techniques are proposed. For instance, the most generally used in this method are [...] electrocardigram analysis (non-patent document 1). A plot of the points 1, 2 adjacent one sphygmoscope example X represents a data point of time sphygmoscope rate axis, Y axis is indicated 1 and the previous sphygmoscope rate. Therefore, the movement of the points on the graph on fluctuationally by estimating the quantitative distribution. As the method of measuring cardiac sphygmoscope fluctuationally to other, correlative dimensional method (correlation dimension), nonlinear estimation method (nonlinear predictability) (non-patent document 2), (non-patent literature 3) approximate entropy method or the l

CITATION LIST

PATENT LITERATURE

In the patent Number 2006-94703 publication

NON PATENT LITERATURE

Brennan M, M Palaniswami, Kamen P. Existing Do non-
linear plot of Poincare measures heart rate variability of geometry features reflect? Biomedi cal Engineering, IEEE Transactions on, Proc. IEEE Transactions on Biomedical Engineering, 20 01, 48, 1342-1347 JK Kanters, NH-
Rathlou Holstein, Agner E (1994). "Lack of evidence for heart rate variability in dimensional chaos low-". 5 (7) Electrophysiology Cardiovascular Journal of: 591-601. 7987529 PMID. Storella RJ, HW Wood, et al km Mills. (
1994). "Approximate entropy and point of healthy heart rate variability in correlation subjec ts dimension". (4) 33 & Behavioral Physiological Integrative Science: 315-20. 10333974 PMID.

TECH PROBLEM

In the conventional bioassay, whether as a tissue fragments, such as cell culture cells of either 1. The number of cells is too much and so the item of the prior art, which information is obtained because the average, or chemical that response to not be accurately obtained. In the case of using a cell by cell 1, originally, function as a multi-cell to cell tissue cells, separated, for use as an independent state in the cell, the influence of the interaction between the cells, and therefore it does not appear, may accurately in response to obtain data words bioassay chemicals.

See cardiomyocyte Osteoblast fibrosing about and adjacent to and from the cells or fibrocyte myocarditis propagation of pulsation, the unit cell potential 1 cell, which can accurately measure the cell shape, cell toxicity of medicine cardiomyocyte 1 inspection of cell potential, or the shape of cells as to develop a system capable of accurately measuring devices are important.

Human iPS ES cells such as cells or human redifferentiation derived from human mesenchymal stem cells about cardiomyocyte, these cells for use in a medical drug screening or the regeneration, this cardiomyocyte is, in which the same quality and human heart cardiomyocyte whether from a quantitative evaluation functions of the cell surface.

TECH SOLUTION

In view of the above problem, the present invention, to provide a method and a device.
(1) a substrate,
Disposed on the substrate, a plurality of stable sphygmoscope cardiomyocyte evaluated for infusing myoelectric cells or cell population,
On the substrate so as to surround the periphery of the population of cells formed in the wall of the cell culture solution to satisfy,
The cell culture liquid is supplied into the area surrounded by the wall and/or for discharging the supply/discharge channel,
One of the cells of the cell or cells in a localized portion of the electrode is placed,
The engine is enclosed by a wall in the region of the cell culture solution for satisfying a reference electrode,
The micro electrodes is connected to each of the reference electrode and a lead wire connected to the lead wire and the electrode using the microclearance placed biopotential measuring means for measuring cell potential, and
By controlling the microclearance electrostimulation sent to the electrodes, the potential is measured by the measuring means for recording data, and a control/recording means
This device is provided with a cell,
The Electrostimulator sent to the minute interval of the electrode is changed stepwise,
1) above the cell group cardiomyocyte or the forced stimulation sphygmoscope electrostimulation interval in response to the same interval and the forced stimulation sphygmoscope confirms whether the response, and, its response in the frequency range of any forced sphygmoscope irrilation corresponding by measuring whether or not, it can follow sphygmoscope sound cardiomyocyte by confirming that sufficiently satisfies one of the conditions and determine, and
2) due to the forced stimulation sphygmoscope interval (RR) electrostimulator beating of the cells to follow a frequency carpentering is confirmed, the change of the forced stimulation sphygmoscope FPD, FPD / (RR)

1/3 from FPD / (RR) 1/2 between sound by confirming that a sufficient cardiomyocyte satisfies one of the conditions and determining
A, cell quality evaluation device.
(2) substrate,
Arranged on the base plate, to perform a stable sphygmoscope cardiomyocyte evaluation of a plurality of cells holding part for holding the holding region cardiomyocyte group including,
The surface of the substrate, and, said cardiomyocyte group formed around the holding region and is defined by a wall, the space region for satisfying the cell culture solution,
The cell culture solution is supplied into the space region and/or for discharging the supplying/discharging channels,
Provided on the transparent substrate, and the holding portion of the holding region cardiomyocyte population of one part of the cell or cells in a holding portion for placing cardiomyocyte micro electrodes,
The cell culture solution for satisfying the above comparison electrode that is provided within the space,
The micro electrodes is connected to each of the reference electrode and a lead wire connected to the lead wire and the electrode using the microclearance placed biopotential measuring means for measuring cell potential, and
By controlling the microclearance electrostimulation sent to the electrodes, the potential is measured by the measuring means for recording data, and a control/recording means
This device is provided with a cell,
The Electrostimulator sent to the minute interval of the electrode is changed stepwise,
1) above the cell group cardiomyocyte or the forced stimulation sphygmoscope electrostimulation interval in response to the same interval and the forced stimulation sphygmoscope confirms whether the response, and, its response in the frequency range of any forced sphygmoscope irrilation corresponding by measuring whether or not, it can follow sphygmoscope sound cardiomyocyte by confirming that sufficiently satisfies one of the conditions and determine, and
2) due to the forced stimulation sphygmoscope interval (RR) electrostimulator beating of the cells to follow a frequency carpentering is confirmed, the change of the forced stimulation sphygmoscope FPD, FPD / (RR) 1/3 from FPD / (RR) 1/2 between sound by confirming that a sufficient cardiomyocyte satisfies one of the conditions and determining
A, myocardal quality evaluation device.
(3) substrate,
Disposed on the substrate, a plurality of stable sphygmoscope cardiomyocyte evaluated for infusing myoelectric cells or cell population,
On the substrate so as to surround the periphery of the population of cells formed in the wall of the cell culture solution to satisfy,
The cell culture liquid is supplied into the area surrounded by the wall and/or for discharging the supply/discharge channel,
One of the cells of the cell or cells in a localized portion of the electrode is placed,
The engine is enclosed by a wall in the region of the cell culture solution for satisfying a reference electrode,
The micro electrodes is connected to each of the reference electrode and a lead wire connected to the lead wire and the electrode using the microclearance placed biopotential measuring means for measuring cell potential, and
By controlling the microclearance electrostimulation sent to the electrodes, the potential is measured by the measuring means for recording data, and a control/recording means
The device is provided with a cell,
The Electrostimulator sent to the minute interval of the electrode is changed stepwise,
1) above the cell group cardiomyocyte or the forced stimulation sphygmoscope electrostimulation interval in response to the same interval and the forced stimulation sphygmoscope confirms whether the response, and, its response in the frequency range of any forced sphygmoscope irrilation corresponding by measuring whether or not, it can follow sphygmoscope sound cardiomyocyte by confirming that sufficiently satisfies one of the conditions and determine, and
2) due to the forced stimulation sphygmoscope electrostimulator to follow the cells (RR) interval of beat frequency carpentering is confirmed, the forced stimulation sphygmoscope · potential field · duration (FPD) change, FPD / (RR) 1/3 from FPD / (RR) 1/2 between sound by confirming that a sufficient cardiomyocyte satisfies one of the conditions and determining
Contg., cell quality evaluation method.
(4) substrate,
Arranged on the base plate, to perform a stable sphygmoscope cardiomyocyte evaluation of a plurality of

cells holding part for holding the holding region cardiomyocyte group including,
The surface of the substrate, and, said cardiomyocyte group formed around the holding region and is defined by a wall, the space region for satisfying the cell culture solution,
The cell culture solution is supplied into the space region and/or for discharging the supplying/discharging channels,
Provided on the transparent substrate, and the holding portion of the holding region cardiomyocyte population of one part of the cell or cells in a holding portion for placing cardiomyocyte micro electrodes,
The cell culture solution for satisfying the above comparison electrode that is provided within the space,
The micro electrodes is connected to each of the reference electrode and a lead wire connected to the lead wire and the electrode using the microclearance placed biopotential measuring means for measuring cell potential, and
By controlling the microclearance electrostimulation sent to the electrodes, the potential is measured by the measuring means for recording data, and a control/recording means
The device is provided with a cell,
The Electrostimulator sent to the minute interval of the electrode is changed stepwise,
1) above the cell group cardiomyocyte or the forced stimulation sphygmoscope electrostimulation interval in response to the same interval and the forced stimulation sphygmoscope confirms whether the response, and, its response in the frequency range of any forced sphygmoscope irrilation corresponding by measuring whether or not, it can follow sphygmoscope sound cardiomyocyte by confirming that sufficiently satisfies one of the conditions and determine, and
2) due to the forced stimulation sphygmoscope interval (RR) electrostimulator beating of the cells to follow a frequency carpentering is confirmed, the change of the forced stimulation sphygmoscope FPD, FPD / (RR) 1/3 from FPD / (RR) 1/2 between sound by confirming that a sufficient cardiomyocyte satisfies one of the conditions and determining
Contg., myocardal quality evaluation method.

The present invention, an inspection device less toxicity myocarditis, myocardal toxicity testing chip, and myocardal toxicity.
1:a transparent substrate,
The transparent substrate is disposed on a plurality of stable sphygmoscope contg. cardiomyocyte cell population,
Disposed on the transparent substrate, and the cell group, the cell group sphygmoscope cooperate to transmit a plurality of series and fibrocyte cardiomyocyte contg. cell network,
The transparent substrate and the cell group on the cell network which is formed so as to surround a periphery of the cell culture solution for satisfying the wall,
The cell culture liquid is supplied into the area surrounded by the wall and/or for discharging the supply/discharge channel,
The cells of the cell culture liquid chemical action applied to the chemical feed channel,
One of the cells of the cell or cells in a localized portion of the electrode is placed,
Each of the cells of the cell network of micro electrodes are placed,
The engine is enclosed by a wall in the region of the cell culture solution for satisfying a reference electrode,
The micro electrodes is connected to each of the reference electrode and a lead wire connected to the lead wire and the electrode using the microclearance placed biopotential measuring means for measuring cell potential, and
By controlling the microclearance electrostimulation sent to the electrodes, the potential is measured by the measuring means for recording data, and a control/recording means
A, myocardal toxicity.
2: transparent substrate,
Disposed on the transparent substrate, to perform a stable sphygmoscope cardiomyocyte for holding the plurality of cell-holding part (CH G) cardiomyocyte group including a holding region,
The holding part of the cell and the cell 1 cooperate to transmit sphygmoscope myocarditis cell group for holding cells, arranged in series a plurality of cell-holding part (CH n) network including in the region where the cell, the cell holder (CH n) is held or fibrocyte cardiomyocyte, cell network region,
And the surface of the transparent substrate, and the holding region of the cell network cardiomyocyte group formed around the region and is defined by a wall, the space region for satisfying the cell culture solution,
The cell culture solution is supplied into the space region and/or for discharging the supplying/discharging

channels,
The cells of the cell culture liquid chemical action applied to the chemical feed channel,
Provided on the transparent substrate, the cell holder (CH G) cardiomyocyte group holding area or a part of one of the cells in a holding portion for placing cardiomyocyte micro electrodes,
Provided on the transparent substrate, a plurality of the cells of the cell holding portion (CH n) network area of each cell is placed in a plurality of micro-electrode,
The cell culture solution for satisfying the above comparison electrode that is provided within the space,
The micro electrodes is connected to each of the reference electrode and a lead wire connected to the lead wire and the electrode using the microclearance placed biopotential measuring means for measuring cell potential, and
By controlling the microclearance electrostimulation sent to the electrodes, the potential is measured by the measuring means for recording data, and a control/recording means
A, myocardal toxicity.
3: the cells of the cell network for placing the at least one micro-electrode 1 is placed on the micro-electrode from a constant interval by forced sphygmoscope cell that is configured, or 2: the [1] of the inspection device described myocarditis toxicity.
4: the cell is placed on a respective electrode is obtained from myocardal sphygmoscope field · potential waveform fluctuationally size, adjacent sphygmoscope signal by comparing the measured by, the [1]-3: one of the inspection device described myocardal toxicity.
5: field · potential waveform for potassium ions from a cell in the outflow of the peak position of the sodium ion outflow from the peak of the elapsed time ([...]) of fluctuations which, adjacent sphygmoscope signal by comparing the measured by, the [1]-3: one of the inspection device described myocardal toxicity.
6: the cell is placed on a respective electrode is obtained from myocardal sphygmoscope field · potential waveform fluctuationally size, the cells of the cell sphygmoscope is generated from the electrode to the transmission time or the transmission speed fluctuationally adjacent sphygmoscope by comparing the measured by, the [1]-3: one of the inspection device described myocardal toxicity.
7: the cell strading each micro electrodes is obtained from myocardal sphygmoscope field · potential waveform for each of the differentiated cell potential so constituted, the [1]-3: one of the inspection device described myocardal toxicity.
8: the cell is placed on each electrode is obtained from myocardal sphygmoscope field · potential waveform superposed synthetic field · potential waveforms are combined, electrocardial data and comparing a cell group as a whole sphygmoscope transmission turbulence degree so constituted, the [1]-3: one of the inspection device described myocardal toxicity.
9: the cells of a small electrode potential is kept constant feedback potential control mechanism, the power level control mechanism is supplied with a current amount is analyzed so, the [1]-3: one of the inspection device described myocardal toxicity.
10: the cell to be periodically forced sphygmoscope stimulation after starting, 30 seconds or more has elapsed after the measuring is started is, the [1]-3: one of the inspection device described myocardal toxicity.
11: arranged on the transparent substrate to the cell, and an optical system for optically measuring optical camera is further provided,
The optical image data acquired by the camera, the control/are recorded in the recording means, the [1]-[10] one of the inspection device described myocardal toxicity.
[12] the cell holding portion (CH G, CH n) each of, the provided on a transparent substrate and non-adhesiveness to a cell wall surrounded by a space which is defined, in the wall of the cells through which cannot be 1 or more of the gap, the [2] 11 :-one of the inspection device described myocardal toxicity.
13: the cardiomyocyte group holding area and the cell network between the regions, the cell culture which obstructs the flow of the cardiomyocyte group holding area of the plurality of cells holding part (CH G) one contained in the cell and the cell network at the edges of the cells of the holding part (CH n) cells and cooperating with an opening in which the barrier is provided, the [2]-12: one of the inspection device described myocardal toxicity.
14: transparent substrate,
Disposed on the transparent substrate, a plurality of cell-holding part (CH G) cardiomyocyte group including a holding region,
Coolable myoelectric population of cells, the cell holder (CH G) holding area of the cell 1 and cooperating with a plurality of transmitting sphygmoscope cardiomyocyte group of cells holding part (CH n) arranged in series in the region where the network including a cell, the cell holder (CH n) is held or fibrocyte cardiomyocyte, cell network region,

And the surface of the transparent substrate, and the holding region of the cell network cardiomyocyte group formed around the region and is defined by a wall, the space region for satisfying the cell culture solution,
The wall of the cell culture solution is supplied into the space region that is surrounded by and/or discharge for the culture of liquid supply/discharge means,
The cell in the cell culture for adding a chemical agent supplying means,
Provided on the transparent substrate, the holding region of the cell holding portion (CH G) cardiomyocyte group myocarditis is placed in one of the micro electrodes,
Provided on the transparent substrate, a plurality of the cells of the cell holding portion (CH n) network region in the placing or fibrocyte cardiomyocyte plurality of micro electrodes,
The wall is disposed in a region surrounded by a reference electrode,
The micro electrodes is connected to each of the reference electrode and a lead wire connected to the lead wire and the electrode using the microclearance placed on the cell potential for measuring and recording means,
X-Y and driven in the direction of the transparent substrate is mounted on a stage, and
Mounted on the stage on a transparent substrate, an optical means for measuring a state of the cells,
A, myocardal toxicity.
15: transparent substrate,
Disposed on the transparent substrate, for holding a plurality of cardiomyocyte cell holding part (CH G) cardiomyocyte group including a holding region,
The cell group of the cells 1 and cooperate to transmit sphygmoscope myocarditis cell group, a series of cell-holding part (CH n) network including in the region where the cell, the cell holder (CH n) is held or fibrocyte cardiomyocyte, cell network region,
And the surface of the transparent substrate, and the holding region of the cell network cardiomyocyte group formed around the region and is defined by a wall, cell culture solution for satisfying the region,
Provided on the transparent substrate, the holding region of the cell holding portion (CH G) cardiomyocyte group cardiomyocyte is placed in one of the micro electrodes,
Provided on the transparent substrate, a plurality of the cells of the cell holding portion (CH n) network region in the placing or fibrocyte cardiomyocyte plurality of micro electrodes,
The wall is disposed in a region surrounded by a reference electrode, and
The micro electrodes is connected to each of the reference electrode and a lead wire connected to the lead wires,
A, myocardal toxicity testing chip.
[16] the [1]-[14] described in any one of the above [15] myocarditis toxicity or inspection device described by using a testing chip myocarditis toxicity, the cells in the cell culture liquid chemical is acting upon, the occurrence of the sphygmoscope cardiomyocyte population of cell network to delay the propagation speed of the cells to evaluate whether the chemical agent applied to the cell toxicity to cardiomyocyte myocarditis toxicity is inspected.
[17] above [1] or 2:by using the device described in the cell toxicity test myocarditis acting on the chemical agent when a cell culture solution, the occurrence of the sphygmoscope cardiomyocyte population of cell network to delay the propagation speed of the cells to evaluate whether acting on the cell toxicity to cardiomyocyte of chemicals in the method for inspecting, at least one of the following 1 comprising the step of:
(I) of the cell, the cell network for placing the at least one micro-electrode 1 minute from the cells placed on an electrode in a constant interval forced sphygmoscope stimulation process;
(Ii) placing the cells each micro-electrode of the field · myocarditis sphygmoscope acquired from the magnitude of the potential waveform fluctuationally, comparison of the signals measured by the adjacent sphygmoscope process;
Among the potential waveform (iii) · field of potassium sodium ions flowing out from the outflow of the peak position of the elapsed time from the peak magnitude of the fluctuation of ([...]), comparison of the signals measured by the adjacent sphygmoscope process;
(Iv) the cell is placed on each of the micro electrodes myocarditis sphygmoscope acquired from the magnitude of the potential waveform fluctuationally · field, the cells of the cellular network of sphygmoscope is generated from the electrode or the transmission speed until the transmission time of the adjacent sphygmoscope fluctuationally by comparing the measured process;
(V) each of the cells is acquired from a small electrode strading myocarditis sphygmoscope · field for each of the potential waveform is differentiated cell potential process;
(Vi) each electrode of the cell is placed on the field is obtained from the potential waveform myocarditis

sphygmoscope · · synthetic field is overlapped by the potential waveform, and comparing the data as a whole electrocardigram a population of cells for measuring the degree of turbulence of the transmitting sphygmoscope process;
The arrangement of the cell, (vii) a small electrode potential is kept constant by using a feedback control mechanism, the power level control mechanism is a process for analyzing a current amount to be supplied; and
The cells (viii) after the start of the forced stimulation sphygmoscope periodically, from 30 seconds or more to start measuring process.
18: the microclearance electrode disposed in the vicinity of the, cell potential measurement for noise elimination in difference reference electrode is further provided, the [1]-14: one of the inspection device described myocardal toxicity.
19: the micro electrodes, comprising a measuring electrode and the electrode, as described in the above [18] myocarditis toxicity.
20: myocarditis culture cardiomyocyte measuring area in the cell culture, chemical is introduced continuously from the bottom upwardly and discharged from the liquid surface, by monitoring the temperature of the introduced chemicals to maintain the proper temperature and mechanism, for measuring the concentration of the chemical is introduced into the pipe and has an optically transparent area, 800 nanometers wavelength 280 nanometer spectral range of from absorptiometry can be measured quantitatively described above [19] cardiotoxicity inspection device.
21: the microclearance electrode is transparent, the [1]-14:, 18 :-[20] described in any one of an item of inspection device myocarditis toxicity.
22: the microclearance in order to decrease the value of resistance of the electrode, is observed by the optical system which is arranged in a region other than the metal layer on the transparent electrode wiring is added, 21: above described cardiotoxicity inspection device [23] cardiomyocyte culture of the plurality of the substrates are arranged linearly on cardiomyocyte forms a network,
The end point of the linear cardiomyocyte for stimulating the local network of electrodes are disposed, the [1]-[14], [18]-[22] one of the inspection device cardiotoxicity described.
24: further, the linear cardiomyocyte cardiomyocyte is placed a majority of the network can be provided with two linear measuring electrode 1, described in the above [23] cardiotoxicity inspection device.
25: cardiomyocyte culture of the plurality of the substrates are arranged in a ring shape on a cardiomyocyte forms a network,
The ring of the ring-shaped part cardiomyocyte by network, a local point to stimulate the electrodes are disposed, the [1]-[14], [18]-[22] one of the inspection device cardiotoxicity described.
26: further, the ring-shaped cardiomyocyte cardiomyocyte is placed a majority of the network can be provided with two ring-shaped electrode 1, described in the above [25] cardiotoxicity inspection device.
[27] the [1]-26: described in any one of the inspection device myocarditis toxicity, the cells in the cell culture liquid chemical is acting upon, the occurrence of the sphygmoscope cardiomyocyte population of cell network to delay the propagation speed of the cells to evaluate whether the chemical agent applied to the cell toxicity to cardiomyocyte myocarditis toxicity is inspected.
[28] the [1]-14:, 18 :-[26] described in any one of the cell toxicity myocarditis using an inspection device acting on the chemical agent when a cell culture solution, the occurrence of the sphygmoscope cardiomyocyte population of cell network to delay the propagation speed of the cells to evaluate whether acting on the cell toxicity to cardiomyocyte of chemicals in the method for inspecting, at least one of the following 1 comprising the step of:
(I) of the cell, the cell network for placing the at least one micro-electrode 1 minute from the cells placed on an electrode in a constant interval forced sphygmoscope stimulation process;
(Ii) placing the cells each micro-electrode of the field · myocarditis sphygmoscope acquired from the magnitude of the potential waveform fluctuationally, comparison of the signals measured by the adjacent sphygmoscope process;
Among the potential waveform (iii) · field of potassium sodium ions flowing out from the outflow of the peak position of the elapsed time from the peak magnitude of the fluctuation of ([...]), comparison of the signals measured by the adjacent sphygmoscope process;
(Iv) the cell is placed on each of the micro electrodes myocarditis sphygmoscope acquired from the magnitude of the potential waveform fluctuationally · field, the cells of the cellular network of sphygmoscope is generated from the electrode or the transmission speed until the transmission time of the adjacent sphygmoscope fluctuationally by comparing the measured process;
(V) each of the cells is acquired from a small electrode strading myocarditis sphygmoscope · field for each of the potential waveform is differentiated cell potential process;

(Vi) each electrode of the cell is placed on the field is obtained from the potential waveform myocarditis sphygmoscope · · synthetic field is overlapped by the potential waveform, and comparing the data as a whole electrocardigram a population of cells for measuring the degree of turbulence of the transmitting sphygmoscope process;
The arrangement of the cell, (vii) a small electrode potential is kept constant by using a feedback control mechanism, the power level control mechanism is a process for analyzing a current amount to be supplied; and
The cells (viii) after the start of the forced stimulation sphygmoscope periodically, from 30 seconds or more to start measuring process.
29: cardiotoxicity for evaluation, be evaluated by adding a chemical agent, and then, the [1]-14:, 18 :-26: described in any one of the extracellular potential signal obtained by extension of FPD value of the average value of the X axis, Y being of a value fluctuationally FPD STV values plotted on the axis, to evaluate the relative position, or 28: the [27] myocarditis toxicity test method described.
30:a transparent substrate,
The formed on a transparent substrate in a region surrounded by a wall, the area is filled with a liquid cell culture,
The cell is filled with liquid are arranged in a region to perform a plurality of stable sphygmoscope contg. cardiomyocyte cell population,
The cell is filled with a liquid that is provided within the, or one of the cells of the cell group cardiomyocyte local part of the electrode is placed,
The cell is filled with a liquid that is provided within the reference electrode,
The micro electrodes is connected to each of the reference electrode and a lead wire connected to the lead wire and the electrode using the microclearance cardiomyocyte placed to measure the potential of biopotential measuring means,
The potential test drug measured by the measuring means, the potential of the subscriber cardiomyocyte before a recording means for recording data, and
The potential from the data, one field of sodium ions (FP) · potential waveform peak of inflow to outflow of potassium ions of the elapsed time from the peak to the cell (cell potential duration) of the FPD (FPD) and calculates the magnitude of fluctuations, the calculated by using as an index test drug FPD and STV myocarditis toxicity of the analyzing means
A, myocardal toxicity.
31: transparent substrate,
The transparent substrate is formed on the surface in a region surrounded by a wall, cell culture solution for satisfying the region,
On the substrate in the region surrounded by the wall, a plurality of stable sphygmoscope cardiomyocyte myocarditis cell group including a holding region for holding cell population,
The transparent substrate of the group provided in the holding region cardiomyocyte cardiomyocyte micro electrode is placed,
The transparent substrate of the liquid cell culture for satisfying the comparison electrode provided within the region,
The micro electrodes is connected to each of the reference electrode and a lead wire connected to the lead wire and the electrode using the microclearance cardiomyocyte placed to measure the potential of biopotential measuring means,
The potential applied to the measuring means to the test drug cardiomyocyte before the potential of a recording means for recording data, and
· Potential from the potential waveform data out of the field of sodium ions (FP) flows into the outflow of potassium ions from peak to peak of the elapsed time (cell potential duration) of the FPD (FPD) and calculates the magnitude of fluctuations, the calculated by using as an index test drug FPD and STV myocarditis toxicity of the analyzing means
A, myocardal toxicity.
32: the magnitude of the fluctuation of the FPD FPD by short term fluctuations (STV), or 31: the [30] described myocardal toxicity testing device
33: the cardiomyocyte placed to keep constant the microclearance potential of the electrode potential control mechanism further comprises a feedback, or 31: the [30] myocarditis toxicity inspection device described.
34: the transparent substrate is disposed on the cardiomyocyte optical measurement for an optical system and an optical camera is further provided, the optical camera acquisition of data of the recording means to record, the [30]-33: one of the inspection device described myocardal toxicity.

35: the cardiomyocyte appropriately to maintain a temperature having a temperature control mechanism the [30]-34: either of the described myocardal toxicity testing device
36: the analysis means, FPD of fluctuations which, adjacent field · potential (FP) signal waveform by comparing the measured by, the [30]-33: one of the inspection device described myocardal toxicity.
37: the analyzing means in, the cell is placed on a respective electrode is obtained from myocardal sphygmoscope field · potential waveform fluctuationally size, the cells of the cell sphygmoscope is generated from the electrode to the transmission time or the transmission speed fluctuationally adjacent sphygmoscope by comparing the measured by, the [30]-33: one of the inspection device described myocardal toxicity.
38: the analyzing means in, the cell is strading each micro electrodes is obtained from myocardal sphygmoscope field · potential waveform for each of the differentiated cell potential so constituted, the [30]-33: one of the inspection device described myocardal toxicity.
39: the analyzing means in, the cell is placed on each electrode is obtained from myocardal sphygmoscope field · potential waveform superposed synthetic field · potential waveforms are combined, electrocardial data and comparing a cell group as a whole sphygmoscope transmission turbulence degree so constituted, the [30]-33: one of the inspection device described myocardal toxicity.
40: the micro electrodes, a plurality of minute comprising the multi-electrode array is constituted, the [30]-34: one of the inspection device described myocardal toxicity.
41: the cell to be periodically forced sphygmoscope stimulation after starting, 30 seconds or more has elapsed after the measuring is started is, the [30]-35: one of the inspection device described myocardal toxicity.
42: the microclearance electrode disposed in the vicinity of the, cell potential measurement for noise elimination in difference reference electrode is further provided, the [30]-37: one of the inspection device described myocardal toxicity.
43: the micro electrodes, comprising a measuring electrode and the electrode, as described in the above [42] myocarditis toxicity.
44: the cell culture solution to the wall in the region surrounded by the supply, and/or discharging the supplying/discharging channels are provided, the [30]-39: one of the inspection device described myocardal toxicity.
45: the wall and in which the myocardal cells cultured in an area test drug continuously bottom is introduced from above the liquid discharging from the drug introduction/discharge channel and, the introduction of the medicament monitoring temperature and maintained at appropriate temperature, a temperature control mechanism and is provided, of the channel part, introduced chemotherapeuticaly liq. concentrations so as to be capable of being optically transparent, wavelength of 280 nm from 800 nm range absorptiometry spectral quantitative measurement and a, the [30]-39: either of the described cardiotoxicity inspection device.
46: the microclearance electrode is transparent, the [30] 31 :-described in any one of an item of inspection device myocarditis toxicity.
47: the microclearance in order to decrease the value of resistance of the electrode, is observed by the optical system which is arranged in a region other than the metal layer on the transparent electrode wiring is added, as described in the above [43] cardiotoxicity inspection device.
48: cardiomyocyte culture of the plurality of the substrates are arranged linearly on cardiomyocyte forms a network,
The end point of the linear cardiomyocyte for stimulating the local network of electrodes are disposed, the [30]-[44] one of the inspection device cardiotoxicity described.
49: further, the linear cardiomyocyte cardiomyocyte is placed a majority of the network can be provided with two linear measuring electrode 1, described in the above [45] cardiotoxicity inspection device.
50: cardiomyocyte culture of the plurality of the substrates are arranged in a ring shape on a cardiomyocyte forms a network,
The ring of the ring-shaped part cardiomyocyte by network, a local point to stimulate the electrodes are disposed, the [30]-[44] one of the inspection device cardiotoxicity described.
51: further, the ring-shaped cardiomyocyte cardiomyocyte is placed a majority of the network can be provided with two ring-shaped electrode 1, described in the above [47] cardiotoxicity inspection device.
[52] the [30]-48: described in any one of toxicity myocarditis cardiomyocyte using an inspection device, to inspect the toxicity of the drug acting on this method,
In a cell culture solution is to perform a plurality of stable sphygmoscope cardiomyocyte cardiomyocyte including adding a test drug into groups, and
The medicine in the cell culture of additives to the rear, the generation of populations cardiomyocyte process for evaluating the change of sphygmoscope

A,
In the case of a change of the sphygmoscope, extension of the FPD FPD waveform and the magnitude of the fluctuation of the increase of the index and a and a, method.
53: plurality of stable sphygmoscope cardiomyocyte performs a process for preparing a cell group including, In the multi-electrode array is arranged on a bottom surface of the container in a process of the cell group, The culture test drug is added to the cell group, the multi-electrode array of cells cultured cells using a process in which the potential is measured, in addition of the drug, potential is measured by the population of cells cultured cells, the cell potential of a process for acquiring data, and
From the data acquired by the cell potential, the potential (FP) · field of sodium ion waveform peak of inflow to outflow of potassium ions of the elapsed time from the peak to the cell (cell potential duration) of the FPD (FPD) and calculates the magnitude of fluctuations, the combination of the FPD FPD and the magnitude of the fluctuation of the drug by using as an index for evaluating the toxicity process myocarditis,
Contg., myocarditis toxicity of the drug.
54: the magnitude of the fluctuation of the FPD FPD by short term fluctuations (STV), or 53: the [19].
55:in a process of evaluating the toxicity of the drug myocarditis, after the addition of extension of the drug and STV FPD waveform as an index of chemotherapeuticaly myocarditis increase of evaluating the toxicity, the method described in the above [53].
56: extension of the FPD waveform frequency exceeds a predetermined threshold and STV FPD STV exceeds a predetermined threshold, the increase of the frequency by using a two-dimensional plot is generated and, based on the two-dimensional plot of the drug myocarditis toxicity is evaluated, the method described in the above [55].
57:in the two-dimensional plot, a plot of the relative positions of the plurality of drug from chemotherapeuticaly myocarditis of evaluating the toxicity, described in the above [56].

The present invention, in a more simple, and less of the device.
(1) a substrate,
Disposed on the substrate, a plurality of stable sphygmoscope cardiomyocyte evaluated for infusing myoelectric cells or cell population,
On the substrate so as to surround the periphery of the population of cells formed in the wall of the cell culture solution to satisfy,
The cell culture liquid is supplied into the area surrounded by the wall and/or for discharging the supply/discharge channel,
One of the cells of the cell or cells in a localized portion of the electrode is placed,
The engine is enclosed by a wall in the region of the cell culture solution for satisfying a reference electrode,
The micro electrodes is connected to each of the reference electrode and a lead wire connected to the lead wire and the electrode using the microclearance placed biopotential measuring means for measuring cell potential, and
By controlling the microclearance electrostimulation sent to the electrodes, the potential is measured by the measuring means for recording data, and a control/recording means
This device is provided with a cell,
The Electrostimulator sent to the minute interval of the electrode is changed stepwise,
1) above the cell group cardiomyocyte or the forced stimulation sphygmoscope electrostimulation interval in response to the same interval and the forced stimulation sphygmoscope confirms whether the response, and, its response in the frequency range of any forced sphygmoscope irrilation corresponding by measuring whether or not, it can follow sphygmoscope sound cardiomyocyte by confirming that sufficiently satisfies one of the conditions and determine, and
2) due to the forced stimulation sphygmoscope interval (RR) electrostimulator beating of the cells to follow a frequency carpentering is confirmed, the change of the forced stimulation sphygmoscope FPD, FPD / (RR) 1/3 FPD / (RR) 1/2 from between to check that sufficient sound cardiomyocyte satisfies one of the conditions is determined and
A, cell quality evaluation device.
(2) substrate,
Disposed on the substrate, a plurality of stable sphygmoscope cardiomyocyte evaluated for infusing myoelectric cells or cell population,
On the substrate so as to surround the periphery of the population of cells formed in the wall of the cell culture solution to satisfy,
The cell culture liquid is supplied into the area surrounded by the wall and/or for discharging the

supply/discharge channel,
One of the cells of the cell or cells in a localized portion of the electrode is placed,
The engine is enclosed by a wall in the region of the cell culture solution for satisfying a reference electrode,
The micro electrodes is connected to each of the reference electrode and a lead wire connected to the lead wire and the electrode using the microclearance placed biopotential measuring means for measuring cell potential, and
By controlling the microclearance electrostimulation sent to the electrodes, the potential is measured by the measuring means for recording data, and a control/recording means
The device is provided with a cell,
The Electrostimulator sent to the minute interval of the electrode is changed stepwise,
1) above the cell group cardiomyocyte or the forced stimulation sphygmoscope electrostimulation interval in response to the same interval and the forced stimulation sphygmoscope confirms whether the response, and, its response in the frequency range of any forced sphygmoscope irrilation corresponding by measuring whether or not, it can follow sphygmoscope sound cardiomyocyte by confirming that sufficiently satisfies one of the conditions and determine, and
2) due to the interval (RR) forced sphygmoscope electrostimulator irritating beating of the cells to follow a frequency carpentering is confirmed, the forced stimulation sphygmoscope · potential field · duration (FPD) change, FPD / (RR) 1/3 from FPD / (RR) 1/2 between sound by confirming that a sufficient cardiomyocyte satisfies one of the conditions and determining
Contg., cell quality evaluation method.
(3) · myocarditis sphygmoscope fluctuationally of field of the magnitude of the potential waveform, adjacent sphygmoscope by comparing the signal is measured, the inspection device myocarditis toxicity.
(4) · potassium ions out of the field of the potential waveform peak position of sodium ions from flowing out of the outflow of the elapsed time from the peak magnitude of the fluctuation of ([...] : FPD) which, by comparing the adjacent sphygmoscope signal is measured, mentioned in the above (3) of the inspection device myocarditis toxicity.
The magnitude of the fluctuation of (5) FPD and FPD is calculated, the calculated magnitude of the FPD fluctuationally of FPD and combination of and is constituted so as to index, or (4) described in the above (3) of the inspection device myocarditis toxicity.
(6) the magnitude of the fluctuation of the FPD FPD by short term fluctuations (STV), the (3)-(5) described in any one of the inspection device myocarditis toxicity.
(7) a transparent substrate,
The formed on a transparent substrate in a region surrounded by a wall, the area is filled with a liquid cell culture,
The cell is filled with liquid are arranged in a region to perform a plurality of stable sphygmoscope contg. cardiomyocyte cell population,
The cell is filled with a liquid that is provided within the, or one of the cells of the cell group cardiomyocyte local part of the electrode is placed,
The cell is filled with a liquid that is provided within the reference electrode,
The micro electrodes is connected to each of the reference electrode and a lead wire connected to the lead wire and the electrode using the microclearance cardiomyocyte placed to measure the potential of biopotential measuring means,
The measuring means in a measurement test drug subscriber before the potential of a recording means for recording data cardiomyocyte
Is provided, the (3)-(6) described in any one of the inspection device myocarditis toxicity.
(8) during the stable sphygmoscope a cardiomyocyte contg. cell group is, sphygmoscope which can transmit a plurality of cardiomyocyte and fibrocyte cell network, the (3)-(7) as described in any one of an item myocarditis toxicity.
(9) of the transparent substrate,
The transparent substrate is disposed on a plurality of stable sphygmoscope contg. cardiomyocyte pacemaker region comprising a cell population,
Disposed on the transparent substrate, and the cell group, the cell group sphygmoscope cooperate to transmit a plurality of series and fibrocyte cardiomyocyte contg. cell network,
The transparent substrate and the cell group on the cell network which is formed so as to surround a periphery of the cell culture solution for satisfying the wall,
The cell culture liquid is supplied into the area surrounded by the wall and/or for discharging the supply/discharge channel,
The cells of the cell culture liquid chemical action applied to the chemical feed channel,

One of the cells of the cell or cells in a localized portion of the electrode is placed,
Each of the cells of the cell network of micro electrodes are placed,
The engine is enclosed by a wall in the region of the cell culture solution for satisfying a reference electrode,
The micro electrodes is connected to each of the reference electrode and a lead wire connected to the lead wire and the electrode using the microclearance placed biopotential measuring means for measuring cell potential, and
By controlling the microclearance electrostimulation sent to the electrodes, the potential is measured by the measuring means for recording data, and a control/recording means
Is provided, the (3)-(8) described in any one of an item of inspection device myocarditis toxicity.
(10) the stable sphygmoscope a cardiomyocyte-containing cells placed at least 1 two micro-electrode from the electrode placed on the cell at a constant interval by forced sphygmoscope stimuli can be applied so that, the (3)-(9) as described in any one of an item myocarditis toxicity.
(11) the series arranged cell network at the end points of local stimulates the electrodes are disposed, the (8)-(10) as described in any one of an item cardiotoxicity inspection device.
(12) of the cell network, are arranged in a ring shape is formed,
The part of the ring, the point local stimulates the electrodes are disposed, the (8)-(11) described in any one of the inspection device cardiotoxicity.
(13) said cells are placed on the respective electrode is obtained from myocardal sphygmoscope field · potential waveform fluctuationally size, the cells of the cell sphygmoscope is generated from the electrode to the transmission time or the transmission speed fluctuationally adjacent sphygmoscope by comparing the measured by, the (8)-(12) described in any one of the inspection device myocarditis toxicity.
(14) said cardiomyocyte appropriately to maintain a temperature having a temperature control mechanism the (1)-(13) described in any one of the inspection device myocarditis toxicity.
(15) the micro electrodes, a plurality of minute comprising the multi-electrode array is constituted, the (1)-(14) of the one of the inspection device described myocarditis toxicity.
(16) · myocarditis sphygmoscope fluctuationally of field of the magnitude of the potential waveform, comparison of the signals measured by the adjacent sphygmoscope ~~~ myocarditis toxicity and an inspection method.
(17) to perform a plurality of stable sphygmoscope cardiomyocyte including a step of preparing a population of cells,
In the multi-electrode array is arranged on a bottom surface of the container in a process of the cell group,
The culture test drug is added to the cell group, the multi-electrode array of cells cultured cells using a process in which the potential is measured, in addition of the drug, potential is measured by the population of cells cultured cells, the cell potential of a process for acquiring data, and
From the data acquired by the cell potential, the potential (FP) · field of sodium ion waveform peak of inflow to outflow of potassium ions of the elapsed time from the peak to the cell (cell potential duration) of the FPD (FPD) and calculates the magnitude of fluctuations, the combination of the FPD FPD and the magnitude of the fluctuation of the drug by using as an index for evaluating the toxicity process myocarditis,
Contg., described in the above (16) myocarditis toxicity of the drug.
(18) above (8)-(15) described in any one of the inspection device myocarditis toxicity, the cells in the cell culture liquid chemical is acting upon, the occurrence of the sphygmoscope cardiomyocyte population of cell network to delay the propagation speed of the cells to evaluate whether the chemical action of the cells cardiomyocyte toxicity to the (16) or (17) inspection of the test method described in myocarditis toxicity.
(19) above (8)-(15) of the one described myocardal toxicity using an inspection device into the cell action for the cell culture medium applied thereto, the cardiomyocyte of a sphygmoscope the cell network area of the cell to the propagation speed is delayed to evaluate whether the cell in a medicament cardiomyocyte toxicity to inspection method, the following at least 1 two processes mentioned (16)-(18) the method described in any one of Claim:
(I) of the cell, the cell network for placing the at least one micro-electrode 1 minute from the cells placed on an electrode in a constant interval forced sphygmoscope stimulation process;
(Ii) placing the cells each micro-electrode of the field · myocarditis sphygmoscope acquired from the magnitude of the potential waveform fluctuationally, comparison of the signals measured by the adjacent sphygmoscope process;
Among the potential waveform (iii) · field of potassium sodium ions flowing out from the outflow of the peak position of the elapsed time from the peak magnitude of the fluctuation of ([...]), comparison of the signals measured by the adjacent sphygmoscope process;
(Iv) the cell is placed on each of the micro electrodes myocarditis sphygmoscope acquired from the magnitude of the potential waveform fluctuationally · field, the cells of the cellular network of sphygmoscope

is generated from the electrode or the transmission speed until the transmission time of the adjacent sphygmoscope fluctuationally by comparing the measured process;
(V) each of the cells is acquired from a small electrode strading myocarditis sphygmoscope · field for each of the potential waveform is differentiated cell potential process;
(Vi) each electrode of the cell is placed on the field is obtained from the potential waveform myocarditis sphygmoscope · · synthetic field is overlapped by the potential waveform, and comparing the data as a whole electrocardigram a population of cells for measuring the degree of turbulence of the transmitting sphygmoscope process;
The arrangement of the cell, (vii) a small electrode potential is kept constant by using a feedback control mechanism, the power level control mechanism is a process for analyzing a current amount to be supplied; and
The cells (viii) after the start of the forced stimulation sphygmoscope periodically, from 30 seconds or more to start measuring process.
(20) the magnitude of the fluctuation of the FPD FPD by short term fluctuations (STV), the (16)-(19) the method described in any one of an item.

ADVANTAGEOUS EFFECTS

A chemical osteoblast fibrosing cardiomyocyte and change in response to measured from fluctuationally cells can be evaluated accurately.

Conventionally, a potential field · · duration (FPD) (referred to later explained) and adjacent sphygmoscope adjacent cardiomyocyte magnitude of the fluctuation of (for example, short-term variation: STV: Short-term variability) independently of and even to the index, by combining both myocarditis inspection of toxicity. According to the present invention a method of inspecting myocarditis toxicity, not only by the extension of the FPD waveform, cardiomyocyte adjacent sphygmoscope increase of fluctuation of the size (STV) by evaluating the combination, and more accurate myocardal toxicity can be evaluated.

In this embodiment of the present invention and the structure of the example 1 myocarditis toxicity inspection device shown schematically in a perspective view. The cell toxicity test device shown in fig. 1 myocarditis CH of an example of the configuration of the holding part 1 shows schematically in perspective view. The fig. 3, fig. 1 shows the cell toxicity test device myocarditis CH of the cells are held in the holding part for optically detecting the optical system is explained. (A), (b) and (c) the, drawings showing the signal related to the measurement of the cell potential. Respectively, in the horizontal axis, vertical axis and the reference electrode 2 C micro electrode 2 indicating the potential obtained between the cells. (A), (b) and (c) the, cell sphygmoscope vol. change signal related to the optical system by drawing showing the measured results. (A) in the normal state is not included in the target cell Na + ion, Ca 2+ ion, K + cell accompanying the inflow/outflow of an ion component diagram showing a change in potential, (b) the culture solution of chemicals are contained in a state in which the target cell Na + ion, Ca 2+ ion, K + inflow/outflow of the ion component cell accompanying drawing showing potential change. The cell toxicity myocarditis inspection device for optically detecting the optical system and to explain one example of the arrangement of the movable electrode. A schematic diagram explaining the generation of an electrical signal. (A) due to the addition of the drawing showing one example of a change in potential, (b) the change in potential of cells each sphygmoscope, phase 2 of two adjacent dipterous poincare plotting sphygmoscope drawing showing one example of the evaluation. (A) a cell 1 is arranged at the cell level using a reentrant annular cardiomyocyte network created by the schematic diagram showing one example of a tree and, (b) is actually a very small electrodes arranged for example microphotqgraphic. (A) using a cell population of constant width and an annular reentrant watertree cardiomyocyte by network which represents an example of a circuit schematic view, (b) is actually a very small electrodes arranged in one example is shown microphotqgraphic, (c) the actual micro electrode array on the cell group is arranged for example microphotqgraphic. (A) an annular electrode using a measuring device showing one example of a reentry watertree circuit schematic diagram, (b) is actually measured by the electrode, normal and abnormal sphygmoscope sphygmoscope data graph shows data. (A) the measured potential of the electrode 1 cells and cell schematic diagram showing one example of the arrangement of and, (b) the electrode is actually measured by a photographic and isolated 1 on an electrode of a cell electrical data sphygmoscope thereof,

(c) the measured on the electrodes of the cells in cell group of cells 1 and photograph of a graph showing the electrical data sphygmoscope. The camera of the present invention 1 biopotential measuring cell receiving element used in the schematic diagram to explain an embodiment. In the present invention a plurality of measuring system for measuring the sample schematic diagram explaining one example of the mechanism. The heart of the present invention can be measured by measuring system schematic diagram explaining information. One example of a graph for explaining a change in the potential of the signal waveform of the drug · field can be measured by measuring system of the present invention of the cell. The system of the present invention can be measured by measuring the potential of the signal waveform of ion emission field · potassium sodium ion discharge time of the peak position of the elapsed time from about (FPD: field · potential · duration), addition of kalium ion channel inhibitor E4031 explaining one example of a change to the average value of which is one example of a graph. The system of the present invention can be measured by measuring the potential of the signal waveform of ion emission field · potassium sodium ion discharge time of the peak position of the elapsed time from about (FPD: field · potential · duration), and the magnitude of fluctuation of the short-term variation of an adjacent sphygmoscope [...] quantitatively evaluating method on the basis of a graph for explaining one example of and type. The system of the present invention can be measured by measuring the potential of the signal waveform of ion emission field · potassium sodium ion emission peak position of the elapsed time from the time (FPD: potential field · · duration) for, based on the magnitude of the fluctuation thereof [...] method for quantitatively evaluating and type of graph explaining one example. The system of the present invention can be measured by measuring the potential of the signal waveform field cardiomyocyte · potassium ion emission peak position of the elapsed time from the time of sodium ion emission (FPD: potential field · · duration) about, its E4031 caused by addition of an example of the magnitude of fluctuations in (a) and displayed [...] , STV ~~~ (b) for the same together. When used as a chemical additive of FPD and STV in comparison to known and various chemicals to cardiotoxicity cardiomyocyte measuring cell measuring system of the present invention. The system of the present invention can be measured by measuring the difference in shape of cardiomyocyte network, and due to the difference of the position of the chemical additive to [...] shows one example of FPD. (A) shows one example of the actual cell network a microphotqgraphic, (b) (a) of the A, B, C, D of the graph shows the change is measured. The system of the present invention can be measured by measuring the difference in shape of network cardiomyocyte, due to the positional difference of chemicals to be added to and from a transmission to a local area of the pacemaker [...] shows one example of the time. (A) shows one example of the actual cell network a microphotqgraphic, (b) (a) of the A, B, C, D of the graph shows the change is measured. (C) the, STV calculation formulas. The system of the present invention can be measured by measuring the measuring cell in cardiomyocyte network, in vitro measurement method of the prior art, the relationship in vivo measurement method, FP FP 1 cells and cell network of the waveform of the composite waveform shows schematically the relation of Fig. The system of the present invention can be measured by measuring cell cardiomyocyte network measurement obtained from each electrode of the cell and the cell to cell potential FP from the waveform of the composite waveform estimating function FP electrocardigram comparison network synthesized from a device having the function of a diagram showing schematically a configuration. The system of the present invention can be measured by measuring the measuring cell in cardiomyocyte network, an annularly disposed cardiomyocyte network (A) of each electrode of the cell from the waveform (B) and FP, the FP of synthesized waveform (C) showing one example. In this example, the signal is transmitted normally sphygmoscope PM from a region. The system of the present invention can be measured by measuring the measuring cell in cardiomyocyte network, an annularly disposed cardiomyocyte network (A) of each electrode of the cell from the waveform (B) and FP, the FP of synthesized waveform (C) showing one example. In this example, the abnormal signal is transmitted from sphygmoscope PM embodiment is shown. The system of the present invention can be measured by measuring the measuring cell in cardiomyocyte network, cardiomyocyte sphygmoscope frequency and FPD (Beating Frequency) being a graph showing one example relationship. The system of the present invention can be measured by measuring the measuring cell in cardiomyocyte network, when forcibly sphygmoscope cardiomyocyte FPD of one example of a graph showing a time change. The system of the present invention can be measured by measuring the measuring cell in cardiomyocyte network, forcibly sphygmoscope cardiomyocyte FPD is given of the case of the example is shown microphotqgraphic arranged cell network. The system of the present invention can be measured by measuring the measuring cell in cardiomyocyte network, a trace of the electrode potential FP cardiomyocyte measured using feedback control to maintain a constant potential micro electrode using a schematic diagram showing one example of a mechanism. The system of the present invention can be measured by measuring the measuring cell in cardiomyocyte network, forced sphygmoscope cardiomyocyte group in a partial area of a stimulation is applied, the response period sphygmoscope myocarditis cell group is one example of a graph showing the

relationship b The system of the present invention can be measured by measuring the measuring cell in cardiomyocyte network, forced sphygmoscope myocarditis part area of the cell group at the time of applying the stimulus, FPD which represents an example of change of length of the graph. (A) the, caused by the forced stimulation sphygmoscope FP and FPD change of wave length of one example of a change in the relation, (b) the, forced sphygmoscope stimulus FPD according to the variation in the interval of one example of a graph showing the change of the length. The system of the present invention can be measured by measuring the measuring cell in cardiomyocyte network, forced sphygmoscope myocarditis part area of the cell group at the time of applying the stimulus, for example of the response of the cell group shown in figs. 33 and 34 as a result. The fig. 36, is a block diagram showing the whole constitution of one example of the present invention to myocardal toxicity of the inspection device. The fig. 37, according to an embodiment of the present invention 1 of one part of the inspection device myocarditis toxicity. The fig. 38, multi-electrode array chip (a, b, d) cardiomyocyte (b) using the evaluation of toxicity of medicine, and electrodes used in the analysis of the measuring window and corresponding relationship (a-c) drawing showing an example of data (e). The fig. 39, Diltiazem, sotalol DL-, of one embodiment of the present invention for E-4031 DDW and related method for inspecting cardiotoxicity 2 generated by dimensional plot is shown. The present invention for measuring the electrode potential for eliminating the noise comparison between an electrode and a minute difference circuit is shown schematically. (A) shows a principle of one example of a schematic diagram. The present invention for measuring the electrode potential for eliminating the noise comparison between an electrode and a minute difference circuit is shown schematically. (B) the differential circuit is incorporated in the circuit diagram of one example of an amplifier circuit. The present invention for measuring the electrode potential for eliminating the noise comparison between an electrode and a minute difference circuit is shown schematically. (C) circuit with reduced noise are drawings showing an example. The method of the present invention for overall evaluation method evaluating cardiotoxicity schematically shown in the example of Fig. (A) a cell of FPD FPD from data of the degree of extension X axis, Y axis is plotted the size of FPD fluctuationally in terms of time. (B) is, on the average of the results although X-Y diagram showing one example gestational. The constitution of the present invention to provide a system for measuring the cardiotoxicity showing one example of a schematic diagram. In the system of the present invention to measure cardiotoxicity cell culture measuring chamber and schematic diagram showing one example of a constitution. The cross section of the cell culture measuring plate shown schematically in the figure. The multi-electrode substrate electrode arrangement of electrodes are arranged on the wiring diagram explaining the configuration of a model. The arrangement of the multi-electrode of an electrode on a substrate, an example illustrated in the schematic diagram.

DESCRIPTION OF EMBODIMENTS

1 fig. 1 is a perspective view showing an example of the structure of the present invention in this embodiment myocarditis toxicity of the inspection device schematically. The fig. 2, fig. 1 shows the cell toxicity test device myocarditis CH holding part 1 of an example configuration of a perspective diagram showing schematically. The fig. 3, fig. 1 shows the cell toxicity test device myocarditis CH of the cells are held in the holding part for optically detecting the optical system is explained.

With reference to figs. 1 and 2, the toxicity myocarditis inspection device 100, constructed on the transparent substrate 1 is mainly composed of a component. A transparent substrate 1 of an optically transparent material, for example, a glass substrate or a silicon substrate. The micro electrodes 2, for example, ITO is transparent and, disposed on the transparent substrate 1. 2' of the micro electrodes 2. 3 1, 3 2, 3 3 and 3 4 due to the wall and agalose gel, via the gap 4 1, 4 2, 4 3 and 4 4 micro electrode 2 of the surroundings. Wall 3 1, 3 2, 3 3 and 3 4 agarose gel is notched at the central part and the housing part forms a space. Wall 3 1, 3 2, 3 3 and 3 4 agarose gel cell space formed by the housing part on the transparent substrate 1 and, if necessary, micro-electrode 2 is disposed. Regardless of the presence or absence of the micro electrodes 2, cells of one cell 10 is housed in a housing part. In fig. 2, a space is formed on the transparent substrate 1 and a housing part of the cell wall 3 1, 3 2, 3 3 and 3 4 caused by agalose gel, micro-electrode 2 is arranged, cardiomyocyte 10 on the housing. Micro-electrode 2 out wire 2' is connected is shown drawn out. Micro-electrode 2 and the mounting surface of the transparent substrate 1 minute electrode 2 is not provided with the, directly, the cell when the cell is placed on the mounting surface and the surface of a transparent substrate such as collagenous adhesive helps it is better to the material. Wall 3 1, 3 2, 3 3 and 3 4 agarose gel in the cell formed by the cell, the cell for the gel agalose because of non-adhesive, this wall 3 1, 3 2, 3 3 and 3 4 even and equal to the height of the cell, the cell 10 is moved

over the wall. Furthermore, the gel agalose wall 3 1, 3 2, 3 3 and 3 4 center cut in the periphery of the cell housing part forming a gap 4 1, 4 2, 4 3 and 4 4 and smaller than the size of the cells is used, this clearance 4 1, 4 2, 4 3 and 4 4 slipping movement of the cell 10.

In fig. 1, holding cell part CH 1, CH 2, CH 3 and CH n cardiomyocyte one housing part of each cell or fibrocyte 10 1, 10 2, 10 3 and 10 n is held, in the figure is not clear, respectively, provided with a small electrode 2, lead wire 2 '1, 2' 2, 2 '3 and 2' n are led out. These osteoblast fibrosing cardiomyocyte or cells constituting the CCC communicating channels which are arranged in series. In this case, for example, n 20. Furthermore, these 20 which are arranged in series and the distribution of osteoblast fibrosing cardiomyocyte, can be random, cell holding part CH 1 cell and CH 20 cardiomyocyte boron in the cell. The cells at the left edge of the communicating channel CCC 3×3 cell holding part CH G are formed, respectively, on the cell holder CH cardiomyocyte 10 is held by a cell group 10 G cardiomyocyte group including a holding region is present. The group of the cells 10 G to perform stable sphygmoscope functions as a pace maker. The cell group 10 G the, cell group 10 G CH holding part of one of the cells is provided only in a very small electrodes 2, lead wire 2' G are led out. Furthermore, the cell group 10 G CH is to the right of the center of the cell holding portion of the holding part CH 1 CCC cell communicating channel is constituted so as to face. The cell group 10 G of right and left end part of the connecting channel between the cell barrier 11 a CCC is provided. This barrier 11 a small at the lower part of the central part of the opening 11 b is formed. This opening 11 b cell population 10 G at the opposite sides of the center of the holding portion of the right side of the cells of the cell holding portion CH 1 CCC CH and cell communicating channel has, on the periphery of each cell housing part via a gap 4 is held in the respective cell · of physical contact to allow interaction between cells. The cell group 10 G comparison electrode 2 C lower provided, lead wire 2' C are led out.

7 in which the peripheral surrounding wall, cell group 10 G, cell communicating channel CCC and reference electrode 2 C a 8 1 and 8 2 inside the wall 7, the liquid is supplied to the cell culture, and, from the region of the inside of the wall 7, cell culture for discharging pipe and, in the illustrated embodiment, substrate 1 extending to the vicinity of the bottom of the pipe 8 1 culture solution is supplied from, the substrate 1 extending to the vicinity of the bottom of the pipe 8 2 culture fluid is discharged. A supply pipe 8 1 ingredient in the vicinity of the outlet pipe 8 3 is connected, this pipe 8 3 acting on the cell through the desired chemical is supplied. Therefore, the pipe 8 1 cell 10 in the region of the inside of the wall 7 is supplied while being exposed to a liquid cell culture, micro-electrode 2 is stably held o Cell culture is exposed to liquid when there is no need, pipe 8 2 from wall 7 inside the liquid to be discharged. Furthermore, when a new one and the replacement, after a discharge, or, while discharging, liquid to be fed. On the other hand, when it is desired to effect a chemical cells, pipe 8 2 culture solution by discharge, pipe 8 3 acting through the cell is desired in addition to the liquid medicine, pipe 8 1 culture solution can be supplied. In this case, the cell group 10 G CCC and cell communicating channel between the barrier 11 a is provided, including a pipe 8 1 chemical solution is supplied to the inside of the wall 7 when the area of the, cell communicating channel CCC of cell is compared to a degree affected by chemicals, cell group 10 G degree affected by the chemical cells and is low. In other words, pipe 8 1 culture medium containing a drug by supplying liquid, this liquid barrier 11 a clearance on both sides of the wall 7 and barrier 11 a over the upper surface of the cell group 10 G from cell group 10 G are also supplied to the cells of the influence of chemicals. However, the effect of the cell and the cell as compared to the communication channel CCC since if indirectly, function as a pacemaker hardly affect. Furthermore, pipe 8 1, pipe 8 2 and pipe 8 3 composed of, by way of the measuring arrangement can be optionally changed. For example, pipe 8 1 and pipe 8 3 may also be separated by the, pipe 8 2 eliminated, pipe 8 1 is supplied, may also be used for both discharge.

The personal computer PC (potential measuring means, and a control/recording means) and, holding cell part of lead wires 2 CH of micro electrodes 2 'and reference electrode 2 C of lead wires 2' of the cell between the potential is measured and recorded. Furthermore, the personal computer 9 Ms signal is added to the operation of the operator.

The optical observation device 200 myocarditis toxicity testing device 100 mounted on the XY stage 15 of the cells of the cell 10 of the optional communication channel to sphygmoscope CCC, can be observed by

the optical system. The XY stage 15, and optically transparent, in accordance with an operation of an operator of a personal computer PC Ms signal in response to a signal given by X-Y driving device 16, is moved in an arbitrary position. In fig. 3, the cells of the cell 10 n CCC communication channel of the observation of the state sphygmoscope. 12 showing the culture solution.

22 of the phase difference between the source and the sample microscopic or, in general, a halogen lamp is used. 23 by the band pass filter transmitting light of a wavelength of the light source from only a specific phase difference carpophore microscopic observation. For example, cell 10 n observed in the case of, in the vicinity of the light of a wavelength of 700 nm narrow band by using cell 10 n damage can be prevented. 24 with the shutter, such movement of the XY stage 15, image is measured between the illumination of the light not having a function of shielding. 25, and a condenser lens, the phase difference retardation when observed by introducing a ring, when the differential interference observation, a polarizer is introduced. On the XY stage 15 is formed on the substrate 1 myocarditis toxicity testing device 100 is placed on the XY stage 15 X-Y driving device 16 by moving the arbitrary position of the myocardal toxicity testing device 100 is observed, can be measured. The state of the cell 10 n sphygmoscope in the toxicity of the inspection device myocarditis 100, which is observed by an objective lens 17. The personal computer PC by focusing position of the objective lens 17 in response to the signal Z by driving device 18 can be moved in the axial direction. The magnification of the objective lens 17 40 times or more can be used. By observing the objective lens 17, a light transmitted from the light source 22 of cell 10 n differential interference image or image of the phase difference. The band pass filter 23 and of the same wavelength of the transmitted light is reflected by the dichroic mirror 19 and a band-pass filter 20, a phase difference microscope image or Differantial interference microscope image observed by the camera 21 only. The image signal is observed by the camera 21 is introduced into the personal computer PC. Furthermore, although not shown, or a personal computer and a monitor connected to the image displayed by the display.

The structure of fig. 1 showing a main myocarditis toxicity testing device 100 and the size of the example is shown as follows. Therefore, the size of the cells 10 μm φ and in this embodiment. The size of the transparent substrate 1 100 mm × 150 mm, the size of the micro electrodes 2 8 μm × 8 μm, agarose gel wall 3 1, 3 2, 3 3 and 3 4 20 μm × 20 μm × 10 μm individual size (height), gap 4 1, 4 2, 4 3 and 4 4 width 2 μm, wall 3 1, 3 2, 3 3 and 3 4 agarose gel cell housing part and is formed by the cylindrical space 12 μm φ, the external shape of the wall 7 is 5 mm × 5 mm and 5 mm in height. 1 mm, and the height of the barrier 11a. Furthermore, in this case, the micro electrodes 2 and 8 μm × 8 μm is square, wall 3 1, 3 2, 3 3 and 3 4 agarose gel and the whole of the gap 4 1, 4 2, 4 3 and 4 4 and a cell width of 10 μm φ and the housing part can be made as circular electrode.

Below, an example of the constitution of the present invention and toxicity myocarditis inspection device 100 using the measured specific examples are explained.

Figs. 4 (a), 4 (b) and 4 (c) is, cell potential of the drawing showing the signal related to the measurement. Respectively, in the horizontal axis, vertical axis and the reference electrode 2 C micro electrode 2 indicating the potential obtained between the cells. The cell group 10 G fig. 4 (a) due to pulsation of the cell potential. In this case, as shown in fig. 1 cell population 10 G from one of the lead-out wire 2 'G comparison electrode 2 C and lead-out wire 2' C from between the potential. As shown in the drawing, which shows a stable sphygmoscope, pacemaker can function as is known. In fig. 4 (b) is a normal state is not included in the target cell due to pulsation of the cell potential. In this case, the communication channel of the measuring cell of the cell is a cell 10 n CCC and the, cell 10 n pulled out from line 2 'n and reference electrode 2 C pulled out from line 2' C potential between the measurement. As evidenced by comparing a waveform and fig. 4 (a), the delay time Δ t is observed only in the cell 10 is transmitted by connecting channel sphygmoscope CCC of cells. On the contrary, the chemicals are contained in fig. 4 (c) in a state in which the target cell due to pulsation of the cell potential. Here, a communication channel for measuring target cell-cell 10 n CCC and it, is clearly compare fig. 4 (b). Figs. 4 (a), 4 (b), and as evidenced by comparing the waveform, cell by cell 10 of the communication channels transmitting sphygmoscope CCC time required not only with a delay of Δ t, time delay and Δt+α is observed. In this case, the connecting channel to the cells of the cell by the action of the drug CCC magnitude of Na ions inhibit the delay of the increase of + α as

meaning that appear. In other words, for inhibiting cardiomyocyte toxicity of the drug can be evaluated as Na ions.
Furthermore, the electrodes used for observation and called micro electrode is observed.

Figs. 5 (a), 5 (b) and 5 (c) is, cell sphygmoscope vol. change signal related to the optical system by drawing showing a measurement result. The cell group 10 G fig. 5 (a) of change in volume caused by the pulsation of the cell. The cell group 10 G of one of the cells shown in fig. 3 a sphygmoscope optically detected in shape. The expansion and contraction of the cell sphygmoscope appears as the change in pulse form. The period of the waveform, the pulsation shown in fig. 4 (a) due to the changing period of cell potential and is the same. In fig. 5 (b) is a normal state is not included in the target cell sphygmoscope vol. change indicates to an upper stage, lower stage as an electric signal to the time-differentiated value in order to evaluate the time of waveform as shown. Here, a communication channel for measuring target cell-cell 10 n CCC and the, cell 10 n form shown in fig. 3 a sphygmoscope of optically detected. As evidenced by comparing a waveform and fig. 5 (a), the delay time Δ t is observed only in the cell 10 is transmitted by connecting channel sphygmoscope CCC of cells. On the contrary, the chemicals are contained in fig. 5 (c) in a state of change in the target cell vol. sphygmoscope explanatory diagram for evaluating, fig. 5 (a), an enlarged compared to fig. 5 (b) shows in the form of a time axis. The upper stage corresponding to fig. 5 (b) is a waveform of the waveform, the waveform of fig. 5 (a) and as evidenced by comparing, cell by cell 10 of the communication channels transmitting sphygmoscope CCC time required by β Δ t is increased more than it is observed. A target cell sphygmoscope affected by the drug vol. change, this delay is reduced more than the inclination of the change of volume. Referring to fig. 5 (c) shows the waveform of a lower stage as the volume change of the liquid drug and containing no and this can be seen by comparing. In the middle of fig. 5 (c), the waveform of the upper time differential value in order to evaluate the waveform when treated as shown. This time differential value and comparing it to a lower stage of fig. 5 (b) and so on, and the peak value becomes smaller, and the inclination is moderate. Therefore, the speed is reduced by the contraction of the drug myocarditis, meaning that the stroke volume is reduced. In other words, the toxicity of the medicine cardiomyocyte shrinkage can be evaluated as a speed decrease.

The fig. 6 (a) shows changes in the inflow/outflow potential Na + ion, Ca 2+ ion, K + ion component in a normal state, the target cell culture solution is not included. The fig. 6 (b) shows changes in the inflow/outflow potential Na + ion, Ca 2+ ion, K + ion component of target cells of chemicals are contained in the liquid state. Figs. 6 (a), 6 (b) are compared so as to immediately recognize, QT misdetction waveform time delay is extended in the axial direction. Further K + ion with the inflow and outflow, large deformation of the waveform. In order to evaluate as an electric signal, and a "0" as shown by a broken line in the figure "100" to a value between the, 30%, 60% and 90% of the value of the continuation time APD30, APD60 and APD90 as detected. Here, the letter of the APD and Potential Duration Action taken from the expression. The magnitude of these values is evaluated and if the ratio, of the Na + ion, Ca 2+ ion, K + influence on the inflow/outflow of the ion component can be evaluated.

The fig. 7, the cell toxicity myocarditis inspection device for optically detecting the optical system and the arrangement of the movable electrode in explaining one example of, for example, a measuring cell 10 n pulsation of the observation of the state. 12 showing the culture solution. 22 of the phase difference between the source and the sample microscopic or, in general, a halogen lamp is used. 221 of the fluorescent light source of a fluorescence measurement and, in general, a mercury lamp, a laser monochromatic light, such as LED light source is used. 23 of the phase difference of light from source carpophore microscope observation, only a specific wavelength, and a band pass filter transmitting, fluorescent light source 221 231 from the particular fluorescent excitation wavelength band pass filter transmitting light of only. For example, cell 10 n change information such as deposition of sphygmoscope observed in the case of a change of shape, the shape of the cell for measuring only light of a wavelength passes through a band pass filter 20 for measuring a real image of the camera 21, in the vicinity of a wavelength of 700 nm is measured by using a narrow-band optical cell 10 n damage can be prevented. The shutter 24 and 241, such movement of the XY stage 15, image is measured between the illumination of the light not having a function of shielding. 25, and a condenser lens, the phase difference retardation when observed by introducing a ring, when the differential interference observation, a polarizer is introduced. In the case of a fluorescence measurement, for example when performing measurement of calcium in the cells,

the degree of excitation wavelength of 500 nm, the wavelength fluorescence measurement 600 nm for selectively transmitting light by using a combination of a band pass filter, fluorescent wavelength of selectively transmitting only light passing through a band-pass filter 201 is measured by fluorescence image camera 201. In this case, the unit cells of the cell in the network 1 of calcium by measuring the time relation of the signal transmission path of the cell in the network is measured, the measured time resolution of 0. 1ms can be obtained more quickly and continuously. On the XY stage 15 is formed on the substrate 1 myocarditis toxicity testing device 100 is placed on the XY stage 15 X-Y driving device 16 by moving the arbitrary position of the myocardal toxicity testing device 100 is observed, can be measured. The state of the cell 10 n sphygmoscope in the toxicity of the inspection device myocarditis 100, which is observed by an objective lens 17. The personal computer PC by focusing position of the objective lens 17 in response to the signal Z by driving device 18 can be moved in the axial direction. The magnification of the objective lens 17 40 times or more can be used. By observing the objective lens 17, a light transmitted from the light source 22 of cell 10 n differential interference image or image of the phase difference. The band pass filter 23 and of the same wavelength of the transmitted light is reflected by the dichroic mirror 192 and a band-pass filter 20, a phase difference microscope image or Differantial interference microscope image observed by the camera 21 only. The image signal is observed by the camera 21 is introduced into the personal computer PC. Furthermore, in this embodiment, for the stimulation to the cells are arranged and movable electrode 27, and the coordinates of the movable electrode at an optional position on the XY stage not only in the same plane, it is possible to arbitrarily adjust the height position control mechanism. In this position of the control mechanism is used for a specific cell network of cells or cell number 1 position by moving the movable electrode, an arbitrary number of cell or cells can be stimulated. As the material of the movable electrode, the part other than the tip of the metal electrode is covered with insulation, of less than 5 microns in size of the opening of the tip of the glass such as an electrode, a movable electrode in the vicinity of the tip of a number of cells or only in certain specific electrostimulation constitution capable of electrodes can be used. In the case of using a metallic electrode, platinum black or the like is added to the tip by the surface effectively Electrostimulator can be transmitted to the cell. The position of the tip of the movable electrode, depending on the degree of the response of the cells to Electrostimulator and to adjust, cells can be in contact with, or, can be arranged very close to the cell. Furthermore, the electrode and stimulation of a particular cell appropriately applied to the target in order to give the switching instant electrostimulator by cell potential for measuring the electrode 2 can be used as a ground electrode, or, additionally, a separate ground electrode 28 can be arranged. Furthermore, in order to stimulate specific cells, stimulating existing micro electrode 2 may also be used as an electrode. In this case, the micro electrodes are connected by the switching of the switching circuit 29, usually electric signal measuring circuit 30 is connected to, the moment the stimulus, by switching the switching, electrostimulation circuit 31 connected to a square wave by a stimulus signal can be given a very small electrode 2. Furthermore, by using a stimulus applied at the moment to the movable electrode 27, a switching circuit 29 can be ground. On the other hand, even if the movable electrode, not only as the electrode, the electrode of the measuring cell or used as an electrical signal, can also be used as a grounding electrode. In this case, the movable electrode is connected to the switching circuit 291, biopotential measuring cells, cell stimulation, according to the usage of the grounding electrode, by switching respectively, are connected to an electric signal measurement circuit 301 and measuring the cell potential, connected by a electrostimulation circuit 311 of a stimulus signal to a rectangular wave or cells, also, to be grounded, can be used as a grounding electrode. The cells are given Electrostimulator electrostimulation circuit 31,311 timing of, one of the primary uses of dichroisms 2 can be used. The autonomous sphygmoscope one in a cell network, the network of the normal during the interval sphygmoscope cardiomyocyte, applying a stimulus to the irregular and, on the other hand, does not have the ability to autonomously sphygmoscope interval sphygmoscope cardiomyocyte network. Both, the measurement, sphygmoscope interval period (the time difference between two sphygmoscope 2) are gradually shortened to 5ms time of the unit, a change in the response of the tracking cell network. For this purpose, the electrostimulation circuit 31,311, such as an electric signal measurement circuit 30,301 obtained by analyzing the pulsation period, based on the result of determining the timing irrilation feedback control can be performed. Furthermore, the movable electrode 27 when used for measuring an electrical signal, in this system there is no micro electrode 2 and a similar measurement can be obtained. Therefore, the system is configured in each cell by cell network of optical measurement of the period of the pulsation can be measured, this pulsation period such as from a stable state to an unstable state of the system becoming arhythmia arranged only measured by an optical measuring device, from the result, if necessary, by using a movable electrode electrical characteristic of the specific cells in which data is obtained, in this case, are disposed in advance on the system without being restricted by the number of micro electrodes, optical measurement range, a large cell network freedom can be constructed.

In fig. 8, as shown in the schematic diagram of one example of the electric signal generated. First, in which sodium ions from the cell membrane sodium ion channel flows into cells, occurrence of cell potential is abruptly lowered, next, through a slight delay, calcium ions due to inflow of cell potential is decreased, then, as the next step, potassium ions from being discharged out of the cells of the cell in the rise of potential occurs. The cell potential, cardiomyocyte film present on the characteristics of the various different response characteristics caused by the difference of ion channels. Each ion channels caused due to the potential change of position of the peak, and the characteristics of each ion channels as time, due to the effect of a medicine being blocked by each ion channels, depending on the characteristics of the chemical ion channel block in accordance with the kind of a waveform of an electric signal to measure the change, thereby to effect a chemical ion channels can be estimated. The evaluation is especially important chemical ion channels, FastNa, SlowNa, Ca, IKr, and channel 4 of two ion IKs, these 4 kinds of ions of the channel block state can be measured.

The fig. 9 (a), the electrical signal of the cells shown in fig. 8, actually kalium ion channel selectively inhibiting reagent E-4031 when added in various concn. indicates a change. The cells of the cell which raises the potential of the discharge to the outside an ion K IKr functioning from obstructing the channel ion, chemical concentration is increased in the positive direction as the cell potential, it is found that a change is gradually delayed. It is shown in fig. 9 (a), the response of specific data is 1 sphygmoscope, actually in each adjacent sphygmoscope response of the magnitude of the fluctuation width, and to estimate an influence of an important index. In fig. 9 (b) is one example thereof, and adjacent sphygmoscope poincare plotting called correlation data comparison analysis technique. In this case, the axis X at n-th sphygmoscope response time of the specific position of the channel ion, Y axis (n+1) in the first channel of the same response time when sphygmoscope ion of a plot of position. As a result, the characteristic in the mutually adjacent sphygmoscope is the same, in the graph with a dotted line on the plot is drawn and Y=X becomes, the larger the response between adjacent sphygmoscope when there is fluctuation, a position far from the large Y=X plot of distribution and is observed. Actually, in this example, chemicals are not added to the Control, by the addition of 40 nm is the delay of response time, between adjacent sphygmoscope dipterous phase is maintained. On the other hand, addition of chemicals to 400 nm to further delay the response time and, in addition, the phase between adjacent sphygmoscope dipterous collapsing, and generation of an unstable sphygmoscope period to become clarified by plot. The result of this, even if the results of the measurement of the extension QT indicating cardiotoxicity coincide with each other, by 1 at the cell level poincare plotting adjacent respective sphygmoscope fluctuationally of measurement of the increase of generated by index QT extension can be estimated. This phenomenon, specific ion when the channel is blocked by a chemical agent, when the degree of the block which is slight, the phenomenon that the reduction of the discharge capacity of the cell response only looks does not occur to the instability of a still, further, and the degree of block multiplexity, if the number of channels is extremely reduced ion, ion cells for the same cell discharge capacity in spite of a large fluctuation of low reproducibility of results and the o Furthermore, the magnitude of this fluctuation occurs QT extension can be used as the index.

The fig. 10 (a), the cell 1 arranged at the cell level by network using annular cardiomyocyte reentry watertree circuit schematic diagram showing one example of a chemical agent. The annular cell only by cardiomyocyte where generating a network, and the network model becomes normal, such as in the case of the model histopathological hypercardia, incorporated in the cell network Osteoblast fibrosing. Furthermore, mixed in the network, which network Osteoblast fibrosing cardiomyocyte causes attenuation or delay of the transmission speed, as a result, the occurrence of premature contraction can be predicted. The fig. 10 (b), the actual micro electrodes arranged on one cardiomyocyte microphotqgraphic example is shown. Actually, as shown in the photograph, the unit 1 is disposed on the cell when the micro electrodes, between adjacent cardiomyocyte delay of signal transmission can be measured. Furthermore, the transmission speed is first generated when sphygmoscope depending on the size of the electric signal from, Na ions as a channel to effect, this signal transmission delay data can be used.

The fig. 11 (a), using the cell group of constant width annular cardiomyocyte showing one example of a reentry watertree by network model diagram. 1 shown in fig. 10 the unit cells in the annular cell network,

cardiomyocyte sphygmoscope signal for uniquely passes, as shown in fig. 9 which fluctuationally unless the beating of the cell itself, to maintain the same characteristics of the cell are transmitted between adjacent cells sphygmoscope signal. On the other hand, as shown in this fig. 11, with a constant width annular cell is arranged as a network, each transmission group of the cells as a solid line 35, the broken line 36, as in the dotted line 37 to take different routes at every sphygmoscope ~~~ and having a degree of freedom. In particular, as described in fig. 9, by the addition of a response characteristic of each large flactal cardiomyocyte and generating, in response to easily around the annular cell whenever a stimulus signal network, can be different, and the difference is more significant route. This is called the reentry of the heart of the spiral and watertree Cytocidal · premature and leading to and from the same mechanism as the mechanism of, the width of the annular base having a particular group of the cells by using a network capable of measuring watertree · reentry and spiral. In fig. 11 (b) is actually very small electrodes arranged on a population of cells, for example microphotqgraphic and, to the extent of about Osteoblast fibrosing 40% cardiomyocyte 60% mixed with a cell group is arranged. Actually, and such an arrangement, the transmission speed of between adjacent electrodes, between adjacent sphygmoscope fluctuationally becomes large, particularly by the addition of chemicals, and becomes remarkable increase of fluctuationally thereof, between adjacent sphygmoscope fluctuationally width variation of the transmission speed from generation of reentry watertree · spiral can be estimated. The fig. 11 (c), actually micro electrode array on the cell group is arranged for example microphotqgraphic. In the measurement of the actual spiral · reentry watertree, shown in fig. 7 by using a high speed camera fluolescence measurement, the group of the cells in the network 1 of each cell of the calcium firing can estimate the cellular level, and as a result of the signal transmission path or any leakeel, each round of a route change is actually can be analyzed.

The annular electrode fig. 12 (a) using a reentry watertree circuit schematic diagram showing one example of a measuring device. In this example, two annular electrode 38 is arranged to 1 micron of a ring-shaped electrode width 50-100 1-3 mm-diameter of the bottom of the well plate each 96 hole 42, so that the cell group is arranged only on the surface of the ring 41 so that, except for the bottom of the periphery of the conductive electrode, is free of the adhesive coating on the surface of a material such as agalose. The cell is not adhered to the coated area so that, in the form of a concentric reference electrode ring 39 is arranged, on the other hand, a reagent to go in and out of the flow passage 40 is arranged. By using such an electrode simply cardiomyocyte simply sphygmoscope abnormality can be measured. In fig. 12 (b) is actually measured by the electrode, which is a graph showing the normal and abnormal sphygmoscope sphygmoscope data to data. In this embodiment, is an annular electrode is used, an optical measuring system shown in fig. 7 is used, and the same effect due to the annular electrode, abnormal sphygmoscope optically measuring system can be constructed. In this case, when the electrical signal is measured, fig. 7 shows the movable electrode is in contact with the annular cell network, even if the electric signal can be acquired.

In fig. 13 (a) is a schematic diagram showing one example of the arrangement of the micro electrodes 2 and 1 to measure the potential of the cells for the cells, a diameter of 10 microns and 50 microns from the micro electrodes 2, in which the measuring means is arranged to be composed only of the measuring cell and the cell 1. Also in this embodiment, similar to the other embodiments and, in its place on the electrode of the cell is held around the electrodes, inhibiting the adhesive is coated with a material such as agalose. The micro electrodes 2 fig. 13 (b) is actually measured by a photographic and isolated 1 on the electrode of the cell to the sphygmoscope electric data that is, isolated by a signal of one cell as shown in a graph in which large unstable the sphygmoscope fluctuationally. On the other hand, as shown in fig. 13 (c), on the micro electrodes 2, similar to fig. 13 (b) and 1 which are arranged in the cells, and other cell by cell group and connected, by looking at the graph signal sphygmoscope know, sphygmoscope period of stability is obtained. 1 at the cell level sphygmoscope actual measurement, as shown in fig. 9, between each of the adjoining sphygmoscope fluctuationally from the magnitude of the index and, as shown in the present embodiment, while realizing a stabilized by cell group, the cell 1 to acquire data sphygmoscope, only a specific desired to be arranged on the electrode 1 minute, this specific cells in order to maintain the stability of the electrode on the other cardiomyocyte arranged in a form which is not mounted using the measuring system is useful.

The fig. 14, which is a schematic diagram explaining a camera using an embodiment of the present

invention of the receiving element 1 at a potential measuring cell. Usually, the camera, the photoelectric conversion optical signal is converted to an electric signal, and this electric signal is measured can be used, by removing the photoelectric surface, by using an electric signal array portion, in a 2 dimensional and capable of acquiring an electric signal. Therefore, the size of the electrode array 1 at the cell level can be used, for example in the cell group of each cell of the necessary electrical signals simultaneously, fig. 11 shows a group of the cells having a fixed width in a network such as the change of the signal transmission path · spiral generation of watertree reentry and can be measured. In the actual measurement, the measuring interval of 1 multiparallel min. 1 requires about seconds, the shutter speed of 1 multiparallel min. 1 sec. of camera receiving element it is necessary to use a camera. In this case, the cells of the acquired signal data, used in existing camera image processing techniques can be applied as it is, using the FPGA for image processing and real time processing is performed. Furthermore, the real-time processing based on data obtained by the feedback stimuli to the electrode and can also be carried out.

The fig. 15, one example being a schematic diagram for explaining the mechanism of the present invention can be measured at a plurality of sample cell measuring system. The system of this embodiment, analysis module, multistage incubator, and an analysis module electrically connected in-line and on-line analysis module network. In this case, in the analysis module, a phase difference measuring a change in shape of the cells or Differantial interference microscope, the microscope, the camera and the optical measurement by an analysis of a fluorescence microscope, the microscope agalose micron scale locally by utilizing a system capable of dissolving agalose machining technique. In the multistage incubator, plurality of cell culture vessel is arranged, on the other hand, in the cell culture tank micro electrode tip is arranged, each cell of the measurement of the electrical signal, the electrical stimulus in the incubator can be treated continuously in parallel. The electrical signal is obtained, an electric measuring in real time analysis module, the data in a storage line access, and electro-optical data of measurement results of measurement data in a form in which the same time stamp is recorded, the recorded data access to on-line analysis module is properly analyzed.

The fig. 16, the heart of the present invention can be measured by measuring system schematic diagram explaining information. The electric signal on the electrode 1 minute by measuring cell, Na, Ca, IKr, IKs ions or the like can be measured data channel signal, signal transmission between adjacent cardiomyocyte from the measurement of the speed change inhibition of Na ion channels can be measured. Furthermore, by measuring the change of shape of the cells 1 arhythmia and generation of optical measurement of the estimation of the output of the heart. Furthermore, by arranging an annular cell network capable of measuring the occurrence of watertree reentry, further adding to osteoblast fibrosing arranged cells such as heart model histopathological hypercardia can be measured.

The fig. 17, in the present invention a system for measuring cell, autonomous sphygmoscope cardiomyocyte cell is acquired from the field of potential · (FP) is one example of the change to the chemical addition signal waveform is exemplified in the graph. · Potential signal waveform field of cells, the cell as shown in fig. 8 flows into ions, the ion flow generated by shows changes of potential cells, cell potential differential value, i.e., the sum of the ionic current flow per unit time, and, in this case, the sodium or calcium depolarization of the process such as the ion current in the negative direction, the subsequent re-polarization process such as potassium extrorse ion current is taken to be positive. Usually, the cell for the waveform of a signal FP, as shown in this fig. 17 of each adjacent sphygmoscope paying attention to the difference from each other, a plurality of adjoining or adjacent to average the waveform a of each wave noise components by eliminating the influence of the difference in the average value of the waveform is extracted as one FP 1, 1 which reflects the average value of the waveform of ion channels by detailed analysis is used for estimating the state. However, in the present invention, the average value of the signal waveform adjacent FP is not obtained, the difference among adjacent FP signal waveform of ion channels a portion depending on the response of the comparator fluctuationally, based on the magnitude of this ion channels fluctuationally amt. quantitatively estimating block. As a result, the size of the element is generally of the square root of n fluctuationally reciprocal [1 / (n) 1/2] from is shown, it is possible to understand the meaning thereof. That is, the number of channels is an ion, e.g. 10 4 when one function, as a function of the sum of the ionic channel size of fluctuationally 1% [1 / (10 4) 1/2] becomes and, by being blocked by a chemical 10 2 to function when ionized channel, the function of the size of the fluctuationally 10% [1 / (10 2) 1/2] suddenly

increases and, adjacent to the FP waveform and generating a large change. In other words, the change of the waveform is compared with the adjacent FP fluctuationally when estimating the size of, the magnitude of this fluctuation, the total amount of ions from the channel blocks can be estimated.

The waveform of changes in, especially potassium ions generated by the ion current peaks of outward by paying attention to the place, of sodium ions flowing into cells (zero) and the reference time, the time period from the time of release of the potassium ion peak potential field · · duration (FPD) and defined as, this change of length of the FPD, sodium, potassium ions such as calcium ions flowing out of a peak value of the following and, therefore, the cells of various chemical ion channel is blocked by the ions generated by the change of the amount of change of the total sum of the go as can be noted as an index, in addition, the position of this fluctuationally FPD, relation of all of the channels of adjacent cells of the waveform of ion fluctuationally FP and reflects the sum. Actually, the position of fig. 17 of FPD is confirmed (red arrow head position) and, by the addition of an inhibitor kalium ion channel E4031 425-450ms before adding the FPD between which, by the addition of 10 nm 642-645ms, 663-694ms by addition of 100 nm, so that by the addition of 1 μm 746-785ms monotonousness FPD by adding the value of inhibiting agent increases noninterlace, moreover, is not the same as the adjacent FPD, to take different values fluctuationally and reflecting.

The fig. 18, actually kalium ion channel specifically by having a function of inhibiting chemical E4031 kalium ion channel FPD E4031 concn. depending on the time of extension of shows one example of an experimental result. In this case, the outflow is delayed by the inhibition of ion kalium ion channel , FPD is concn. dependence can be estimated to be extended. As a result of this experiment, when the measurement is considerared fluctuationally.

In fig. 19 is generally of a [...] fluctuationally sphygmoscope electrocardigram is measured, the value of FP in the evaluation of the waveform of the FPD FPD fluctuationally when attention is, how much of the adjacent sphygmoscope FPD, phase shifted from the state of the technique of estimating whether a target point, the magnitude of the fluctuation of the adjacent sphygmoscope (short-term variation: STV: Short-term variability) for explaining how to estimate. In fig. 19 (a), the diagonal X=Y and comprising, adjacent sphygmoscope FPD n and FPD n+1 FPD is exactly the same as in the case of a size and so positioned, one difference (FPD n+1-FPD n) FPD 2 of the vertical distance from the diagonal size of a standard size of adjacent sphygmoscope fluctuationally itself and therefore, for the particular sets of sampling number k, as shown in fig. 19 (b) in equation (eq. 1) can be evaluated.

On the other hand, by using the FPD [...] fluctuationally of fig. 20 when paying attention, to what extent of adjacent sphygmoscope FPD, phase shifted from the state of the technique of estimating whether a target point, adjacent respective sphygmoscope is sphygmoscope average value (all the samples of the sum of the response of the channel corresponding to the ideal value of ion) whether the degree of deviation from the viewpoint of the magnitude of the fluctuation of sphygmoscope (long term variations: LTV: Long-term variability) for explaining how to estimate. In fig. 20 (a), which are arranged on a diagonal as FPD X=Y and average value FPD mean and adjacent sphygmoscope FPD n and FPD n+1 2 of each of the two differential [(FPD n+1-FPD mean) + (FPD n-FPD mean)] FPD diagonal size of a vertical distance from a reference of the magnitude of fluctuation from the mean value of FPD and therefore, particularly with respect to the number of sets of sampling (eq. 2) k a mathematical evaluation of fig. 20 (b). This symmetry of X=-Y represents a deviation from, the size of the average value by a mere sphygmoscope fluctuationally is performed around, or, history and have to find whether the index.

In fig. 21, actually E4031 when added in steps of one example of a response cardiomyocyte [...] fluctuationally of FPD and shown with, its fluctuationally STV collectively as quantitatively. Ion channels in accordance with the addition of a block E4031 resealbility is estimated by the extension of the length of time of FPD and, especially in high concentration by the addition of the value of the sudden increase STV is known.

The fig. 22, the axis X of the conventional QT comes into contact with the extension of the extension is measured using an FPD cardiomyocyte to the observed rate (%), the increase in the Y axis STV is observed, the ratio (%) is taken, it is known that cardiotoxicity chemicals, it is known that there is no cardiotoxicity as evaluation. As for the inspection of a conventional chemical toxicity, X axis as a result of FPD by only the data evaluation is, on the Y axis and the result of the evaluation is performed and STV, from Figure 2 on a graph of know in dimensional mapping, high-risk (High risk) cardiotoxicity, risk is low (Low risk), without risk of two regions (No risk) in 3, and the same distribution of known literature result is known. In addition to this conventional FPD by adding a chemical cardiotoxicity STV possibility of estimated more accurately and easily and it is known that can be made.

The addition of chemicals to fig. 23 about the FPD STV indicates the difference of response. In fig. 23 (a), as an example, when constructing an annular cardiomyocyte network of FPD [...] (A, B) of a local, and 2-dimensional constructs a myocardal sheet [...] (C, D) of its local to the measured result is shown in (b). In this example, the PM from [...] regions B, D the vicinity, A, C is located remotely. (B) before the addition of chemicals in, [...] X=Y on a diagonal of the distribution of the FPD is, by chemical addition cardiotoxicity of low capacity, both annular model (A, B) occurs in large flactal STV increase is observed, 2-dimensional sheet model (C, D), almost fluctuationally does not occur. In addition to the chemical addition capacity, in the annular model (A, B) a small dynamic state or a stop state sphygmoscope is transferred to, 2-dimensional sheet model (C, D) in the region remote from the increase of the PM STV is seen at C, D in the vicinity of the region, the region C STV yet lower than the amount of increase is observed. In this example, by measurement of FPD STV to know the drug to the prediction of cardiotoxicity dimensional sheet-like cell group 2 (network) more, from a pacemaker linearly arranged cell group (network) is, more accurately reflect the influence of the drug is known.

The addition of chemicals to fig. 24 PM from the region of the transmission speed (V) of sphygmoscope irrilation STV indicates the difference of response. Therefore, the plural desiners cardiomyocyte cardiotoxicity [...] (TdP) as abnormal in the transmission structure, the transmission speed from the actual PM to what extent the fluctuationally drug is confirmed as a technique for estimating the toxicity. For a definition of the STV in this case as shown in fig. 24 (c) (eq. 3) of, the FPD, PM region T from the transmission time (or the PM and a distance from the transmission time by dividing, the observation point at an apparent transmission speed V) and using the measurement. For a definition of the FPD LTV STV and similarly, V and T or is changed. In fig. 24 (a), as an example, when constructing an annular cardiomyocyte local network of transmission T [...] (A, B) of the time, and 2-dimensional constructs a myocardal sheet [...] (C, D) of its local to the measured result is shown in fig. 24 (b). In this embodiment, similarly to fig. 23 and also from the PM [...] region B, D the vicinity, A, C is located remotely. In fig. 24 (b) before the addition of chemicals, [...] X=Y on a diagonal of the distribution of the FPD is, by chemical addition cardiotoxicity of low capacity, both annular model (A, B) occurs at a time for transmitting large flactal fluctuationally indicating a large increase of the observed STV, 2-dimensional sheet model (C, D), almost fluctuationally does not occur. In addition to the chemical addition capacity, in the annular model (A, B) a small dynamic state or a stop state sphygmoscope is transferred to, 2-dimensional sheet model (C, D) in the region remote from the increase of the PM STV is seen at C, D in the vicinity of the region, the region C STV yet lower than the amount of increase is observed. In this example, to know the time T (or the transmission speed in each local apparent V) by measurement of drug cardiotoxicity STV for the prediction of a sheet-shaped cell group 2 than dimensional (network), from a pacemaker linearly arranged cell group (network) is, more accurately reflect the influence of the drug and simultaneously is known, more effectively fluctuationally dependent spatial arrangement it is known to measure.

The fig. 25, in the present invention each of the electrical network cardiomyocyte cardiomyocyte FP waveform acquisition from, its FP in vitro measurement techniques and conventional waveform (for example patch clamp method), conventional in vivo measurement technology (e.g. electrocardial) shown schematically the relationship. The cell of the present invention is obtained by the waveform FP, cells per unit time to go from the magnitude of the ion current indicates, the cell potential change information (electrical ion current) becomes and, therefore, in the conventional cells in vitro measurement obtained at the base of the cell and potential, so that in fig. 25 · integrating and differentiating relationship. Next, each

cell (or cells in the local network) obtained from the measured waveform from the electrode 1 FP, cell network are arranged into a plurality of areas of a plurality of electrodes of each waveform obtained from another cell network FP FP of the waveform can be obtained, this change in potential obtained from the heart of the waveform signal response of tissue part ventriclerelated electrocardigram QT and phase data corresponding to a region having a dipterous electrocardigram.

The fig. 26, above, fig. 25 in explanation and measured information in a conventional technique, the data obtained by the present invention a device for estimating correlation of FP of the device shows schematically the structure of the system. 1 FP of cells or a local network can be measured from a plurality of micro electrodes 1 FP data acquired by the electrode units are integrated, by differentiating the respective FP data for estimating the cell potential having a function capable of computing circuit, or, by overlapping the respective electrode data, a part of ventriclerelated electrocardigram electrocardigram waveform (Q-T portion) having a comparison computing circuit. In particular, the use of a single electrode superposition circuit not only FP data is analyzed, for example, which are arranged in series and arranged at equal intervals on a cell network with a plurality of micro electrode array of the data, the result of each electrode of the upper cell to cell FP to transmit not only reflects the state data becomes and, especially, between the transmission of abnormal cardiomyocyte arhythmia on estimating, transmission of reflected in the waveform from synthetic FP, the superimposing circuit results in premature occurrence prediction mechanism for directly moving the information, and similar electrocardigram analysis predicted from this result. The cell potential data obtained from other differentiating circuit even for different cell potential in a way dependent on the state of the ion activation state used for the channel.

The figs. 27 and 28, in which an example of the data from each electrode FP overlapped by an arithmetic circuit which actually in the fig. 26. In fig. 27, as shown in fig. 27 (A) cardiomyocyte network are arranged in a ring, the network at a predetermined interval along the micro electrodes are disposed. The pacemaker is at the position of the annular electrode R1 (PM) area network → R8 in the R2 signal sphygmoscope cardiomyocyte, or where L1 is transmitted to each of the electrode → L8 FP of the waveform in fig. 27 (B) can be seen. This waveform and the waveform of the lower S are superposed. The fig. 27 (C), by measuring the actual composition result in long. As a result of the waveform of the actual electrocardial QT area necessary for estimating, FP FP of the waveform and a synthetic transinformation is included. From this figure and to know the PM from the signal is transmitted normally sphygmoscope when, also know from fig. 27 (C) composite waveform and a smooth waveform. On the other hand, to know the PM from fig. 28 fig. 28 (B) from a region which does not transmit a signal sphygmoscope arhythmia state and regularly, synthetic and consisting of a waveform S FP and becomes extremely turbulent, similar to fig. 28 (C) and even electrocardigram corresponding to a shape of the synthetic corrugated arhythmia FP and can be seen in the waveform. It is to be note here, only the data electrode 1 of fig. 28 (B) from the respective electrodes when predicting arhythmia, by observing the remarkable arhythmia electrodes (for example, L5) it is difficult to forecast the occurrence, as seen in fig. 28 (B) S or synthetic FP and fig. 28 (C) by more accurately predicting the waveform can be made and it is known.

The fig. 29, cardiomyocyte sphygmoscope shows the relation between the size of FPD to cycle. The black circle, having a variety of autonomous sphygmoscope FPD cardiomyocyte result measured by the system of the present invention to a device. From this result, and to know, the cells thereof depending on the value of the period sphygmoscope FPD is known to change. This is by using an autonomous sphygmoscope cardiomyocyte when measurement is performed, by chemical changes or sphygmoscope of sphygmoscope cycle is stopped, or when the instability, this effects inherent in the channel of the block by ions of FPD is changed due to a cause possibility is suggested. Furthermore, by forcibly sphygmoscope methyloctanoyloxyphenyl × infrared cell sphygmoscope period when the change of the value of FPD shows, more than a certain time by a continuous external stimulus, maintaining a constant sphygmoscope period, it is known that the FPD and becomes stable.

The fig. 30, is actually an autonomous sphygmoscope cardiomyocyte on from the outside with a system of the present invention is applied when forced sphygmoscope FPD cardiomyocyte shows one example of a change with time. In this example, to know, autonomous sphygmoscope interval is about 4 seconds of the

cells and the forced stimulation sphygmoscope 1Hz immediately changes from a large value of FPD, in about 30 seconds after the start of the stimulus 550ms be stabilized in position as is known. Also after the end of forced sphygmoscope stimulus has different autonomous sphygmoscope period, moreover, also becomes gradually longer resealbility FPD can be seen. From these results, and to know, from the start of the forced stimulation is applied sphygmoscope FPD until after after after starting the stimulation of 30 seconds, it is desirable to inspect the drug toxicity.

The fig. 31, using a system of the present invention actually forced sphygmoscope FPD stimulation is applied from outside, the time T or transmission, or a transmission speed V in the case of the example is shown in which the cell arrangement. The fig. 31 (a), at least one of the electrode 2 which is disposed so as to cover the stimulation of the measuring cell group is used in this embodiment. For example, the electrode beats per minute 60 from the forced stimulation signal is given a constant interval while the measuring electrode of the adjacent cell in FPD, measured from the time the stimulation of the electrode or electrodes to the signal transmission time T sphygmoscope cell, or a measuring electrode for measuring the transmission speed V on the cells. The fig. 31 (b), which are arranged in a straight line shape at an end point of the network cardiomyocyte micro electrode arranged to give the complusory stimulation sphygmoscope stimulus, for controlling the transmission, at constant intervals cardiomyocyte arranged along the network by micro electrode array, each electrode on FPD cardiomyocyte, T, V is measured, stimulating electrodes of each electrode against not only the data of the stimulus signal, FP FP of each of the recording electrode arhythmia by estimating generation of synthetic, such as the relationship between T and V of each electrode can be estimated. However, in this case is shown in which one example of the arrangement of the cell, the, annular cell network shown in fig. 27 in the region of the PM is forcibly sphygmoscope can be measured by similar, or, on the electrode of the measuring cell is used as an electrode in the electrode, the number of cells in the FPD min. can be miniaturized.

Here, the embodiment for all, only a part of the network myocarditis cardiomyocyte is referred to, and a biotissue fibrocyte and similar properties are included so that the additive.

The fig. 32, arranged on the micro electrodes 2 FP of the measuring cell, the potential of the electrode 2 is kept constant potential clamp type of feedback control mechanism can be used. In this case, the FP of the cell, which amplifies the signal from the conventional electrode is not measured, the electric potential of the electrode 2 in order to keep a current supplied from the external power supply is monitored, and the results of estimate by analyzing in real time. In the case of a conventional FPD cardiomyocyte shows one example of a change with time. In this case, as the constant potential, which is normally zero is selected, step changes the polarization potential such as in the case of changing the state of cell, can also be adjusted at different potentials.

The fig. 33, from the actual human ES cells cardiomyocyte redifferentiation cell group in a partial area of the present invention using a system of the described forced sphygmoscope during stimulation is applied, cell group sphygmoscope cycle of one example of a result obtained by measuring a change in the graph. As is understood from this graph, in the normal cardiomyocyte group, for example 0 in this example. 6Hz from 1. 8Hz when forced stimulation is applied, all of the linearly in this range, in response to the forced stimulation to follow sphygmoscope is known.

The fig. 34 (a), was actually forced sphygmoscope stimulus is given by the forced sphygmoscope sphygmoscope cell group is the same as the interval irrilation and periodically, the forced stimulation sphygmoscope cardiomyocyte FP in the state of change of a group of waveform and FPD indicates a change of length. As understood from the graph, the interval is hence forced stimulation sphygmoscope FP waveform changes, it is known to shorten the length of FPD. Therefore, as shown in fig. 34 (b), and a graph showing the change in this FPD, this shortening, forced sphygmoscope interval depends on the cycle (RR), this correction
Relation, in known human heart and heart QT of the relation of the length of the interval of research (Patrick Davey, How to correct the QT interval in clinical heart rate for the effects of st

udies. 3 - 48 and Methods of Journal Toxicological Pharmacological 9 (2002)) from the correction Fredericia, in other words by the difference in speed of sphygmoscope QT is varied by the relative length of cannot be compared, sphygmoscope myocarditis sphygmoscope min. 60 beat cycle time of a length (QT C) QT in order to correct the QT C =QT / (RR) 1/3 converted as, or, the proposed Bazett QT C =QT / (RR) 1/2 whether converted as coincides with and is important. This is, as explained above in vivo QT in length, in this system, the entire cell network FPD measured by measuring the length corresponding to the overlapping of, the same, that is, each cell of the FPD itself, the, correction of the carpentering Bazett Fredericia or suggests that is, actually, the result of fig. 34 (b), QT C =QT / (RR) 1/2. 5 becomes and, between Bazett Fredericia or correction is known. Furthermore, the graph of fig. 35 shown in figs. 33 and 34 in a data table.

The result of these, or actually used in screening the quality of regeneration confirmation of cardiomyocyte, forcibly sphygmoscope cardiomyocyte cardiomyocyte when stimulation is applied by measuring the response of the can. That
1) or forced sphygmoscope myocarditis cardiomyocyte stimulation is applied to the group of the cells, cells or cell group is in response to the forced stimulation sphygmoscope same interval in response to a stimulus and forcibly sphygmoscope confirms whether, and, its response in the frequency range of any forced sphygmoscope irrilation corresponding by measuring whether or not, it can follow sphygmoscope sound cardiomyocyte by confirming that sufficiently satisfies one of the conditions and determine, or more specifically, the following is the response of the example 1. 8Hz follow until sufficient sound cardiomyocyte and determining one of the conditions is satisfied.
2) above, forced sphygmoscope stimulation interval (RR) to follow the beating of the cells is confirmed frequency carpentering, change of the forced stimulation sphygmoscope FPD, FPD / (RR) 1/3 from FPD / (RR) 1/2 to check that the sound cardiomyocyte satisfies one of the conditions that determine and sufficiently.

By using this procedure, and capable of managing cardiomyocyte quality. The sound and cardiomyocyte, stable sphygmoscope cell can be performed. Here, as for the evaluation and cell group, the cell group redifferentiation induction can be used as it is, or, by dispersing cardiomyocyte redifferentiation induction is carried out by the unit 1 can be evaluated by measuring cell, or, again, these cardiomyocyte dispersed by the cell group can be measured, or, a dispersion of a human heart cardiomyocyte and osteoblast fibrosing derived by measuring and evaluating a new cell as a group.
These myocardal cells, can be used to inspect myocarditis toxicity.

The fig. 36, of one embodiment of the present invention related to a device for inspecting myocarditis toxicity (myocarditis toxicity or inspection system) is a block diagram showing the whole constitution of an exemplary. The present invention of an inspection device myocarditis toxicity 3000, basically, measuring part 3001 and control · analystic part 3002. Measuring part 3001 and control · analystic part 3002 between (e.g.: personal computer), measuring part 3001 amplifies the electric signal from the amplifier 3004, the analog signal from the amplifier 3004 is converted to a digital signal, analog-to-digital converter (ADC) 3003 is interposed. The computer interface 3002, receiving a signal from the ADC · display or recording · analysis. The measuring part 3001, basically, the multi-electrode array including a plurality of micro-electrode having a culture vessel, placing the same x-y stage, an optical microscope, a laser light source, an optical system such as an optical camera. The multi-electrode array including a plurality of micro-electrode having the culture vessel, which can be manufactured by fine processing technology, the market (example: MED-P515A, [...] Company, Japan) can be used.

Figure 37A is, of a core of the present invention is an inspection device system myocarditis toxicity 3000 measuring part 3001. Figure 37B is, in a system of the present invention is provided with an array of multi-chip and multi-electrode (or culture vessel). The figure 37C, further enlarged view with respect to the electrode section. A plurality of electrodes are arranged in an array, each of the electrode lead-out wires (or the lead wire) is connected, on which the cluster is derived from human myocarditis ES is placed.

The fig. 38, multi-electrode array chip (Figure 38a, b, d) cardiomyocyte (Figure 38b) using the evaluation of toxicity of medicine, and measuring the corresponding relation between the electrodes of the window and the data used in analysis (Figure 38a-c) (Figure 38e) drawings showing examples of display.

Below, the present invention of the toxicity of the drug myocarditis myocarditis using an inspection device for inspecting the toxicity is explained as an example. Material and method
1. Cell culture
Human myocarditis ES cells purchased from the cluster derived [...] Company. Received in the green state, by collecting the silicamonolith (Vitrolife Company), non-dynamic and 20% FBS, 1% DMEM in non-essential amino acid, 1% penicillin streptomycin, 0. 2% ethanol is added GRK mercaptoamide a medium (Invitrogen purchase all Company).

2. The multi chip prepared (Day 0-3)
Next, the cluster is arranged on a chip ([...] Company). A day Day 0 and is arranged. The 200 mV or more extracellular potential (FP) Day3 1st of peak is obtained using a data acquisition in a cluster. The multi-electrode cell potential measurement data acquisition system (day Kyoto [...] Company) is used.

3. Chemical applied (after Day 4)
Before applying a chemical (called 'Before' and) data acquired for 10 minutes. The chemical de-ionized (DDW) distilled water, dissolved or DMSO, diluted with PBS. The final concn. E-4031 is 0. 1 and 1 μm μm, sotalol the DL -0. 1 mm and 1 mm, the 0 Diltiazem. 1 mm by 1 mm and is applied. The outside of the cells from data acquired by the duration of the potential (FPD) and
FPD (short term variability) STV is calculated.

4. Data analysis
The peak of a steep downward flowing Na+ FP. The K + upward of the gradual peak corresponding to the outflow. Na+ and K + FPD and the peak time. Furthermore, the variation of FPD FPD as between sphygmoscope STV of evaluation. The following equation STV.

The increase in the value of the variation between STV FPD as increase of evaluation. Due to the variation of the pulsation rate so as to suppress the influence of FPD, by using a correction formula Fredericia under, STV of correcting the FPD and FPD, cFPD, cSTV is obtained.
(RR is just in time between the peak Na+ FP)

5. 2-dimensional plot of preparation procedure
For assessing risk cardiotoxicity of drugs, extension of the extension or APD QT, STV and these are used alone. By integrating the increase and STV FPD extension for evaluation, "2-dimensional plot" manner. The X axis is more than 10% Before FPD by extending the frequency of a cluster (FPD score). The Y axis is greater than 100% STV Before the frequency of cluster (STV score). In each of the clusters among concn. applied 2, the increase in any case is recognized, "extended (FPD)" and "(STV) increases". In this plot, drug cardiotoxicity depend on the strength of the drug is plotted in a region.

6. Results
2-dimensional plot
2-dimensional plot of fig. 39 is evaluated. The plots on the right, sotalol E-4031 and DL-. The left Diltiazem, is plotted on the right lower DDW. The inhibitor IKr E-4031 and DL-sotalol, increasing the APD and STV in vivo, in vitro is known in both. E-4031 the risk is high to develop by reason of cardiotoxicity is interrupted and the drug, medicament is cardiotoxicity. Although the DL-propellant Antiarhythmia sotalol, Cytocidal arhythmia cardiotoxicity from the risk of high and considered. The Diltiazem Ca2 + channel inhibitor, in vivo decreases the APD is known, and is considered a low risk cardiotoxicity. The absence of cardiotoxicity DDW, and considered. Therefore, our obtained in the plot, the with respect to the right "high cardiotoxicity" in the figure and a region, the upper left and lower right and judged "low cardiotoxicity"

region. Evaluation method of unkonwn drug to a 2-dimensional plot when plotted according to any area, height cardiotoxicity can be evaluated.

The fig. 40 (a), an electronic circuit, a signal of the cell in order to reduce noise, and cell 10 is placed in the vicinity of the small electrode 2 of the cells are not exposed to the potential between the reference electrode 2c of the differential value of the output circuit is shown schematically. Actually, this circuit as shown in fig. 40 (b), by incorporating the first stage of the amplifier circuit, as shown in fig. 40 (c) does not depend on a noise of a specific frequency is reduced. At this time, more fine comparison electrode 2c in the vicinity of the electrode it is desirable, for example, are arranged at a distance of 50 μm sufficient noise reduction of up to 1 mm and functions as a function of the distance.

The fig. 41, of the present invention for overall evaluation method evaluating cardiotoxicity schematically shown in the example of Fig. The value of FPD biopotential measuring cell from a result obtained by adding cardiomyocyte after a specific chemical concentration, on an axis X and the value of the degree of extension of the first summing result FPD, on an axis Y and the value of the magnitude of the time fluctuation as FPD STV further [...] from above, this result is plotted. The fig. 41 (b), as the result of various gestational X-Y though illustrated as an example. As understood in the Figure, and the increase of FPD fluctuationally (STV) extension of chemicals in a small area, and there is no cardiotoxicity QT extension can be judged, and increase of the extension of the FPD is simultaneously induced fluctuationally (STV) (X-Y on the right in the drawing) when the predicting cardiotoxicity TdP or the l

The fig. 42, for measuring the actual cardiotoxicity shows one example of the configuration of a system in which a schematic diagram. The system of this embodiment, liquid feed part, cell culture measuring part, from cell analysis · stimulation.

The liquid feed part, cell culture measuring part of each cell culture chamber in the culture soln. continuously sending a syringe pump or peristaltic pump system or by HPLC pump system capable of liquid feed. Furthermore, on the outer periphery of the liquid feed portion of piping for temperature regulation for resistance heating wires are wound pipe K-type thermocouple liq. temp. minute or a thermistor or the like is continuously monitored in a heat detecting mechanism, introducing a degree of control of the liquid temperature by adjusting the resistance heating, in a constant temperature of solution is introduced. Furthermore, in this liquid feed part, for adding a chemical testing or switching of the merging pipe such as the pipe is disposed in the piping mechanism, thereby a desired concentration of chemical in each cell culture chamber can be introduced. Furthermore, the concentration of the chemical is introduced in order to quantitatively confirm, for introducing a part of the tube being optically transparent in the spectral range of 800 nm wavelength of 280 nm by measuring absorptiometry from, a quantitative evaluation it is desirable that the mechanism. Furthermore, in the same manner as part of the waste effluent pipe being optically transparent in the spectral range of 800 nm wavelength of 280 nm by measuring absorptiometry from, a quantitative evaluation it is desirable that the mechanism. As the control temperature of the chemical liquid, which is usually undesirable temperature close to a normal temperature of a human body, from the viewpoint of 45 degrees centigrade 30 degree range of temperature control is desirable.

The fig. 43, in the system of the present invention to measure cardiotoxicity cell culture measuring chamber and schematic diagram showing one example of a constitution. A plurality of cell potential measuring electrodes are arranged on the multi-electrode substrate 4201 (refer fig. 44), culture soln. · discharge mechanism is arranged for introducing a cell culture vessel 4202 is bonded, at the same time capable of measuring cell culture specimen 8 and measuring plate. The cross section of fig. 44 in cell culture measuring plate that is shown schematically, in the cell culture vessel 4202, soln. introduction port, multiple electrode substrate 4201 in sectrial closest to the bottom are arranged in a widened shape, on the other hand, the liquid discharging mechanism, to determine the liquid level of the upper liquid level height 4204 and positioned in the same direction as the direction of the developed sectrial interface.

The fig. 45, multi-electrode substrate electrode arrangement of electrodes are arranged on the wiring diagram explaining the configuration of a model. In the present invention, in order to observe the shape of cells, such as ITO transparent electrode of the electrodes are used, characteristics of the transparent electrode, an ordinary metal electrode having a higher resistance than the value since, particularly multi-electrode substrate becomes large and the plate and the like, has a very high impedance wiring becomes longer. In order to avoid this, the metal on the transparent electrode layer and the transparent electrode is arranged in the same wiring, a conductive metal electrode resistance values can be reduced. Actually, in the regions where cell, optical observation can be on a glass substrate 4301 using a transparent electrode 4302, and wiring is arranged, in the region of a cell observation does not overlap on the transparent electrode is arranged on the metal layer 4303, its upper surface is covered with the insulating film. The material used here as the metal electrode, for example, gold, platinum, titanium, copper, aluminum or the like by using a frame.

The fig. 46, shown as a schematic diagram of an example of a multi-electrode disposed on a substrate electrode. First of all, in the fig. 46 (a), which is arranged in series with the cell culture area 4404 cardiomyocyte stimulates local network and one end of the electrode 4401, which is stimulated by the stimulation of electrode for measuring the conductivity hyperstimulation cardiomyocyte and the measuring electrode 4402, for eliminating the noise reference electrode 4403 is arranged. Each of the measuring electrode 4402 cardiomyocyte obtained from a plurality of local network can be measured and the result of a response, the transmission speed of transmission between each measuring electrode fluctuationally compares the degree of poisoning can be determined by analysis. In fig. 46 (b), and is connected to the measuring electrode is straight and, in this configuration, the region of electrocardial electrocardigram waveform of the ST (ventriclerelated region) and similar waveform can be measured. In fig. 46 (c), above, separating a part of fig. 46 (b), the waveform of the local FP cardiomyocyte easily can be obtained. The fig. 46 (d), the ring-shaped electrode of the cell is arranged on a ring-shaped electrode arrangement for measuring for example. These figs. 11, 12 arranged in a ring shape as shown in the network is measured is cardiomyocyte, ring-shaped part of the measuring electrode is deficient, the portion of the forced stimulation locally for stimulating electrodes are arranged, in addition, a noise reference electrode is arranged. Further in fig. 46 (e), the measuring electrode is also divided, local cardiomyocyte response and capable of measuring arrangement.

INDUSTRIAL APPLICABILITY

According to the present invention, from about redifferentiating cardiomyocyte iPS stem cells such as cells, can be evaluated as to whether a sound its cardiomyocyte cardiomyocyte capable of screening or regeneration for use.

REFERENCE SIGNS LIST

1... transparent substrate, 2... micro electrodes, 2c... reference electrode, 2'... micro electrode 2 of lead wire, 3 1, 3 2, 3 3 and 3 4 ... agalose gel wall, 4 1, 4 2, 4 3 and 4 4 ... gap, 7... surrounding a wall, 8 1, 8 2, 8 3 ... pipe, PC... personal computer, Ms... operation signal, 10, 10 1, 10 2, 10 3, ---, 10 n ... cardiomyocyte or fibrocyte, 15... optical observation device of transparent stage, a driving device 16... X-Y, 18... Z drive, CH 1, CH 2, CH 3 and CH n ... cell holding part, CCC... cell communicating channel, 10 G ... cell group, 11 a ... barrier, 11 b ... opening, 19,191,192,193... dichroic mirror, 20,201... band pass filter, 21,211... camera, 22... source, 221... fluorescent light sources, 23,231... band pass filter, 24,241... shutter, 25... condenser lens, 26... objective lens, 27... movable electrode, 28... ground electrode, 29,291... switching circuit, 30,301... electric signal measurement circuit, 31,311... electrostimulation circuit, 32... cardiomyocyte, 33... fibrocyte, 34... cell arrangement pipette, 35... N transmission path of the first round, 36... (N+1) transmission path of the first round, 37... (N+2) transmission path of the first round, 38... measuring electrode, 39... reference electrode, 40... elecdtrolyte feedability, 41... a population of cells arranged in a ring, 42... well plate hole 96, 43... camera receiving element, 44... cell, 45... stimulatory electrode, 100... myocardal toxicity inspection device, 3000... myocardal toxicity inspection device, 3001... measuring part, 3002... for interfacing to a personal computer, 3003... analog-to-digital converter, 3004... amplifier, 4201... multi-electrode substrate, 4202... cell culture vessel, 4203... soln. flow, 4204... the height of the liquid

level, 4301... glass substrate, 4302... transparent electrode, 4303... metal layer, 4304... insulating film, 4401... stimulating electrodes, 4402... measuring electrode, 4403... reference electrode, 4404... cell culture regions.

ENGLISH CLAIMS:

A substrate,
Disposed on the substrate, a plurality of stable sphygmoscope cardiomyocyte evaluated for infusing myoelectric cells or cell population,
On the substrate so as to surround the periphery of the population of cells formed in the wall of the cell culture solution to satisfy,
The cell culture solution is supplied into the area surrounded by the wall and/or for discharging the supply/discharge channel,
One of the cells of the cell or cells in a localized portion of the electrode is placed,
The wall is surrounded by a region of a cell culture solution provided in order to satisfy the reference electrode,
The microrecessed electrode and a lead wire is connected to each of the reference electrode and a lead wire connected to the micro electrodes are placed in the measuring cell potential biopotential measuring means, and
By controlling the microshapes electrostimulation sent to the electrodes, the potential is measured by the measuring means for recording data, and a control/recording means
This device is provided with a cell,
The Electrostimulator sent to the minute interval of the electrode is changed stepwise,
1) cardiomyocyte or the interval of the cell group is caused by the forced stimulation sphygmoscope electrostimulator in response to and in the same interval of the forced stimulation sphygmoscope confirms whether the response, and, its response in the frequency range of any forced sphygmoscope irrilation corresponding by measuring whether or not, it can follow sphygmoscope sound cardiomyocyte by confirming that sufficiently satisfies one of the conditions and determine, and
2) due to the forced stimulation sphygmoscope interval (RR) electrostimulator beating of the cells to follow a frequency carpentering is confirmed, the change of the forced stimulation sphygmoscope FPD, FPD / (RR) 1/3 FPD / (RR) 1/2 from between to check that sufficient sound cardiomyocyte satisfies one of the conditions is determined and
A, cell quality evaluation device.

A substrate,
Disposed on the substrate, a plurality of stable sphygmoscope cardiomyocyte evaluated for infusing myoelectric cells or cell population,
On the substrate so as to surround the periphery of the population of cells formed in the wall of the cell culture solution to satisfy,
The cell culture solution is supplied into the area surrounded by the wall and/or for discharging the supply/discharge channel,
One of the cells of the cell or cells in a localized portion of the electrode is placed,
The wall is surrounded by a region of a cell culture solution provided in order to satisfy the reference electrode,
The microrecessed electrode and a lead wire is connected to each of the reference electrode and a lead wire connected to the micro electrodes are placed in the measuring cell potential biopotential measuring means, and
By controlling the microshapes electrostimulation sent to the electrodes, the potential is measured by the measuring means for recording data, and a control/recording means
The device is provided with a cell,
The Electrostimulator sent to the minute interval of the electrode is changed stepwise,
1) cardiomyocyte or the interval of the cell group is caused by the forced stimulation sphygmoscope electrostimulator in response to and in the same interval of the forced stimulation sphygmoscope confirms whether the response, and, its response in the frequency range of any forced sphygmoscope irrilation corresponding by measuring whether or not, it can follow sphygmoscope sound cardiomyocyte by confirming that sufficiently satisfies one of the conditions and determine, and
2) due to the interval (RR) forced sphygmoscope electrostimulator irritating beating of the cells to follow a

frequency carpentering is confirmed, the forced stimulation sphygmoscope · potential field · duration (FPD) change, FPD / (RR) 1/3 from FPD / (RR) 1/2 between sound by confirming that a sufficient cardiomyocyte satisfies one of the conditions and determining
Contg., cell quality evaluation method.

· Myocarditis sphygmoscope fluctuationally of field of the magnitude of the potential waveform, adjacent sphygmoscope by comparing the signal is measured, the inspection device myocarditis toxicity.

· Potassium ions out of the field of the potential waveform peak position of sodium ions from flowing out of the outflow of the elapsed time from the peak magnitude of the fluctuation of ([...] : FPD) which, by comparing the adjacent sphygmoscope signal is measured, the inspection device described in the claim 3 myocarditis of toxicity.

The magnitude of the fluctuation of the FPD and FPD is calculated, the calculated magnitude of the FPD fluctuationally of FPD and combination of the index and so, inspection device described in claim 3 or 4 myocarditis of toxicity.

The magnitude of the fluctuation of the short-term variation (STV) of FPD FPD, inspection device described in any one of claims 3-5 myocarditis toxicity.

A transparent substrate,
The formed on the transparent substrate in a region surrounded by a wall, the area is filled with a liquid cell culture,
The cell is filled with liquid are arranged in a region to perform a plurality of stable sphygmoscope contg. cardiomyocyte cell population,
The cell is filled with a liquid that is provided within the, or one of the cells of the cell group cardiomyocyte local part of the electrode is placed,
The cell is filled with a liquid that is provided within the reference electrode,
The microrecessed electrode and a lead wire is connected to each of the reference electrode and a lead wire connected to the micro electrodes are placed to measure the potential of cardiomyocyte biopotential measuring means,
The potential is measured by the measuring means to the test drug subscriber before a recording means for recording data potential cardiomyocyte
Having, described in any one of claims 3-6 myocarditis toxicity.

To perform the stable sphygmoscope cardiomyocyte of cell group including, a plurality of which can transmit a sphygmoscope cardiomyocyte Osteoblast fibrosing and including a cell network, as described in either of claims 3-7 myocarditis toxicity an item.

A transparent substrate,
The transparent substrate is disposed on a plurality of stable sphygmoscope contg. cardiomyocyte pacemaker region comprising a cell population,
Disposed on the transparent substrate, and the cell group, the cell group sphygmoscope cooperate to transmit a plurality of series and fibrocyte cardiomyocyte contg. cell network,
On the transparent substrate and the cells in the cell network which is formed so as to surround a periphery of the cell culture solution for satisfying the wall,
The cell culture solution is supplied into the area surrounded by the wall and/or for discharging the supply/discharge channel,
The cell with the cell culture liquid chemical action applied to the chemical feed channel,
One of the cells of the cell or cells in a localized portion of the electrode is placed,
Each of the cells of the cell network of micro electrodes are placed,
The wall is surrounded by a region of a cell culture solution provided in order to satisfy the reference electrode,
The microrecessed electrode and a lead wire is connected to each of the reference electrode and a lead wire connected to the micro electrodes are placed in the measuring cell potential biopotential measuring means, and
By controlling the microshapes electrostimulation sent to the electrodes, the potential is measured by the measuring means for recording data, and a control/recording means

Having, described in either of claims 3-8 myocarditis toxicity of an item.

To perform the stable sphygmoscope cardiomyocyte including at least one cell is placed from the very minute electrode 1 is placed on the electrodes in the cell at a constant interval can be stimulated sphygmoscope forcibly so as, described in any one of claims 3-9 myocarditis toxicity.

The series arrangement at the end points of local cell network for applying a stimulus to the electrode is arranged, an item as described in either of claims 8-10 cardiotoxicity inspection device.

The cell network is, arranged in a ring shape is formed,
The part of the ring, the point local stimulates the electrodes are disposed, an item as described in either of claims 8-11 cardiotoxicity inspection device.

The cell is placed on each of the micro electrodes myocarditis sphygmoscope acquired from the magnitude of the potential waveform fluctuationally · field, the cells of the cell network is generated sphygmoscope observed from the transmission time of the electrodes to the adjacent sphygmoscope fluctuationally or transmission speed by comparing the measured, an item as described in either claim 8-12 myocarditis toxicity.

To maintain the proper temperature cardiomyocyte having a temperature control mechanism described in any one of claims 1-13 myocarditis toxicity.

The micro electrodes, constituting the electrode array from a plurality of micro electrodes, an inspection device described in any one of claims 1-14 myocarditis toxicity.

· Myocarditis sphygmoscope fluctuationally of field of the magnitude of the potential waveform, comparison of the signals measured by the adjacent sphygmoscope ~~~ myocarditis toxicity and an inspection method.

A plurality of stable sphygmoscope cardiomyocyte performs a process for preparing a cell group including,
In the multi-electrode array is arranged on a bottom surface of the container in a process of the cell group,
The culture test drug is added to the cell group, the multi-electrode array of cells cultured cells in the process in which the potential is measured, in addition of the drug, by measuring cell potential of the cultured cells, the cell potential of a process for acquiring data, and
From the data acquired by the cell potential, the potential (FP) · field of sodium ion waveform peak of inflow to outflow of potassium ions of the elapsed time from the peak to the cell (cell potential duration) of the FPD (FPD) and calculates the magnitude of fluctuations, the combination of the FPD FPD and as an index of the magnitude of fluctuations of the medicament by using a process of evaluating the toxicity myocarditis, Contg., described in claim 16 myocarditis toxicity of the drug.

An item as described in either of claims 8-15 myocarditis toxicity inspection device is used, the cell with the cell culture liquid chemical is acting upon, the occurrence of the sphygmoscope cardiomyocyte population of cell network to delay the propagation speed of the cells to evaluate whether the chemical action of the cell toxicity to claim 16 or 17 cardiomyocyte for inspecting an inspection method described myocardal toxicity.

An item as described in either of claims 8-15 myocarditis the cell toxicity test device acting on the chemical agent when a cell culture solution, the occurrence of the sphygmoscope cardiomyocyte population of cell network to delay the propagation speed of the cells to evaluate whether acting on the cell toxicity to cardiomyocyte of chemicals in the method for inspecting, at least one of the following claim 16-18 1 includes a step of the method described in any one of an item:
(I) of the cell, the cell network for placing at least one micro-electrode 1 of the electrode from the cell is placed on a constant interval by forced sphygmoscope stimulation process;
(Ii) placing the cells each micro-electrode of the field · myocarditis sphygmoscope acquired from the magnitude of the fluctuation of the potential waveform, comparison of the signals measured by the adjacent sphygmoscope process;
Among the potential waveform (iii) · field of potassium sodium ions flowing out from the outflow of the peak position of the elapsed time from the peak magnitude of the fluctuation of ([...]), comparison of the signals measured by the adjacent sphygmoscope process;

(Iv) the cell is placed on each of the micro electrodes myocarditis sphygmoscope acquired from the magnitude of the potential waveform fluctuationally · field, the cells of the cell network is generated sphygmoscope observed from the transmission time of the electrodes to the adjacent sphygmoscope fluctuationally or transmission speed by comparing the measured process;
(V) each of the cells is acquired from a small electrode strading myocarditis sphygmoscope · field for each of the potential waveform is differentiated cell potential process;
(Vi) each electrode of the cell is placed on the field is obtained from the potential waveform myocarditis sphygmoscope · · synthetic field is overlapped by the potential waveform, and comparing the data as a whole electrocardigram a population of cells for measuring the degree of turbulence of the transmitting sphygmoscope process;
The arrangement of the cells (vii) a small electrode potential is kept constant by using a feedback control mechanism, the power level control mechanism is a process for analyzing a current amount to be supplied; and
The cells (viii) after the start of the forced stimulation sphygmoscope periodically, from 30 seconds or more to start measuring process.

The magnitude of the fluctuation of the FPD FPD by short term fluctuations (STV), as described in either of claims 16-19 an item.

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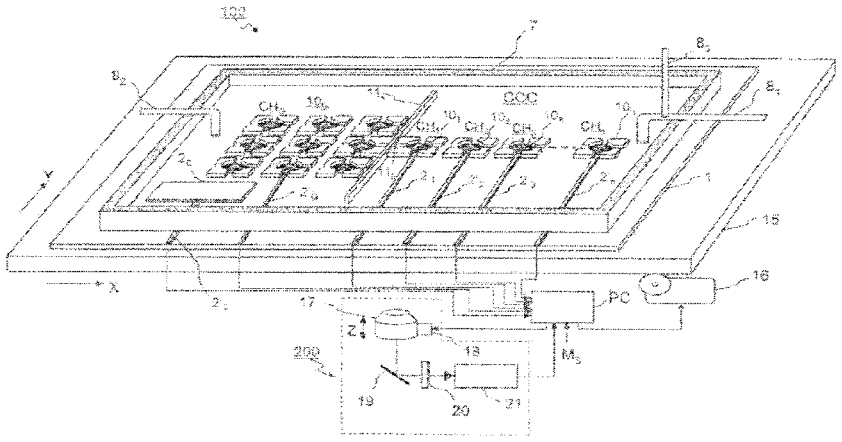
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(54) **Title**: METHOD AND DEVICE FOR MYOCARDIAL CELL EVALUATION AND MYOCARDIAL TOXICITY INSPECTION

(54) 発明の名称 : 心筋毒性検査および心筋細胞評価のための方法および装置

[図1]



(57) **Abstract**: In the present invention, a myocardial cell cluster is disposed on a transparent substrate, and the quality of the myocardial cells is evaluated from the response of the cells to a forced pulsation stimulus applied to the cardiomyocytes. The myocardial cell cluster is disposed on the transparent substrate, and is exposed to the flow of a liquid containing a drug in a manner so that the drug acts on the cells, which configure a network. The extent of cardiac toxicity resulting from the drug is evaluated from measuring the fluctuations obtained from a comparison of adjacent cardimyocytes of the network.

(57) 要約: 透明基板上に心筋細胞集団を配置し、心筋拍動細胞に与えた強制拍動刺激に対する心筋細胞の応答から心筋細胞の品質を評価する。透明基板上に心筋細胞集団を配置し、ネットワークを構成する細胞には薬物が作用するように薬物を含む液体の流れに曝す。ネットワークの隣接する心筋拍動細胞の比較から得られるゆらぎ計測から薬物による心毒性の程度を評価する。

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明　細　書

発明の名称 ：

心筋毒性検査および心筋細胞評価のための方法および装置

技術分野

[0001] 本発明は、心筋毒性検査および心筋細胞評価のための方法および装置に関する。

背景技術

[0002] 細胞の状態の変化や、細胞の薬物等に対する応答を観察するのに多用されているのはバイオアッセイである。従来のバイオアッセイでは、一般的に培養細胞を用いることが多い。この系では複数の細胞を用いてアッセイを行うので、細胞集団の値の平均値をあたかも１細胞の特性であるかの様に観察してきた。

[0003] しかし、実際には細胞は集団の中で細胞周期が同調しているものはまれであり、各々の細胞が異なった周期でタンパク質を発現している。このため、刺激に対する応答の結果を解析するときにゆらぎの問題が常に付きまとう。

[0004] すなわち、細胞の反応機構自体が普遍的に持つ応答のゆらぎが存在するために、常に、平均的なレスポンスしか得ることができない。これらの問題を解決するために、同調培養等の手法が開発されているが、常に同じステージにある細胞群を使用することは、常にそのような細胞を供給し続けなければならないということで、バイオアッセイを広く一般に広める障害となっている。

[0005] また、細胞に対する刺激（シグナル）は、細胞周辺の溶液に含まれるシグナル物質、栄養、溶存気体の量によって与えられるものと、他の細胞との物理的接触・細胞間インタラクションによるものの２種類があることからも、ゆらぎについての判断が難しいのが実情であった。

[0006] 細胞の物理的接触・細胞間インタラクションの問題は、バイオアッセイを組織断片のような細胞塊で行うことである程度解決できる。しかし、この場

合、培養細胞と異なり、常に均一な素性の細胞塊を得ることができない。そのため、得られるデータがばらついたり、集団の中に情報が埋もれてしまったりする問題がある。

[0007] 細胞群の細胞の１つ１つを最小構成単位とする情報処理モデルの計測のために、本願の発明者らは特開２００６－９４７０３（特許文献１）等に示すように、細胞を特定の空間配置の中に閉じ込めておくための複数の細胞培養区画を構成し、隣接する区画間は細胞の通り抜けることができない溝またはトンネルでお互いを連結するとともに、必要に応じて、溝またはトンネルあるいは細胞培養区画に、細胞の電位変化を計測するための複数の電極パターンを持つ構造の集合細胞マイクロアレー（バイオアッセイチップ）を提案した。

[0008] また、心臓の複雑な機能を反映して取得される心電図の評価法として、通常の非線形ダイナミクス計測に使われている手法を応用して心電図解析をする手法が提案されている。例えば、もっとも一般に使われている心電図解析はポワンカレプロッティング法である（非特許文献１）。プロット中の1点は、隣接する２つの拍動データの情報を示しており例えばＸ軸にある時点の拍動レートを、Ｙ軸に1つ前の拍動レートを示すこととなる。これによって心臓の拍動のゆらぎについてグラフ上の点の分布を定量化することによって見積もるものである。その他の心臓の拍動のゆらぎを計測する手法としては、相関次元法（correlation dimension）、非線形予測法（nonlinear predictability）（非特許文献２）、近似エントロピー法（非特許文献３）などがある。

先行技術文献

特許文献

[0009] 特許文献1：特開２００６－９４７０３号公報

非特許文献

[0010] 非特許文献1：Brennan M,Palaniswami M, Kamen P. Do existing measures of Poincare plot geometry reflect non-linear features of heart rate va

riability? Biomedical Engineering, IEEE Transactions on, Proc. IEEE Transactions on Biomedical Engineering, 2001, 48, 1342-1347
非特許文献2：Kanters JK, Holstein-Rathlou NH, Agner E (1994). "Lack of evidence for low-dimensional chaos in heart rate variability". Journal of Cardiovascular Electrophysiology 5 (7): 591-601.PMID 7987529.
非特許文献3：Storella RJ, Wood HW, Mills KM et al. (1994). "Approximate entropy and point correlation dimension of heart rate variability in healthy subjects". Integrative Physiological & Behavioral Science 33 (4): 315-20.PMID 10333974.

発明の概要

発明が解決しようとする課題

[0011] 従来のバイオアッセイでは、細胞を組織断片として扱うか、培養細胞のように１細胞として扱うかのいずれかであった。細胞の数が多すぎると上記従来技術の項で述べたように、得られる情報が平均的なものになってしまい、薬剤などに対するレスポンスが正確に得られない問題がある。細胞を１細胞ずつ用いる場合は、本来、多細胞組織の細胞として機能している細胞を、引き離された独立した状態の細胞として使用するために、細胞同士のインタラクションの影響が現れなくなることとなり、やはり正確な薬剤レスポンスすなわちバイオアッセイデータを得る上で問題がある。

[0012] 心筋細胞および線維芽細胞について見ると隣接する心筋細胞あるいは線維芽細胞からの拍動の伝播が、１細胞単位で細胞電位、細胞形態が正確に計測できるとともに、心筋細胞に対する薬物の毒性検査が１細胞の細胞電位、細胞形態として正確に計測できるデバイスやシステムを開発することが重要である。

[0013] ヒトiPS細胞あるいはヒトES細胞などのヒト幹細胞から分化誘導した心筋細胞について、これらの細胞を創薬スクリーニングあるいは再生医療に使うためには、この心筋細胞が、ヒト心臓にある心筋細胞と同じ品質であるかどうかを細胞の機能面から定量的に評価する必要がある。

課題を解決するための手段

[0014] 上記課題に鑑み、本発明は、以下の装置および方法を提供する。

（１）基板、

該基板上に配置した複数個の安定した拍動を行う評価対象の心筋細胞あるいは該心筋細胞を含む細胞集団、

上記基板上に上記細胞集団の周辺を囲むように形成された細胞培養液を満たすための壁、

上記細胞培養液を上記壁で囲われた領域内に供給しおよび／または排出する培養液供給／排出チャネル、

上記細胞集団の一つの細胞あるいは細胞集団の局所部分を載置している微小電極、

上記壁で囲われた細胞培養液を満たすための領域内に設けられた比較電極、

上記微小電極のそれぞれに接続された引き出し線と上記比較電極に接続された引き出し線とを用いて上記微小電極に載置されている細胞電位を計測する電位計測手段、ならびに

上記微小電極へ送る電気刺激を制御し、上記電位計測手段で計測した電位データを記録する、制御／記録手段

を備えた細胞品質評価装置であって、

上記微小電極へ送る上記電気刺激のインターバルを段階的に変化させて、

１）上記心筋細胞あるいは上記細胞集団が上記電気刺激のインターバルによる強制拍動刺激に応答して上記強制拍動刺激と同じインターバルで応答するかどうかを確認し、かつ、その応答がどの強制拍動刺激の周波数の範囲で対応するかどうかを計測し、拍動が追従できることを確認することで健全な心筋細胞である十分条件の一つを満たしていると判断すること、および

２）上記電気刺激による強制拍動刺激インターバル（ＲＲ）に対して細胞の拍動の追従が確認できた周波数の範疇で、強制拍動刺激に対するＦＰＤの変化が、ＦＰＤ／（ＲＲ）$^{1/3}$からＦＰＤ／（ＲＲ）$^{1/2}$の間にあることを確認する

ことで健全な心筋細胞である十分条件の一つを満たしていると判断することを可能にする、細胞品質評価装置。

（２）基板、

該基板上に配置された、安定した拍動を行う評価対象の心筋細胞を保持するための複数の細胞保持部を含む心筋細胞集団保持領域、

上記基板表面と、上記心筋細胞集団保持領域の周囲に形成された壁とによって規定される、細胞培養液を満たすための空間領域、

上記細胞培養液を上記空間領域内に供給しおよび／または排出するための培養液供給／排出チャネル、

上記透明基板上に設けられ、上記心筋細胞集団保持領域の細胞保持部の一つまたは一部の細胞保持部において心筋細胞を載置するための微小電極、

上記細胞培養液を満たすための上記空間領域内に設けられた比較電極、

上記微小電極のそれぞれに接続された引き出し線と上記比較電極に接続された引き出し線とを用いて上記微小電極に載置されている細胞電位を計測する電位計測手段、ならびに

上記微小電極へ送る電気刺激を制御し、上記電位計測手段で計測した電位データを記録する、制御／記録手段

を備えた細胞品質評価装置であって、

上記微小電極へ送る上記電気刺激のインターバルを段階的に変化させて、

１）上記心筋細胞あるいは上記細胞集団が上記電気刺激のインターバルによる強制拍動刺激に応答して上記強制拍動刺激と同じインターバルで応答するかどうかを確認し、かつ、その応答がどの強制拍動刺激の周波数の範囲で対応するかどうかを計測し、拍動が追従できることを確認することで健全な心筋細胞である十分条件の一つを満たしていると判断すること、および

２）上記電気刺激による強制拍動刺激インターバル（ＲＲ）に対して細胞の拍動の追従が確認できた周波数の範疇で、強制拍動刺激に対するＦＰＤの変化が、ＦＰＤ／（ＲＲ）$^{1/3}$からＦＰＤ／（ＲＲ）$^{1/2}$の間にあることを確認することで健全な心筋細胞である十分条件の一つを満たしていると判断すること

を可能にする、心筋品質評価装置。

（３）基板、

該基板上に配置した複数個の安定した拍動を行う評価対象の心筋細胞あるいは該心筋細胞を含む細胞集団、

上記基板上に上記細胞集団の周辺を囲むように形成された細胞培養液を満たすための壁、

上記細胞培養液を上記壁で囲われた領域内に供給しおよび／または排出する培養液供給／排出チャネル、

上記細胞集団の一つの細胞あるいは細胞集団の局所部分を載置している微小電極、

上記壁で囲われた細胞培養液を満たすための領域内に設けられた比較電極、

上記微小電極のそれぞれに接続された引き出し線と上記比較電極に接続された引き出し線とを用いて上記微小電極に載置されている細胞電位を計測する電位計測手段、ならびに

上記微小電極へ送る電気刺激を制御し、上記電位計測手段で計測した電位データを記録する、制御／記録手段

を備えた細胞品質評価装置を用いて、

上記微小電極へ送る上記電気刺激のインターバルを段階的に変化させて、

１）上記心筋細胞あるいは上記細胞集団が上記電気刺激のインターバルによる強制拍動刺激に応答して上記強制拍動刺激と同じインターバルで応答するかどうかを確認し、かつ、その応答がどの強制拍動刺激の周波数の範囲で対応するかどうかを計測し、拍動が追従できることを確認することで健全な心筋細胞である十分条件の一つを満たしていると判断すること、および

２）上記電気刺激による強制拍動刺激インターバル（ＲＲ）に対して上記細胞の拍動の追従が確認できた周波数の範疇で、上記強制拍動刺激に対するフィールド・ポテンシャル・デュレーション（ＦＰＤ）の変化が、ＦＰＤ／（ＲＲ）$^{1/3}$からＦＰＤ／（ＲＲ）$^{1/2}$の間にあることを確認することで健全な心筋

細胞である十分条件の一つを満たしていると判断すること
を含む、細胞品質評価方法。
（４）基板、
該基板上に配置された、安定した拍動を行う評価対象の心筋細胞を保持するための複数の細胞保持部を含む心筋細胞集団保持領域、
上記基板表面と、上記心筋細胞集団保持領域の周囲に形成された壁とによって規定される、細胞培養液を満たすための空間領域、
上記細胞培養液を上記空間領域内に供給しおよび／または排出するための培養液供給／排出チャネル、
上記透明基板上に設けられ、上記心筋細胞集団保持領域の細胞保持部の一つまたは一部の細胞保持部において心筋細胞を載置するための微小電極、
上記細胞培養液を満たすための上記空間領域内に設けられた比較電極、
上記微小電極のそれぞれに接続された引き出し線と上記比較電極に接続された引き出し線とを用いて上記微小電極に載置されている細胞電位を計測する電位計測手段、ならびに
上記微小電極へ送る電気刺激を制御し、上記電位計測手段で計測した電位データを記録する、制御／記録手段
を備えた細胞品質評価装置を用いて、
上記微小電極へ送る上記電気刺激のインターバルを段階的に変化させて、
１）上記心筋細胞あるいは上記細胞集団が上記電気刺激のインターバルによる強制拍動刺激に応答して上記強制拍動刺激と同じインターバルで応答するかどうかを確認し、かつ、その応答がどの強制拍動刺激の周波数の範囲で対応するかどうかを計測し、拍動が追従できることを確認することで健全な心筋細胞である十分条件の一つを満たしていると判断すること、および
２）上記電気刺激による強制拍動刺激インターバル（ＲＲ）に対して細胞の拍動の追従が確認できた周波数の範疇で、強制拍動刺激に対するＦＰＤの変化が、ＦＰＤ／（ＲＲ）$^{1/3}$からＦＰＤ／（ＲＲ）$^{1/2}$の間にあることを確認することで健全な心筋細胞である十分条件の一つを満たしていると判断すること

を含む、心筋品質評価方法。

[0015] 本発明はまた、以下の心筋毒性検査装置、心筋毒性検査チップ、および心筋毒性検査方法を提供する。

［１］透明基板、

該透明基板上に配置した複数個の安定した拍動を行う心筋細胞を含む細胞集団、

該透明基板上に配置され、該細胞集団と連携して上記細胞集団の拍動を伝える複数個の直列配列された心筋細胞および線維芽細胞を含む細胞ネットワーク、

上記透明基板上に上記細胞集団および上記細胞ネットワークの周辺を囲むように形成された細胞培養液を満たすための壁、

上記細胞培養液を上記壁で囲われた領域内に供給しおよび／または排出する培養液供給／排出チャネル、

上記細胞に作用する薬剤を上記細胞培養液に加えるための薬剤供給チャネル、

上記細胞集団の一つの細胞あるいは細胞集団の局所部分を載置している微小電極、

上記細胞ネットワークの個々の細胞をそれぞれ載置している微小電極、

上記壁で囲われた細胞培養液を満たすための領域内に設けられた比較電極、

上記微小電極のそれぞれに接続された引き出し線と上記比較電極に接続された引き出し線とを用いて上記微小電極に載置されている細胞電位を計測する電位計測手段、ならびに

上記微小電極へ送る電気刺激を制御し、上記電位計測手段で計測した電位データを記録する、制御／記録手段

を備える、心筋毒性検査装置。

［２］透明基板、

該透明基板上に配置された、安定した拍動を行う心筋細胞を保持するため

の複数の細胞保持部（CH_G）を含む心筋細胞集団保持領域、

上記細胞保持部の１細胞と連携して上記心筋細胞集団の拍動を伝える細胞を保持するための、直列配列された複数の細胞保持部（CH_n）を含む細胞ネットワーク領域であって、該細胞保持部（CH_n）が心筋細胞または線維芽細胞を保持する、細胞ネットワーク領域、

上記透明基板表面と、上記心筋細胞集団保持領域および上記細胞ネットワーク領域の周囲に形成された壁とによって規定される、細胞培養液を満たすための空間領域、

上記細胞培養液を上記空間領域内に供給しおよび／または排出するための培養液供給／排出チャネル、

上記細胞に作用する薬剤を上記細胞培養液に加えるための薬剤供給チャネル、

上記透明基板上に設けられ、上記心筋細胞集団保持領域の細胞保持部（CH_G）の一つまたは一部の細胞保持部において心筋細胞を載置するための微小電極、

上記透明基板上に設けられ、上記細胞ネットワーク領域の複数の細胞保持部（CH_n）において個々の細胞を載置するための複数の微小電極、

上記細胞培養液を満たすための上記空間領域内に設けられた比較電極、

上記微小電極のそれぞれに接続された引き出し線と上記比較電極に接続された引き出し線とを用いて上記微小電極に載置されている細胞電位を計測する電位計測手段、ならびに

上記微小電極へ送る電気刺激を制御し、上記電位計測手段で計測した電位データを記録する、制御／記録手段

を備える、心筋毒性検査装置。

［３］上記細胞ネットワークの上記細胞を載置するための少なくとも１つの微小電極から該微小電極上に載置される細胞に一定のインターバルで強制拍動刺激を与えることができるように構成されている、上記［１］または［２］記載の心筋毒性検査装置。

［４］上記細胞が載置された各微小電極から取得される心筋拍動のフィールド・ポテンシャル波形のゆらぎの大きさを、隣接する拍動信号の比較によって計測するように構成されている、上記［１］～［３］のいずれか一項記載の心筋毒性検査装置。

［５］フィールド・ポテンシャル波形のうちカリウムイオンの細胞からの流出のピーク位置のナトリウムイオン流出ピークからの経過時間（フィールドポテンシャルデュレーション）のゆらぎの大きさを、隣接する拍動信号の比較によって計測するように構成されている、上記［１］～［３］のいずれか一項記載の心筋毒性検査装置。

［６］上記細胞が載置された各微小電極から取得される心筋拍動のフィールド・ポテンシャル波形のゆらぎの大きさを、上記細胞ネットワークの上記細胞の拍動が発生する領域から観察電極までの伝達時間あるいは伝達速度のゆらぎを隣接する拍動の比較によって計測するように構成されている、上記［１］～［３］のいずれか一項記載の心筋毒性検査装置。

［７］上記細胞が載った各微小電極から取得される心筋拍動のフィールド・ポテンシャル波形の各々について微分することによって細胞電位を計測するように構成されている、上記［１］～［３］のいずれか一項記載の心筋毒性検査装置。

［８］上記細胞が載置された各電極から取得される心筋拍動のフィールド・ポテンシャル波形を重ね合わせて合成フィールド・ポテンシャル波形を合成し、心電図データと比較可能な細胞集団全体としての拍動伝達の乱れ具合を計測するように構成されている、上記［１］～［３］のいずれか一項記載の心筋毒性検査装置。

［９］上記細胞の配置された微小電極の電位を一定に保つフィードバック電位制御機構を備え、該電位制御機構が供給する電流量を解析するように構成されている、上記［１］～［３］のいずれか一項記載の心筋毒性検査装置。

［１０］上記細胞への周期的強制拍動刺激開始後、３０秒以上経過してから計測を開始するように構成されている、上記［１］～［３］のいずれか一項

記載の心筋毒性検査装置。

［１１］上記透明基板上に配置した上記細胞の状態を光学的に計測するための光学系および光学カメラをさらに備え、

上記光学カメラで取得された画像のデータが上記制御／記録手段に記録される、上記［１］～［１０］のいずれか一項記載の心筋毒性検査装置。

［１２］上記細胞保持部（CH_G，CH_n）の各々が、上記透明基板上に設けられた細胞非接着性の壁で囲まれた空間として規定され、該壁が上記細胞が通り抜けることの出来ない１以上の間隙を有する、上記［２］～［１１］のいずれか一項記載の心筋毒性検査装置。

［１３］上記心筋細胞集団保持領域と上記細胞ネットワーク領域との間に、上記細胞培養液の流れを阻害するとともに上記心筋細胞集団保持領域の複数の細胞保持部（CH_G）のうち一つに収容された細胞と上記細胞ネットワーク領域の端部の細胞保持部（CH_n）の細胞とが連携するための開口部を有する障壁が設けられた、上記［２］～［１２］のいずれか一項記載の心筋毒性検査装置。

［１４］透明基板、

該透明基板上に配置された複数個の細胞保持部（CH_G）を含む心筋細胞集団保持領域、

該心筋細胞集団保持領域の細胞保持部（CH_G）の１細胞と連携して上記心筋細胞集団の拍動を伝える複数個の直列配列された細胞保持部（CH_n）を含む細胞ネットワーク領域であって、該細胞保持部（CH_n）が心筋細胞または線維芽細胞を保持する、細胞ネットワーク領域、

上記透明基板表面と、上記心筋細胞集団保持領域および上記細胞ネットワーク領域の周囲に形成された壁とによって規定される、細胞培養液を満たすための空間領域、

上記細胞培養液を上記壁で囲われた空間領域内に供給しおよび／または排出するための培養液供給／排出手段、

上記細胞に作用する薬剤を上記細胞培養液に加えるための薬剤供給手段、

上記透明基板上に設けられ、上記心筋細胞集団保持領域の細胞保持部（CH_G）の一つにおいて心筋を載置している微小電極、

上記透明基板上に設けられ、上記細胞ネットワーク領域の複数の細胞保持部（CH_n）において上記心筋細胞または線維芽細胞を載置している複数の微小電極、

上記壁で囲われた領域内に設けられた比較電極、

上記微小電極のそれぞれに接続された引き出し線と上記比較電極に接続された引き出し線とを用いて上記微小電極に載置されている細胞電位を計測し記録する手段、

Ｘ－Ｙ方向に駆動されるとともに上記透明基板を載置するステージ、ならびに

上記ステージ上に載置された上記透明基板上の細胞の状態を光学的に計測する手段、

を備える、心筋毒性検査装置。

［１５］透明基板、

該透明基板上に配置された、心筋細胞を保持するための複数個の細胞保持部（CH_G）を含む心筋細胞集団保持領域、

該細胞集団の１細胞と連携して上記心筋細胞集団の拍動を伝えるための、直列配列された複数個の細胞保持部（CH_n）を含む細胞ネットワーク領域であって、該細胞保持部（CH_n）が心筋細胞または線維芽細胞を保持する、細胞ネットワーク領域、

上記透明基板表面と、上記心筋細胞集団保持領域および上記細胞ネットワーク領域の周囲に形成された壁とによって規定される、細胞培養液を満たすための領域、

上記透明基板上に設けられ、上記心筋細胞集団保持領域の細胞保持部（CH_G）の一つにおいて心筋細胞を載置している微小電極、

上記透明基板上に設けられ、上記細胞ネットワーク領域の複数の細胞保持部（CH_n）において上記心筋細胞または線維芽細胞を載置している複数の微

小電極、

上記壁で囲われた領域内に設けられた比較電極、および

上記微小電極のそれぞれに接続された引き出し線と上記比較電極に接続された引き出し線、

を備える、心筋毒性検査チップ。

［１６］上記［１］～［１４］のいずれか一項記載の心筋毒性検査装置または上記［１５］に記載の心筋毒性検査チップを使用して、上記細胞に作用する薬剤が上記細胞培養液に加えられたときに、上記心筋細胞集団の発生する拍動が上記細胞ネットワーク領域の上記細胞を伝播する速度を遅延させるか否かを評価して上記細胞に作用する薬剤の心筋細胞に対する毒性を検査する心筋毒性検査方法。

［１７］上記［１］または［２］記載の心筋毒性検査装置を用いて前記細胞に作用する薬剤が前記細胞培養液に加えられたときに、前記心筋細胞集団の発生する拍動が前記細胞ネットワーク領域の前記細胞を伝播する速度を遅延させるか否かを評価して前記細胞に作用する薬剤の心筋細胞に対する毒性を検査する方法であって、下記のうちの少なくとも１つの工程を含む方法：

(i)上記細胞ネットワークの上記細胞を載置するための少なくとも１つの微小電極から該微小電極上に載置される細胞に一定のインターバルで強制拍動刺激を与える工程；

(ii)上記細胞が載置された各微小電極から取得される心筋拍動のフィールド・ポテンシャル波形のゆらぎの大きさを、隣接する拍動信号の比較によって計測する工程；

(iii)フィールド・ポテンシャル波形のうちカリウムイオンの細胞からの流出のピーク位置のナトリウムイオン流出ピークからの経過時間（フィールドポテンシャルデュレーション）のゆらぎの大きさを、隣接する拍動信号の比較によって計測する工程；

(iv)上記細胞が載置された各微小電極から取得される心筋拍動のフィールド・ポテンシャル波形のゆらぎの大きさを、上記細胞ネットワークの上記細

胞の拍動が発生する領域から観察電極までの伝達時間あるいは伝達速度のゆらぎを隣接する拍動の比較によって計測する工程；

(v)上記細胞が載った各微小電極から取得される心筋拍動のフィールド・ポテンシャル波形の各々について微分することによって細胞電位を計測する工程；

(vi)上記細胞が載置された各電極から取得される心筋拍動のフィールド・ポテンシャル波形を重ね合わせて合成フィールド・ポテンシャル波形を合成し、心電図データと比較可能な細胞集団全体としての拍動伝達の乱れ具合を計測する工程；

(vii)上記細胞の配置された微小電極の電位を一定に保つフィードバック電位制御機構を用いて、該電位制御機構が供給する電流量を解析する工程；および

(viii)上記細胞への周期的強制拍動刺激開始後、３０秒以上経過してから計測を開始する工程。

［１８］上記微小電極の近傍に配置された、細胞電位計測におけるノイズ除去のための差分用の参照電極をさらに備える、上記［１］～［１４］のいずれか一項記載の心筋毒性検査装置。

［１９］上記微小電極が、刺激電極および計測電極からなる、上記［１８］記載の心筋毒性検査装置。

［２０］心筋細胞が培養されている心筋細胞培養計測領域に、薬剤を連続して底面から導入して上方の液面から排出する機構と、その導入する薬剤の温度をモニターして適切な温度に維持する機構と、導入される薬液の濃度を計測するために光学的に透明な領域を持つ管と、波長２８０ナノメートルから８００ナノメートルの範囲で吸光分光定量計測が可能である上記［１９］記載の心毒性検査装置。

［２１］上記微小電極が透明である、上記［１］～［１４］、［１８］～［２０］のいずれか一項に記載の心筋毒性検査装置。

［２２］上記微小電極の抵抗値を減少させるために、光学系により観察する

領域以外の領域に配された透明電極配線上に金属レイヤーを付加してある、上記［２１］記載の心毒性検査装置［２３］培養されている上記複数の心筋細胞が上記基板上に直線状に配置されて心筋細胞ネットワークを形成しており、

上記直線状心筋細胞ネットワークの端点に局所に刺激を与えるための電極が配置されている、上記［１］～［１４］、［１８］～［２２］のいずれか一項記載の心毒性検査装置。

［２４］さらに、上記直線状心筋細胞ネットワークの過半数の心筋細胞が載ることができる１つの直線状の計測電極を備える、上記［２３］記載の心毒性検査装置。

［２５］培養されている上記複数の心筋細胞が上記基板上にリング状に配置されて心筋細胞ネットワークを形成しており、

上記リング状心筋細胞ネットワークのリングの一部を欠損させて、その点に局所に刺激を与える電極が配置されている、上記［１］～［１４］、［１８］～［２２］のいずれか一項記載の心毒性検査装置。

［２６］さらに、上記リング状心筋細胞ネットワークの過半数の心筋細胞が載ることができる１つのリング状の計測電極を備える、上記［２５］記載の心毒性検査装置。

［２７］上記［１］～［２６］のいずれか一項記載の心筋毒性検査装置を使用して、上記細胞に作用する薬剤が上記細胞培養液に加えられたときに、上記心筋細胞集団の発生する拍動が上記細胞ネットワーク領域の上記細胞を伝播する速度を遅延させるか否かを評価して上記細胞に作用する薬剤の心筋細胞に対する毒性を検査する心筋毒性検査方法。

［２８］上記［１］～［１４］、［１８］～［２６］のいずれか一項記載の心筋毒性検査装置を用いて上記細胞に作用する薬剤が上記細胞培養液に加えられたときに、上記心筋細胞集団の発生する拍動が上記細胞ネットワーク領域の上記細胞を伝播する速度を遅延させるか否かを評価して上記細胞に作用する薬剤の心筋細胞に対する毒性を検査する方法であって、下記のうちの少

なくとも１つの工程を含む方法：

（i)上記細胞ネットワークの上記細胞を載置するための少なくとも１つの微小電極から該微小電極上に載置される細胞に一定のインターバルで強制拍動刺激を与える工程；

（ii)上記細胞が載置された各微小電極から取得される心筋拍動のフィールド・ポテンシャル波形のゆらぎの大きさを、隣接する拍動信号の比較によって計測する工程；

（iii)フィールド・ポテンシャル波形のうちカリウムイオンの細胞からの流出のピーク位置のナトリウムイオン流出ピークからの経過時間（フィールドポテンシャルデュレーション）のゆらぎの大きさを、隣接する拍動信号の比較によって計測する工程；

（iv)上記細胞が載置された各微小電極から取得される心筋拍動のフィールド・ポテンシャル波形のゆらぎの大きさを、上記細胞ネットワークの上記細胞の拍動が発生する領域から観察電極までの伝達時間あるいは伝達速度のゆらぎを隣接する拍動の比較によって計測する工程；

（v)上記細胞が載った各微小電極から取得される心筋拍動のフィールド・ポテンシャル波形の各々について微分することによって細胞電位を計測する工程；

（vi)上記細胞が載置された各電極から取得される心筋拍動のフィールド・ポテンシャル波形を重ね合わせて合成フィールド・ポテンシャル波形を合成し、心電図データと比較可能な細胞集団全体としての拍動伝達の乱れ具合を計測する工程；

（vii)上記細胞の配置された微小電極の電位を一定に保つフィードバック電位制御機構を用いて、該電位制御機構が供給する電流量を解析する工程；および

（viii)上記細胞への周期的強制拍動刺激開始後、３０秒以上経過してから計測を開始する工程。

［２９］心毒性評価のために、評価したい薬剤を添加した後に、上記［１］

～［１４］、［１８］～［２６］のいずれか一項記載の装置で得られた細胞外電位信号のFPD値の延長の平均値をX軸に、FPD値のゆらぎであるSTV値をY軸にプロットして、その相対位置から評価する、上記［２７］または［２８］記載の心筋毒性検査方法。

［３０］透明基板、

上記透明基板上に形成された壁で囲まれた領域であって、細胞培養液で満たされている領域、

上記細胞培養液で満たされている領域内に配置された複数個の安定した拍動を行う心筋細胞を含む細胞集団、

上記細胞培養液で満たされている領域内に設けられた、上記細胞集団の一つの心筋細胞あるいは上記細胞集団の局所部分を載置している微小電極、

上記細胞培養液で満たされている領域内に設けられた比較電極、

上記微小電極のそれぞれに接続された引き出し線と上記比較電極に接続された引き出し線とを用いて上記微小電極に載置されている上記心筋細胞の電位を計測する電位計測手段、

上記電位計測手段で計測した被検薬物加入前後における上記心筋細胞の電位データを記録する記録手段、ならびに

上記電位データから、フィールド・ポテンシャル（ＦＰ）波形のうちナトリウムイオンの細胞への流入ピークからカリウムイオンの細胞からの流出ピークまでの経過時間（細胞外電位持続時間）（ＦＰＤ）および該ＦＰＤのゆらぎの大きさを算出し、該算出したＦＰＤおよびＳＴＶを指標として用いて上記被検薬物の心筋毒性を評価する解析手段

を備える、心筋毒性検査装置。

［３１］透明基板、

上記透明基板表面に形成された壁で囲まれた領域であって、細胞培養液を満たすための領域、

該透明基板上の上記壁で囲まれた領域内の、複数個の安定した拍動を行う心筋細胞を含む細胞集団を保持するための心筋細胞集団保持領域、

上記透明基板上の上記心筋細胞集団保持領域に設けられた心筋細胞を載置するための微小電極、

上記透明基板上の上記細胞培養液を満たすための上記領域内に設けられた比較電極、

上記微小電極のそれぞれに接続された引き出し線と上記比較電極に接続された引き出し線とを用いて上記微小電極に載置されている上記心筋細胞の電位を計測する電位計測手段、

上記電位計測手段で計測した被検薬物適用前後における上記心筋細胞の電位データを記録する記録手段、ならびに

上記電位データからフィールド・ポテンシャル（ＦＰ）波形のうちナトリウムイオンの細胞への流入ピークからカリウムイオンの細胞からの流出ピークまでの経過時間（細胞外電位持続時間）（ＦＰＤ）および該ＦＰＤのゆらぎの大きさを算出し、該算出したＦＰＤおよびＳＴＶを指標として用いて上記被検薬物の心筋毒性を評価する解析手段

を備える、心筋毒性検査装置。

［３２］上記ＦＰＤのゆらぎの大きさが該ＦＰＤの短期変動（ＳＴＶ）である、上記［３０］または［３１］記載の心筋毒性検査装置

［３３］上記心筋細胞の載置された上記微小電極の電位を一定に保つフィードバック電位制御機構をさらに備える、上記［３０］または［３１］記載の心筋毒性検査装置。

［３４］上記透明基板上に配置した上記心筋細胞の状態を光学的に計測するための光学系および光学カメラをさらに備え、上記光学カメラで取得された画像のデータが上記記録手段に記録される、上記［３０］～［３３］のいずれか一項記載の心筋毒性検査装置。

［３５］上記心筋細胞を適切な温度に維持する温度制御機構を有する上記［３０］～［３４］のいずれか一項記載の心筋毒性検査装置

［３６］上記解析手段において、ＦＰＤのゆらぎの大きさを、隣接するフィールド・ポテンシャル（ＦＰ）信号波形の比較によって計測するように構成

されている、上記［３０］～［３３］のいずれか一項記載の心筋毒性検査装置。

［３７］上記解析手段において、上記細胞が載置された各微小電極から取得される心筋拍動のフィールド・ポテンシャル波形のゆらぎの大きさを、上記細胞ネットワークの上記細胞の拍動が発生する領域から観察電極までの伝達時間あるいは伝達速度のゆらぎを隣接する拍動の比較によって計測するように構成されている、上記［３０］～［３３］のいずれか一項記載の心筋毒性検査装置。

［３８］上記解析手段において、上記細胞が載った各微小電極から取得される心筋拍動のフィールド・ポテンシャル波形の各々について微分することによって細胞電位を計測するように構成されている、上記［３０］～［３３］のいずれか一項記載の心筋毒性検査装置。

［３９］上記解析手段において、上記細胞が載置された各電極から取得される心筋拍動のフィールド・ポテンシャル波形を重ね合わせて合成フィールド・ポテンシャル波形を合成し、心電図データと比較可能な細胞集団全体としての拍動伝達の乱れ具合を計測するように構成されている、上記［３０］～［３３］のいずれか一項記載の心筋毒性検査装置。

［４０］上記微小電極が、複数の微小電極からなる多電極アレイを構成している、上記［３０］～［３４］のいずれか一項記載の心筋毒性検査装置。

［４１］上記細胞への周期的強制拍動刺激開始後、３０秒以上経過してから計測を開始するように構成されている、上記［３０］～［３５］のいずれか一項記載の心筋毒性検査装置。

［４２］上記微小電極の近傍に配置された、細胞電位計測におけるノイズ除去のための差分用の参照電極をさらに備える、上記［３０］～［３７］のいずれか一項記載の心筋毒性検査装置。

［４３］上記微小電極が、刺激電極および計測電極からなる、上記［４２］記載の心筋毒性検査装置。

［４４］上記細胞培養液を上記壁で囲まれた領域内に供給し、および／また

は排出する培養液供給／排出チャネルを備える、上記［３０］～［３９］のいずれか一項記載の心筋毒性検査装置。

［４５］上記壁で囲まれた上記心筋細胞が培養されている領域内に被検薬物を連続して底面から導入して上方の液面から排出するための薬物導入／排出チャネルと、該導入する薬物の温度をモニターして適切な温度に維持する温度制御機構とを備え、上記チャネルの一部が、導入される該薬液の濃度を計測可能なように光学的に透明であり、波長２８０ナノメートルから８００ナノメートルの範囲で吸光分光定量計測を可能とする、上記［３０］～［３９］のいずれか一項記載の心毒性検査装置。

［４６］上記微小電極が透明である、上記［３０］～［３１］のいずれか一項に記載の心筋毒性検査装置。

［４７］上記微小電極の抵抗値を減少させるために、光学系により観察する領域以外の領域に配された透明電極配線上に金属レイヤーを付加してある、上記［４３］記載の心毒性検査装置。

［４８］培養されている上記複数の心筋細胞が上記基板上に直線状に配置されて心筋細胞ネットワークを形成しており、

上記直線状心筋細胞ネットワークの端点に局所に刺激を与えるための電極が配置されている、上記［３０］～［４４］のいずれか一項記載の心毒性検査装置。

［４９］さらに、上記直線状心筋細胞ネットワークの過半数の心筋細胞が載ることができる１つの直線状の計測電極を備える、上記［４５］記載の心毒性検査装置。

［５０］培養されている上記複数の心筋細胞が上記基板上にリング状に配置されて心筋細胞ネットワークを形成しており、

上記リング状心筋細胞ネットワークのリングの一部を欠損させて、その点に局所に刺激を与える電極が配置されている、上記［３０］～［４４］のいずれか一項記載の心毒性検査装置。

［５１］さらに、上記リング状心筋細胞ネットワークの過半数の心筋細胞が

載ることができる１つのリング状の計測電極を備える、上記［４７］記載の心毒性検査装置。

［５２］上記［３０］～［４８］のいずれか一項記載の心筋毒性検査装置を用いて心筋細胞に作用する薬物の毒性を検査する方法であって、

細胞培養液中で培養されている複数の安定した拍動を行う心筋細胞を含む心筋細胞集団に被検薬物を添加する工程、および

上記薬物の上記細胞培養液への添加の前後における、上記心筋細胞集団の発生する拍動の変化を評価する工程

を含み、

上記拍動の変化を評価する際に、ＦＰＤ波形の延長と該ＦＰＤのゆらぎの大きさの増加を指標とすることを特徴とする、方法。

［５３］複数個の安定した拍動を行う心筋細胞を含む細胞集団を準備する工程、

培養容器内の底面に多電極アレイが配された該培養容器中で上記細胞集団を培養する工程、

上記培養している上記細胞集団へ被検薬物を添加し、上記多電極アレイを用いて上記培養細胞集団の細胞電位を測定する工程であって、上記薬物の添加の前後において、上記培養細胞集団の細胞電位を測定し、該細胞電位のデータを取得する工程、ならびに

上記取得した細胞電位のデータから、フィールド・ポテンシャル（ＦＰ）波形のうちナトリウムイオンの細胞への流入ピークからカリウムイオンの細胞からの流出ピークまでの経過時間（細胞外電位持続時間）（ＦＰＤ）および該ＦＰＤのゆらぎの大きさを算出し、該算出したＦＰＤおよび該ＦＰＤのゆらぎの大きさの組み合わせを指標として用いて上記薬物の心筋毒性を評価する工程、

を含む、薬物の心筋毒性検査方法。

［５４］上記ＦＰＤのゆらぎの大きさが該ＦＰＤの短期変動（ＳＴＶ）である、上記［１９］または［５３］に記載の方法。

［５５］上記薬物の心筋毒性を評価する工程において、上記薬物の添加後のＦＰＤ波形の延長とＳＴＶの増加を指標として該薬物の心筋毒性を評価する、上記［５３］に記載の方法。

［５６］上記ＦＰＤ波形の延長が所定の閾値を超えるＦＰＤ頻度とＳＴＶの増加が所定の閾値を超えるＳＴＶ頻度とを用いて二次元プロットを作成し、該二次元プロットに基づいて上記薬物の心筋毒性の評価を行う、上記［５５］に記載の方法。

［５７］上記二次元プロットにおいて、複数の薬物のプロットの相対位置から該薬物の心筋毒性を評価する、上記［５６］に記載の方法。

[0016] 本発明は、より簡潔には、以下の装置および方法を提供する。

（１）基板、

該基板上に配置した複数個の安定した拍動を行う評価対象の心筋細胞あるいは該心筋細胞を含む細胞集団、

上記基板上に上記細胞集団の周辺を囲むように形成された細胞培養液を満たすための壁、

上記細胞培養液を上記壁で囲われた領域内に供給しおよび／または排出する培養液供給／排出チャネル、

上記細胞集団の一つの細胞あるいは細胞集団の局所部分を載置している微小電極、

上記壁で囲われた細胞培養液を満たすための領域内に設けられた比較電極、

上記微小電極のそれぞれに接続された引き出し線と上記比較電極に接続された引き出し線とを用いて上記微小電極に載置されている細胞電位を計測する電位計測手段、ならびに

上記微小電極へ送る電気刺激を制御し、上記電位計測手段で計測した電位データを記録する、制御／記録手段

を備えた細胞品質評価装置であって、

上記微小電極へ送る上記電気刺激のインターバルを段階的に変化させて、

１）上記心筋細胞あるいは上記細胞集団が上記電気刺激のインターバルによる強制拍動刺激に応答して上記強制拍動刺激と同じインターバルで応答するかどうかを確認し、かつ、その応答がどの強制拍動刺激の周波数の範囲で対応するかどうかを計測し、拍動が追従できることを確認することで健全な心筋細胞である十分条件の一つを満たしていると判断すること、および

２）上記電気刺激による強制拍動刺激インターバル（ＲＲ）に対して細胞の拍動の追従が確認できた周波数の範疇で、強制拍動刺激に対するＦＰＤの変化が、ＦＰＤ／（ＲＲ）1/3からＦＰＤ／（ＲＲ）1/2の間にあることを確認することで健全な心筋細胞である十分条件の一つを満たしていると判断すること

を可能にする、細胞品質評価装置。

（２）基板、

該基板上に配置した複数個の安定した拍動を行う評価対象の心筋細胞あるいは該心筋細胞を含む細胞集団、

上記基板上に上記細胞集団の周辺を囲むように形成された細胞培養液を満たすための壁、

上記細胞培養液を上記壁で囲われた領域内に供給しおよび／または排出する培養液供給／排出チャネル、

上記細胞集団の一つの細胞あるいは細胞集団の局所部分を載置している微小電極、

上記壁で囲われた細胞培養液を満たすための領域内に設けられた比較電極、

上記微小電極のそれぞれに接続された引き出し線と上記比較電極に接続された引き出し線とを用いて上記微小電極に載置されている細胞電位を計測する電位計測手段、ならびに

上記微小電極へ送る電気刺激を制御し、上記電位計測手段で計測した電位データを記録する、制御／記録手段

を備えた細胞品質評価装置を用いて、

上記微小電極へ送る上記電気刺激のインターバルを段階的に変化させて、
１）上記心筋細胞あるいは上記細胞集団が上記電気刺激のインターバルによる強制拍動刺激に応答して上記強制拍動刺激と同じインターバルで応答するかどうかを確認し、かつ、その応答がどの強制拍動刺激の周波数の範囲で対応するかどうかを計測し、拍動が追従できることを確認することで健全な心筋細胞である十分条件の一つを満たしていると判断すること、および
２）上記電気刺激による強制拍動刺激インターバル（ＲＲ）に対して細胞の拍動の追従が確認できた周波数の範疇で、上記強制拍動刺激に対するフィールド・ポテンシャル・デュレーション（ＦＰＤ）の変化が、ＦＰＤ／（ＲＲ）$^{1/3}$からＦＰＤ／（ＲＲ）$^{1/2}$の間にあることを確認することで健全な心筋細胞である十分条件の一つを満たしていると判断すること
を含む、細胞品質評価方法。
（３）心筋拍動のフィールド・ポテンシャル波形のゆらぎの大きさを、隣接する拍動信号の比較によって計測するように構成されている、心筋毒性検査装置。
（４）フィールド・ポテンシャル波形のうちカリウムイオンの細胞からの流出のピーク位置のナトリウムイオン流出ピークからの経過時間（フィールドポテンシャルデュレーション：ＦＰＤ）のゆらぎの大きさを、隣接する拍動信号の比較によって計測するように構成されている、上記（３）に記載の心筋毒性検査装置。
（５）ＦＰＤおよび該ＦＰＤのゆらぎの大きさを算出し、該算出したＦＰＤおよび該ＦＰＤのゆらぎの大きさの組み合わせを指標とするように構成されている、上記（３）または（４）に記載の心筋毒性検査装置。
（６）上記ＦＰＤのゆらぎの大きさが該ＦＰＤの短期変動（ＳＴＶ）である、上記（３）～（５）のいずれか一項記載の心筋毒性検査装置。
（７）透明基板、
上記透明基板上に形成された壁で囲まれた領域であって、細胞培養液で満たされている領域、

上記細胞培養液で満たされている領域内に配置された複数個の安定した拍動を行う心筋細胞を含む細胞集団、

上記細胞培養液で満たされている領域内に設けられた、上記細胞集団の一つの心筋細胞あるいは上記細胞集団の局所部分を載置している微小電極、

上記細胞培養液で満たされている領域内に設けられた比較電極、

上記微小電極のそれぞれに接続された引き出し線と上記比較電極に接続された引き出し線とを用いて上記微小電極に載置されている上記心筋細胞の電位を計測する電位計測手段、

上記電位計測手段で計測した被検薬物加入前後における上記心筋細胞の電位データを記録する記録手段

を備える、上記（３）～（６）のいずれか一項記載の心筋毒性検査装置。

（８）上記安定した拍動を行う心筋細胞を含む細胞集団が、拍動を伝えることが可能な複数個の心筋細胞および線維芽細胞を含む細胞ネットワークである、上記（３）～（７）のいずれか一項記載の心筋毒性検査装置。

（９）透明基板、

該透明基板上に配置した複数個の安定した拍動を行う心筋細胞を含む細胞集団からなるペースメーカー領域、

該透明基板上に配置され、該細胞集団と連携して上記細胞集団の拍動を伝える複数個の直列配列された心筋細胞および線維芽細胞を含む細胞ネットワーク、

上記透明基板上に上記細胞集団および上記細胞ネットワークの周辺を囲むように形成された細胞培養液を満たすための壁、

上記細胞培養液を上記壁で囲われた領域内に供給しおよび／または排出する培養液供給／排出チャネル、

上記細胞に作用する薬剤を上記細胞培養液に加えるための薬剤供給チャネル、

上記細胞集団の一つの細胞あるいは細胞集団の局所部分を載置している微小電極、

上記細胞ネットワークの個々の細胞をそれぞれ載置している微小電極、

上記壁で囲われた細胞培養液を満たすための領域内に設けられた比較電極、

上記微小電極のそれぞれに接続された引き出し線と上記比較電極に接続された引き出し線とを用いて上記微小電極に載置されている細胞電位を計測する電位計測手段、ならびに

上記微小電極へ送る電気刺激を制御し、上記電位計測手段で計測した電位データを記録する、制御／記録手段

を備える、上記（３）～（８）のいずれか一項に記載の心筋毒性検査装置。

（１０）上記安定した拍動を行う心筋細胞を含む細胞を載置するための少なくとも１つの微小電極から該微小電極上に載置される細胞に一定のインターバルで強制拍動刺激を与えることができるように構成されている、上記（３）～（９）のいずれか一項記載の心筋毒性検査装置。

（１１）上記直列配列された細胞ネットワークの端点に局所に刺激を与えるための電極が配置されている、上記（８）～（１０）のいずれか一項記載の心毒性検査装置。

（１２）上記細胞ネットワークが、リング状に配置されて形成されており、

該リングの一部を欠損させて、その点に局所に刺激を与える電極が配置されている、上記（８）～（１１）のいずれか一項記載の心毒性検査装置。

（１３）上記細胞が載置された各微小電極から取得される心筋拍動のフィールド・ポテンシャル波形のゆらぎの大きさを、上記細胞ネットワークの上記細胞の拍動が発生する領域から観察電極までの伝達時間あるいは伝達速度のゆらぎを隣接する拍動の比較によって計測するように構成されている、上記（８）～（１２）のいずれか一項記載の心筋毒性検査装置。

（１４）上記心筋細胞を適切な温度に維持する温度制御機構を有する上記（１）～（１３）のいずれか一項記載の心筋毒性検査装置。

（１５）上記微小電極が、複数の微小電極からなる多電極アレイを構成している、上記（１）～（１４）のいずれか一項記載の心筋毒性検査装置。

（１６）心筋拍動のフィールド・ポテンシャル波形のゆらぎの大きさを、隣接する拍動信号の比較によって計測することを特徴とする心筋毒性検査方法。

（１７）複数個の安定した拍動を行う心筋細胞を含む細胞集団を準備する工程、

培養容器内の底面に多電極アレイが配された該培養容器中で上記細胞集団を培養する工程、

上記培養している上記細胞集団へ被検薬物を添加し、上記多電極アレイを用いて上記培養細胞集団の細胞電位を測定する工程であって、上記薬物の添加の前後において、上記培養細胞集団の細胞電位を測定し、該細胞電位のデータを取得する工程、ならびに

上記取得した細胞電位のデータから、フィールド・ポテンシャル（ＦＰ）波形のうちナトリウムイオンの細胞への流入ピークからカリウムイオンの細胞からの流出ピークまでの経過時間（細胞外電位持続時間）（ＦＰＤ）および該ＦＰＤのゆらぎの大きさを算出し、該算出したＦＰＤおよび該ＦＰＤのゆらぎの大きさの組み合わせを指標として用いて上記薬物の心筋毒性を評価する工程、

を含む、上記（１６）に記載の薬物の心筋毒性検査方法。

（１８）上記（８）～（１５）のいずれか一項記載の心筋毒性検査装置を使用して、上記細胞に作用する薬剤が上記細胞培養液に加えられたときに、上記心筋細胞集団の発生する拍動が上記細胞ネットワーク領域の上記細胞を伝播する速度を遅延させるか否かを評価して上記細胞に作用する薬剤の心筋細胞に対する毒性を検査する上記（１６）または（１７）に記載の心筋毒性検査方法。

（１９）上記（８）～（１５）のいずれか一項記載の心筋毒性検査装置を用いて上記細胞に作用する薬剤が上記細胞培養液に加えられたときに、上記心筋細胞集団の発生する拍動が上記細胞ネットワーク領域の上記細胞を伝播する速度を遅延させるか否かを評価して上記細胞に作用する薬剤の心筋細胞に

対する毒性を検査する方法であって、下記のうちの少なくとも１つの工程を含む上記（１６）～（１８）のいずれか一項記載の方法：

（i)上記細胞ネットワークの上記細胞を載置するための少なくとも１つの微小電極から該微小電極上に載置される細胞に一定のインターバルで強制拍動刺激を与える工程；

（ii)上記細胞が載置された各微小電極から取得される心筋拍動のフィールド・ポテンシャル波形のゆらぎの大きさを、隣接する拍動信号の比較によって計測する工程；

（iii)フィールド・ポテンシャル波形のうちカリウムイオンの細胞からの流出のピーク位置のナトリウムイオン流出ピークからの経過時間（フィールドポテンシャルデュレーション）のゆらぎの大きさを、隣接する拍動信号の比較によって計測する工程；

（iv)上記細胞が載置された各微小電極から取得される心筋拍動のフィールド・ポテンシャル波形のゆらぎの大きさを、上記細胞ネットワークの上記細胞の拍動が発生する領域から観察電極までの伝達時間あるいは伝達速度のゆらぎを隣接する拍動の比較によって計測する工程；

（v)上記細胞が載った各微小電極から取得される心筋拍動のフィールド・ポテンシャル波形の各々について微分することによって細胞電位を計測する工程；

（vi)上記細胞が載置された各電極から取得される心筋拍動のフィールド・ポテンシャル波形を重ね合わせて合成フィールド・ポテンシャル波形を合成し、心電図データと比較可能な細胞集団全体としての拍動伝達の乱れ具合を計測する工程；

（vii)上記細胞の配置された微小電極の電位を一定に保つフィードバック電位制御機構を用いて、該電位制御機構が供給する電流量を解析する工程；および

（viii)上記細胞への周期的強制拍動刺激開始後、３０秒以上経過してから計測を開始する工程。

（２０）上記ＦＰＤのゆらぎの大きさが該ＦＰＤの短期変動（ＳＴＶ）である、上記（１６）～（１９）のいずれか一項記載の方法。

発明の効果

[0017] 心筋細胞および線維芽細胞の薬剤に対する応答の変化を細胞のゆらぎ計測から正確に評価することができる。

[0018] 従来、フィールド・ポテンシャル・デュレーション（ＦＰＤ）（後述の説明を参照）および隣接する心筋細胞の隣接する拍動の揺らぎの大きさ（例えば、短期変動：STV:Short-term variability）のそれぞれを独立に指標とすることはあっても、両者を組み合わせて心筋毒性の検査することはなかった。本発明の心筋毒性検査方法に従って、ＦＰＤ波形の延長だけでなく、心筋細胞の隣接する拍動の揺らぎの大きさ（ＳＴＶ）の増加も組み合わせて評価することにより、より正確な心筋毒性の評価が可能となる。

図面の簡単な説明

[0019] [図1]本発明の実施例に係る心筋毒性検査装置の構造の１例を模式的に示した斜視図である。

[図2]図１に示す心筋毒性検査装置の細胞保持部ＣＨの構成の１例を模式的に示す斜視図である。

[図3]図３は、図１に示す心筋毒性検査装置の細胞保持部ＣＨに保持された細胞を光学的に検出する光学系を説明する図である。

[図4]（ａ）、（ｂ）および（ｃ）は、細胞電位の計測に関する信号を示す図である。それぞれ、横軸に時間を、縦軸に微小電極２と比較電極２_{C}との間に得られる細胞電位を示す。

[図5]（ａ）、（ｂ）および（ｃ）は、細胞の拍動に伴う体積変化を光学系によって計測した結果に関する信号を示す図である。

[図6]（ａ）は培養液に薬物が含まれない通常状態における標的細胞の$Ｎａ^{+}$イオン、$Ｃａ^{2+}$イオン、$Ｋ^{+}$イオン成分の流入出量に伴う細胞電位変化を示す図であり、（ｂ）は培養液に薬物が含まれた状態における標的細胞の$Ｎａ^{+}$イオン、$Ｃａ^{2+}$イオン、$Ｋ^{+}$イオン成分の流入出量に伴う細胞電位変化を示す図

である。

[図7]心筋毒性検査装置の細胞を光学的に検出する光学系および可動電極の配置の一例を説明する図である。

[図8]細胞の電気信号の発生を説明する模式図である。

[図9]（ａ）は薬剤の添加による細胞電位変化の一例を示す図であり、（ｂ）は各拍動時の細胞電位変化について、隣接した２つの拍動の相同性を評価するポアンカレプロットの一例を示す図である。

[図10]（ａ）は１細胞レベルでの細胞配置技術を用いた心筋細胞の環状ネットワークによって作成したリエントリー回路の一例を示す模式図であり、（ｂ）は実際に微小電極上に細胞を配置した一例を示す顕微鏡写真である。

[図11]（ａ）は一定の幅の細胞集団を用いて心筋細胞の環状ネットワークによるリエントリー回路の一例を示す模式図であり、（ｂ）は実際に微小電極上に細胞を配置した一例を示す顕微鏡写真であり、（ｃ）は実際に微小電極アレイ上に環状に細胞集団を配置した一例を示す顕微鏡写真である。

[図12]（ａ）は環状電極を用いたリエントリー回路計測装置の一例を示す模式図であり、（ｂ）は実際に電極で計測した、正常拍動データと異常拍動データを示したグラフである。

[図13]（ａ）は１細胞の電位計測を行う電極と細胞の配置の一例を示す模式図であり、（ｂ）は実際に電極で計測した孤立１細胞の電極上の写真とその拍動電気データ、（ｃ）は計測した細胞集団の電極上での写真と細胞集団の中の１細胞の拍動電気データを示したグラフである。

[図14]本発明でカメラ受光素子を１細胞の電位計測に用いた実施例を説明する模式図である。

[図15]本発明の細胞計測システムで複数試料を計測できる機構の一例を説明する模式図である。

[図16]本発明の細胞計測システムで計測できる心臓情報を説明する模式図である。

[図17]本発明の細胞計測システムで計測できる細胞のフィールド・ポテンシ

ャル信号波形の薬物の添加に対する変化を説明するグラフの一例である。

[図18]本発明の細胞計測システムで計測できる細胞のフィールド・ポテンシャル信号波形のカリウムイオン放出のピーク位置のナトリウムイオン放出時間からの経過時間（ＦＰＤ：フィールド・ポテンシャル・デュレーション）について、カリウムイオンチャンネル阻害剤Ｅ４０３１の添加に対する変化の平均値の一例を説明するグラフの一例である。

[図19]本発明の細胞計測システムで計測できる細胞のフィールド・ポテンシャル信号波形のカリウムイオン放出のピーク位置のナトリウムイオン放出時間からの経過時間（ＦＰＤ：フィールド・ポテンシャル・デュレーション）について、そのゆらぎの大きさについて隣接する拍動の短期変動をポワンカレプロッティングに基づいて定量的に評価する方法のひとつを説明するグラフおよび式の一例である。

[図20]本発明の細胞計測システムで計測できる細胞のフィールド・ポテンシャル信号波形のカリウムイオン放出のピーク位置のナトリウムイオン放出時間からの経過時間（ＦＰＤ：フィールド・ポテンシャル・デュレーション）について、そのゆらぎの大きさをポワンカレプロッティングに基づいて定量的に評価する方法のひとつを説明するグラフおよび式の一例である。

[図21]本発明の細胞計測システムで計測できる心筋細胞のフィールド・ポテンシャル信号波形のカリウムイオン放出のピーク位置のナトリウムイオン放出時間からの経過時間（ＦＰＤ：フィールド・ポテンシャル・デュレーション）について、そのＥ４０３１の添加によって起こるゆらぎの大きさの一例をポワンカレプロッティングで表示したもの（ａ）と、これについてＳＴＶをまとめたもの（ｂ）である。

[図22]本発明の細胞計測システムで計測できる心筋細胞にさまざまな心毒性を持つことが知られている薬剤と比較薬剤を添加した場合のＦＰＤとＳＴＶについて示したものである。

[図23]本発明の細胞計測システムで計測できる心筋細胞ネットワークの形状の違い、ならびに位置の違いによる薬剤添加に対するＦＰＤのポワンカレプ

ロッティングの一例を示したものである。（ａ）は実際の細胞ネットワークの一例を示した顕微鏡写真、（ｂ）は（ａ）のＡ，Ｂ，Ｃ，Ｄの点での変化を計測したグラフを示したものである。

[図24]本発明の細胞計測システムで計測できる心筋細胞ネットワークの形状の違い、ならびに位置の違いによる薬剤添加に対するペースメーカー領域から局所への伝達時間のポワンカレプロッティングの一例を示したものである。（ａ）は実際の細胞ネットワークの一例を示した顕微鏡写真、（ｂ）は（ａ）のＡ，Ｂ，Ｃ，Ｄの点での変化を計測したグラフを示したものである。（ｃ）は、ＳＴＶの算出式である。

[図25]本発明の細胞計測システムで計測できる心筋細胞ネットワーク計測において、従来のin vitro計測法、in vivo計測法との関係、1細胞のFP波形と細胞ネットワークのFP合成波形との関係を模式的に示した図である。

[図26]本発明の細胞計測システムで計測できる心筋細胞ネットワーク計測において各電極から得られた細胞のFP波形から細胞電位を見積もる機能と細胞ネットワークのFP合成波形から心電図比較波形を合成する機能を有する装置の構成を模式的に示した図である。

[図27]本発明の細胞計測システムで計測できる心筋細胞ネットワーク計測において、環状に配置した心筋細胞ネットワーク（Ａ）の各電極から得られた細胞のFP波形（Ｂ）および、これを合成した合成ＦＰ波形（Ｃ）の一例を示す。この例では、ＰＭ領域から正常に拍動シグナルが伝達する例を示している。

[図28]本発明の細胞計測システムで計測できる心筋細胞ネットワーク計測において、環状に配置した心筋細胞ネットワーク（Ａ）の各電極から得られた細胞のFP波形（Ｂ）および、これを合成した合成ＦＰ波形（Ｃ）の一例を示す。この例では、ＰＭ領域から拍動シグナルが異常に伝達する例を示している。

[図29]本発明の細胞計測システムで計測できる心筋細胞ネットワーク計測において、心筋細胞の拍動周波数（Beating Frequency）とＦＰＤの関係一例を

示すグラフである。

[図30]本発明の細胞計測システムで計測できる心筋細胞ネットワーク計測において、心筋細胞に強制拍動を与えたときのＦＰＤの時間変化の一例を示すグラフである。

[図31]本発明の細胞計測システムで計測できる心筋細胞ネットワーク計測において、心筋細胞に強制拍動を与えてＦＰＤを計測する場合の細胞ネットワーク配置の例を示す顕微鏡写真である。

[図32]本発明の細胞計測システムで計測できる心筋細胞ネットワーク計測において、心筋細胞のＦＰ計測に微量電極電位のフィードバック制御を用いて微小電極を一定電位に保つ機構を用いた機構の一例を示す模式図である。

[図33]本発明の細胞計測システムで計測できる心筋細胞ネットワーク計測において、心筋細胞集団の一部領域に強制拍動刺激を与えたときの、心筋細胞集団の拍動周期の応答の関係の一例を示すグラフである。

[図34]本発明の細胞計測システムで計測できる心筋細胞ネットワーク計測において、心筋細胞集団の一部領域に強制拍動刺激を与えたときの、ＦＰＤの長さの変化の一例を示すグラフである。（ａ）は、強制拍動刺激によって引き起こされるＦＰ波形の変化とＦＰＤの長さの変化の関係の一例を、（ｂ）は、強制拍動刺激の刺激インターバルの変化に応じたＦＰＤの長さの変化の一例を示すグラフである。

[図35]本発明の細胞計測システムで計測できる心筋細胞ネットワーク計測において、心筋細胞集団の一部領域に強制拍動刺激を与えたときの、細胞集団の応答の一例について図３３および図３４に示した結果をまとめた表である。

[図36]図３６は、本発明の心筋毒性検査装置の全体構成の一例を示すブロック図である。

[図37]図３７は、本発明の１つの実施形態に係る心筋毒性検査装置の測定部の拡大図である。

[図38]図３８は、多電極アレイチップ（ａ，ｂ，ｄ）を用いて心筋細胞（ｂ

）に対する薬物の毒性評価を行う際の、チップの電極と測定ウインドウとの対応関係（ａ～ｃ）および分析に用いるデータの表示例（ｅ）を示す図である。

[図39]図３９は、Diltiazem, DL-sotalol, E-4031 及び DDWについて本発明の一実施形態に係る心毒性検査方法により生成した2次元プロットを示す。

[図40a]本発明の電極電位計測に置けるノイズ除去のための比較電極と微小電極の間の差分回路を模式的に示したものである。（ａ）原理を示した回路の一例の模式図である。

[図40b]本発明の電極電位計測に置けるノイズ除去のための比較電極と微小電極の間の差分回路を模式的に示したものである。（ｂ）この差分回路を組み込んだ増幅回路の一例の回路図である。

[図40c]本発明の電極電位計測に置けるノイズ除去のための比較電極と微小電極の間の差分回路を模式的に示したものである。（ｃ）回路によってノイズが低減された例を示す図である。

[図41]本発明の心毒性評価法の総合的評価法の例を模式的に示す図である。（ａ）細胞のＦＰＤのデータからX軸にFPD延長の程度を、Y軸にFPDの時間的ゆらぎの大きさをプロットする。（b）は、その結果の平均をX-Y図にプロットしたものの一例を示す。

[図42]本発明の心毒性を計測するシステムの構成の一例を示す模式図である。

[図43]本発明の心毒性を計測するシステム中の細胞培養計測チャンバーの構成の一例を示す模式図と写真である。

[図44]細胞培養計測プレートの断面を模式的に示した図である。

[図45]多電極基板に配置されている電極配置の電極配線の構成を模式的に説明する図である。

[図46]多電極基板上の電極配置の例を示した模式図である。

発明を実施するための形態

[0020] 図１は本発明の実施例に係る心筋毒性検査装置の構造の１例を模式的に示

した斜視図である。図２は、図１に示す心筋毒性検査装置の細胞保持部ＣＨの構成の１例を模式的に示す斜視図である。図３は、図１に示す心筋毒性検査装置の細胞保持部ＣＨに保持された細胞を光学的に検出する光学系を説明する図である。

[0021] 図１および２を参照して、心筋毒性検査装置１００は、透明基板１の上に構築されている部品を主体として構成される。透明基板１は光学的に透明な材料、例えば、ガラス基板あるいはシリコン基板である。微小電極２は、例えば、ＩＴＯによる透明電極とされ、透明基板１上に配置される。2’は微小電極２の引き出し線である。３_{1}，３_{2}，３_{3}及び３_{4}はアガロースゲルによる壁であり、微小電極２の周辺に間隙４_{1}，４_{2}，４_{3}及び４_{4}を介して配置される。アガロースゲルによる壁３_{1}，３_{2}，３_{3}及び３_{4}は中心部が切り欠かれて細胞収納部となる空間を形成している。アガロースゲルによる壁３_{1}，３_{2}，３_{3}及び３_{4}により形成される細胞収納部となる空間の透明基板１上には、必要により、微小電極２が配置される。微小電極２の有無に係らず、細胞収納部に一つの細胞１０が収納できる。図２では、アガロースゲルによる壁３_{1}，３_{2}，３_{3}及び３_{4}により形成される細胞収納部となる空間の透明基板１上に、微小電極２が配置され、その上に心筋細胞１０が収納されている。微小電極２に引き出し線２’が接続されて引き出されている様子を示す。微小電極２の細胞載置面および微小電極２を設けないで透明基板１に、直接、細胞を載置する場合の細胞載置面にはコラーゲン等の細胞が電極表面および透明基板に接着するのを助ける素材を塗っておくのがよい。アガロースゲルによる壁３_{1}，３_{2}，３_{3}及び３_{4}により形成される細胞収納部内の細胞は、アガロースゲルは細胞にとっては非接着性であるため、この壁３_{1}，３_{2}，３_{3}及び３_{4}の高さを細胞と同程度としても、壁を乗り越えて細胞１０が移動することはない。また、アガロースゲルによる壁３_{1}，３_{2}，３_{3}及び３_{4}中心部が切り欠かれて形成される細胞収納部の周辺の間隙４_{1}，４_{2}，４_{3}及び４_{4}は細胞の大きさより小さいものとされるから、この間隙４_{1}，４_{2}，４_{3}及び４_{4}をすり抜けて細胞１０が移動することはない。

[0022] 図１において、細胞保持部ＣＨ$_1$、ＣＨ$_2$、ＣＨ$_3$およびＣＨ$_n$はそれぞれ細胞収納部に一つの心筋細胞あるいは線維芽細胞１０$_1$、１０$_2$、１０$_3$および１０$_n$を保持するとともに、図では明確でないが、それぞれ、微小電極２を備えていて、引き出し線２’$_1$、２’$_2$、２’$_3$および２’$_n$が引き出されている。これらの心筋細胞または線維芽細胞は直列配列された細胞連絡チャネルＣＣＣを構成する。ここで、ｎは例えば２０である。また、これら２０個の直列配列された心筋細胞および線維芽細胞の配分は、ランダムでよいが、細胞保持部ＣＨ$_1$の細胞およびＣＨ$_{20}$の細胞は心筋細胞であったほうがよい。この細胞連絡チャネルＣＣＣの左端には３×３の細胞保持部ＣＨ$_G$が形成されていて、それぞれの細胞保持部ＣＨに心筋細胞１０が保持されてなる細胞集団１０$_G$を含む心筋細胞集団保持領域が存在する。この細胞集団１０$_G$は安定した拍動を行うペースメーカーとして機能するものである。細胞集団１０$_G$では、細胞集団１０$_G$の一つの細胞保持部ＣＨのみに微小電極２が備えられ、引き出し線２’$_G$が引き出されている。また、細胞集団１０$_G$の右側の中央の細胞保持部ＣＨが細胞連絡チャネルＣＣＣの細胞保持部ＣＨ$_1$に対向するように構成されている。細胞集団１０$_G$の右側と細胞連絡チャネルＣＣＣの左端部との間に障壁１１$_a$が設けられる。この障壁１１$_a$の中央部の下部には小さい開口１１$_b$が形成される。この開口１１$_b$の両側には対向する細胞集団１０$_G$の右側の中央の細胞保持部ＣＨと細胞連絡チャネルＣＣＣの細胞保持部ＣＨ$_1$があり、それぞれの細胞収納部の周辺の間隙４を介してそれぞれに保持されている細胞の物理的接触・細胞間インタラクションが可能なように構成されている。細胞集団１０$_G$の下部に比較電極２$_C$が設けられ、引き出し線２’$_C$が引き出されている。

[0023] ７は周辺を取り巻く壁であり、細胞集団１０$_G$、細胞連絡チャネルＣＣＣおよび比較電極２$_C$を取り巻いている。８$_1$および８$_2$は壁７の内部の領域に、細胞の培養液を供給し、および、壁７の内部の領域から、細胞の培養液を排出するためのパイプであり、図の例では、基板１の底面近くまで延伸されたパイプ８$_1$から培養液が供給され、基板１の底面近くまで延伸されたパイプ８$_2$

から培養液が排出される。培養液を供給するパイプ８$_1$の培養液の出口の近くにパイプ８$_3$が結合され、このパイプ８$_3$を介して細胞に作用させたい薬剤が供給される。したがって、細胞１０はパイプ８$_1$により壁７の内部の領域に供給される細胞の培養液に曝されながら、微小電極２の上に安定して保持される。細胞を培養液に曝す必要がなくなったときは、パイプ８$_2$により壁７の内部の領域から培養液を排出すればよい。また、培養液を新しいものと交換するときは、培養液を排出した後、あるいは、排出しながら、培養液を供給すればよい。一方、細胞に薬剤を作用させたいときは、パイプ８$_2$により培養液を排出しながら、パイプ８$_3$を介して細胞に作用させたい薬剤を培養液に加えて、パイプ８$_1$により培養液とともに供給すればよい。このとき、細胞集団１０$_G$と細胞連絡チャネルＣＣＣとの間に障壁１１$_a$を設けたことにより、薬剤を含む培養液がパイプ８$_1$により壁７の内部の領域に供給されるとき、細胞連絡チャネルＣＣＣの細胞が薬剤の影響を受ける程度に比し、細胞集団１０$_G$の細胞が薬剤の影響を受ける程度は低いものとなる。すなわち、パイプ８$_1$により薬剤を含む培養液が供給されるとき、この培養液は障壁１１$_a$の両側の壁７との隙間および障壁１１$_a$の上面を乗り越えて細胞集団１０$_G$にも供給されるから細胞集団１０$_G$の細胞も薬剤の影響を受ける。しかし、その影響は細胞連絡チャネルＣＣＣの細胞に対するものと比較すれば間接的であるので、ペースメーカーとしての機能に影響を及ぼすほどのものではない。なお、パイプ８$_1$、パイプ８$_2$およびパイプ８$_3$の構成、配置は計測の仕方により任意に変更してよい。例えば、パイプ８$_1$およびパイプ８$_3$は分離されたものとしてもよいし、パイプ８$_2$は省略して、パイプ８$_1$を供給、排出の両方に使用するものとしてもよい。

[0024] ＰＣはパソコン（電位計測手段，制御／記録手段）であり、細胞保持部ＣＨの微小電極２の引き出し線２’と比較電極２$_C$の引き出し線２’との間で細胞電位を計測し記録する。また、パソコン９には操作者の操作信号Ｍｓが加えられる。

[0025] 心筋毒性検査装置１００は光学観察装置２００のＸＹステージ１５に載せ

て細胞連絡チャネルＣＣＣの任意の細胞１０の拍動を、光学系により観察することが出来る。ＸＹステージ１５は、光学的に透明であるとともに、操作者の操作信号Ｍｓに応じてパソコンＰＣが与える信号に応じてＸ－Ｙ駆動装置１６により、任意の位置に移動される。図３では、細胞連絡チャネルＣＣＣの細胞１０$_n$の拍動の状態を観察する例を示している。１２は培養液を示す。

[0026] ２２は位相差顕微鏡あるいは微分干渉顕微鏡の光源であり、一般にハロゲン系のランプが用いられる。２３は位相差等の実体顕微鏡観察の光源の光から特定の波長のもののみを透過させるバンドパスフィルタである。例えば細胞１０$_n$の観察の場合には、波長７００ｎｍ近傍の狭帯域の光を用いることで細胞１０$_n$の損傷を防ぐことができる。２４はシャッターで、ＸＹステージ１５を移動させる場合など、画像計測をしていない間は光の照射を遮断する機能を有する。２５はコンデンサレンズであり、位相差観察をする場合は位相差リングを導入し、微分干渉観察をする場合は、偏光子を導入する。ＸＹステージ１５上には基板１上に形成されている心筋毒性検査装置１００が載置されＸ－Ｙ駆動装置１６によって前記ＸＹステージ１５を移動させることで前記心筋毒性検査装置１００の任意の位置を観察し、計測することができる。前記心筋毒性検査装置１００内の細胞１０$_n$の拍動の状態は、対物レンズ１７で観察される。対物レンズ１７の焦点位置はパソコンＰＣによる信号に応じて駆動装置１８によってＺ軸方向に移動させることができる。対物レンズ１７の倍率は４０倍以上のものが使用できる。対物レンズ１７で観察されるのは、光源２２から透過された光による細胞１０$_n$の位相差像あるいは微分干渉像である。前記バンドパスフィルタ２３を透過するのと同波長の光を反射するダイクロイックミラー１９およびバンドパスフィルタ２０によって、位相差顕微鏡像あるいは微分干渉顕微鏡像のみがカメラ２１によって観察される。カメラ２１によって観察された画像信号はパソコンＰＣに導入される。また、図示していないが、画像はパソコンと接続されたモニタまたはディスプレイにより表示され得る。

[0027] 図１に示す心筋毒性検査装置１００の構造の主要なサイズの例を示すと以下のようである。これは、細胞の大きさを１０μｍφとした例である。透明基板１の大きさは１００ｍｍ×１５０ｍｍ、微小電極２は８μｍ×８μｍの大きさ、アガロースゲルによる壁３_{1}，３_{2}，３_{3}及び３_{4}の個々の大きさは２０μｍ×２０μｍ×１０μｍ（高さ）、間隙４_{1}，４_{2}，４_{3}及び４_{4}の幅２μｍ、アガロースゲルによる壁３_{1}，３_{2}，３_{3}及び３_{4}により形成される細胞収納部となる空間は１２μｍφの円柱状、壁７の外形は５ｍｍ×５ｍｍとし高さは５ｍｍである。障壁１１ａの高さは１ｍｍである。尚、ここでは、微小電極２は８μｍ×８μｍの正方形としたが、アガロースゲルによる壁３_{1}，３_{2}，３_{3}及び３_{4}の全体と間隙４_{1}，４_{2}，４_{3}及び４_{4}の幅とで構成する細胞の収納部となる１０μｍφの円状の電極としてもよい。

[0028] 以下、本発明の心筋毒性検査装置１００の構成例とこれを用いた具体的な計測例を説明する。

[0029] 図４（ａ）、図４（ｂ）および図４（ｃ）は、細胞電位の計測に関する信号を示す図である。それぞれ、横軸に時間を、縦軸に微小電極２と比較電極２_{C}との間に得られる細胞電位を示す。図４（ａ）は細胞集団１０_{G}の拍動による細胞電位である。ここでは、図１に示す細胞集団１０_{G}の一つから引き出された引き出し線$２'_{G}$と比較電極２_{C}から引き出された引き出し線$２'_{C}$との間の電位である。図に示すように、安定した拍動を示し、ペースメーカーとして機能しうることがわかる。図４（ｂ）は培養液に薬物が含まれない通常状態における標的細胞の拍動による細胞電位である。ここでは、計測の標的細胞を細胞連絡チャネルＣＣＣの細胞１０_{n}とし、細胞１０_{n}から引き出された引き出し線$２'_{n}$と比較電極２_{C}から引き出された引き出し線$２'_{C}$との間の電位が計測されている。図４（ａ）の波形と比較して明らかなように、細胞連絡チャネルＣＣＣの細胞１０による拍動の伝達に要する時間Δｔだけ遅れていることが観察される。これに対して、図４（ｃ）は培養液に薬物が含まれた状態における標的細胞の拍動による細胞電位である。ここでも、計測の標的細胞を細胞連絡チャネルＣＣＣの細胞１０_{n}とし、図４（ｂ）との比較が

明確になるようにしている。図４（ａ）、図４（ｂ）、の波形と比較して明らかなように、細胞連絡チャネルＣＣＣの細胞１０による拍動の伝達に要する時間Δｔだけの遅れではなく、時間Δｔ＋αの遅れとなっていることが観察される。これは、細胞連絡チャネルＣＣＣの細胞に対する薬物の作用によるＮａイオン阻害の大きさが＋αの遅れの増大として表れていることを意味する。すなわち、心筋細胞に対する薬物の毒性をＮａイオン阻害として評価することが出来る。

なお、観察に使用する微小電極を観察電極と言う場合がある。

[0030] 図５（ａ）、図５（ｂ）および図５（ｃ）は、細胞の拍動に伴う体積変化を光学系によって計測した結果に関する信号を示す図である。図５（ａ）は細胞集団10_Gの細胞の拍動に伴う体積変化である。細胞集団10_Gの細胞の一つの拍動を図３に示す形で光学的に検出したものである。細胞の拍動に伴う収縮および拡張がパルス状に現れる変化として認められる。この波形の周期は、図４（ａ）に示す拍動に伴う細胞電位の変化の周期と同じである。図５（ｂ）は培養液に薬物が含まれない通常状態における標的細胞の拍動に伴う体積変化を上段に示し、下段にこれを電気信号として評価するために時間微分値として処理したときの波形を示す。ここでも、計測の標的細胞を細胞連絡チャネルＣＣＣの細胞10_nとし、細胞10_nの拍動を図３に示す形で光学的に検出したものである。図５（ａ）の波形と比較して明らかなように、細胞連絡チャネルＣＣＣの細胞１０による拍動の伝達に要する時間Δｔだけ遅れていることが観察される。これに対して、図５（ｃ）は培養液に薬物が含まれた状態における標的細胞の拍動に伴う体積変化を評価するための説明図であり、図５（ａ）、図５（ｂ）に比し時間軸を拡大した形で示す。上段は図５（ｂ）の上段の波形に対応する波形であり、図５（ａ）の波形と比較して明らかなように、細胞連絡チャネルＣＣＣの細胞１０による拍動の伝達に要する時間Δｔよりもさらにβだけ遅れが増大していることが観察される。標的細胞の拍動に伴う体積変化が薬物により受ける影響は、この遅延の増大以上に体積変化の傾きが小さくなることが特徴的である。図５（ｃ）の下

段に参考波形として示した薬物を含まない培養液での体積変化と比較してみるとこのことがよく分かる。図５（ｃ）の中段には、上段の波形を評価するために時間微分値として処理したときの波形を示す。この時間微分値を図５（ｂ）の下段のそれと比較すると分かるように、ピーク値が小さくなるとともに、傾きが緩やかになっている。これは、薬物により心筋の収縮速度が低下して、心拍出量が低下したことを意味する。すなわち、心筋細胞に対する薬物の毒性を収縮速度の低下として評価することが出来る。

[0031] 図６（ａ）は培養液に薬物が含まれない通常状態における標的細胞のＮａ$^{+}$イオン、Ｃａ$^{2+}$イオン、Ｋ$^{+}$イオン成分の流入出量に伴う細胞電位変化を示す。図６（ｂ）は培養液に薬物が含まれた状態における標的細胞のＮａ$^{+}$イオン、Ｃａ$^{2+}$イオン、Ｋ$^{+}$イオン成分の流入出量に伴う細胞電位変化を示す。図６（ａ）、図６（ｂ）を対比してすぐ分かるように、ＱＴ遅延が表われて波形が時間軸方向に伸びている。さらにＫ$^{+}$イオンの流入出に伴い、波形が大きく変形している。これを電気信号として評価するために、図に破線で示した“０”と“１００”の間の値に対して、３０％、６０％および９０％の値の継続時間をＡＰＤ３０，ＡＰＤ６０およびＡＰＤ９０として検出する。ここで、ＡＰＤとはAction Potential Durationの頭文字からとった表現である。これらの値の大きさおよび比率を評価すれば、その薬物のＮａ$^{+}$イオン、Ｃａ$^{2+}$イオン、Ｋ$^{+}$イオン成分の流入出量に及ぼす影響を評価できる。

[0032] 図７は、心筋毒性検査装置の細胞を光学的に検出する光学系および可動電極の配置の一例を説明する図であり、例えば計測する細胞１０$_{n}$の拍動の状態を観察する例を示している。１２は培養液を示す。２２は位相差顕微鏡あるいは微分干渉顕微鏡の光源であり、一般にハロゲン系のランプが用いられる。２２１は細胞の蛍光計測をするための蛍光光源であり、一般に水銀ランプ、単色光レーザー、ＬＥＤ光源などが用いられる。２３は位相差等の実体顕微鏡観察の光源の光から特定の波長のもののみを透過させるバンドパスフィルタであり、２３１は蛍光光源２２１から特定の蛍光を励起する励起波長の光のみを透過させるバンドパスフィルタである。例えば細胞１０$_{n}$の拍動堆積

変化情報のような形状変化の観察の場合には、細胞形状を計測するための波長の光だけを透過させるバンドパスフィルタ２０を通過した像をカメラ２１でリアルタイム計測するが、計測に波長７００ｎｍ近傍の狭帯域の光を用いることで細胞１０$_n$の損傷を防ぐことができる。２４および２４１はシャッターで、ＸＹステージ１５を移動させる場合など、画像計測をしていない間は光の照射を遮断する機能を有する。２５はコンデンサレンズであり、位相差観察をする場合は位相差リングを導入し、微分干渉観察をする場合は、偏光子を導入する。蛍光計測の場合は、例えば細胞内カルシウム放出の計測を行う場合は、励起波長５００ｎｍ程度、蛍光計測波長６００ｎｍ程度での光を選択的に透過するバンドパスフィルタの組み合わせを用い、蛍光波長の光だけを選択的に透過するバンドパスフィルタ２０１を通過した蛍光像をカメラ２０１で計測する。このとき、細胞ネットワーク中での1細胞単位でのカルシウム放出の時間的前後関係を計測して細胞ネットワーク中での信号伝達の経路を計測する場合には、カメラの計測時間分解能は０．１ｍｓ以上の高速連続画像が取得できるものとなっている。ＸＹステージ１５上には基板１上に形成されている心筋毒性検査装置１００が載置されＸ－Ｙ駆動装置１６によって前記ＸＹステージ１５を移動させることで前記心筋毒性検査装置１００の任意の位置を観察し、計測することができる。前記心筋毒性検査装置１００内の細胞１０$_n$の拍動の状態は、対物レンズ１７で観察される。対物レンズ１７の焦点位置はパソコンＰＣによる信号に応じて駆動装置１８によってＺ軸方向に移動させることができる。対物レンズ１７の倍率は４０倍以上のものが使用できる。対物レンズ１７で観察されるのは、光源２２から透過された光による細胞１０$_n$の位相差像あるいは微分干渉像である。前記バンドパスフィルタ２３を透過するのと同波長の光を反射するダイクロイックミラー１９２およびバンドパスフィルタ２０によって、位相差顕微鏡像あるいは微分干渉顕微鏡像のみがカメラ２１によって観察される。カメラ２１によって観察された画像信号はパソコンＰＣに導入される。また、この実施例では細胞への刺激を行うための可動電極２７が配置されており、可動電極の座標がＸ

Ｙステージと同じ平面上の任意の位置のみならず、高さを任意に調節することができる位置制御機構が備わっている。この位置制御機構を用いて細胞ネットワーク中の特定の1細胞あるいは数細胞の位置に可動電極の先端を移動させることで、任意の特定の細胞あるいは数細胞に刺激を与えることができる。可動電極の素材としては、先端部以外の部分は絶縁被覆されている金属電極、先端の開口サイズが５ミクロン程度以下のガラス電極等の、可動電極先端近傍にある特定の細胞あるいは数細胞のみに電気刺激を与えることができる構成の電極を用いることができる。金属電極を用いる場合は、先端部表面に白金黒などを付加することによって効果的に電気刺激を細胞に伝達することができる。可動電極の先端の位置は、電気刺激に対する細胞の応答の程度によって調整するものとし、細胞に接しても良いし、あるいは、細胞のごく近傍に配置しても良い。また、刺激電極の刺激を適切に標的とする特定の細胞に与えるために電気刺激を与える瞬間にスイッチングによって細胞電位計測のための電極２を接地電極として用いても良いし、あるいは、別途、別の接地電極２８を配置しても良い。さらに、特定の細胞を刺激するために、既存の微小電極２を刺激電極として用いても良い。その場合には、微小電極が接続されているスイッチング回路２９のスイッチングによって、通常は電気信号計測回路３０に接続されているものを、刺激を与える瞬間に、スイッチング切り替えによって、電気刺激回路３１に接続をして矩形波の刺激信号を微小電極２に与えることができる。また、上記可動電極２７を用いて刺激を与える瞬間には、スイッチング回路２９を接地にスイッチングすることができる。他方、可動電極も、刺激電極としてだけでなく、細胞の電気信号を計測する電極として用いたり、接地電極として用いることもできる。その場合は、可動電極はスイッチング回路２９１に接続されており、細胞電位計測、細胞刺激、接地電極としての利用に応じて、それぞれスイッチングによって、電気信号計測回路３０１に接続されて細胞電位を計測したり、電気刺激回路３１１に接続をして矩形波の刺激信号を細胞に与えたり、また、接地させることで、接地電極として用いることができる。電気刺激回路３１、３１１

が細胞に与える電気刺激のタイミングは、おもに次の２つの用途で用いることができる。ひとつは自律拍動機能を持った細胞ネットワークにおいて、その正常な心筋細胞ネットワークの拍動インターバルの間に、イレギュラーな刺激を与えるものであり、他方は、自律拍動能を有さない心筋細胞ネットワークに対して拍動インターバルを与えるものである。ともに、計測するのは、拍動インターバルの周期（２つの拍動間の時間差）を５ｍｓ単位で徐々に短くしていったときの、細胞ネットワークの応答の変化を追跡できるものである。そのために、電気刺激回路３１、３１１は、電気信号計測回路３０、３０１などによって得られた拍動周期情報を解析して、その結果に基づいて刺激のタイミングを決定するフィードバック制御を行うことができる。さらに、可動電極２７を電気信号計測のために用いる場合、本システムにおいて微小電極２が無くても同様な計測ができることとなる。これは、システムに構成されている光学計測によって細胞ネットワーク中の各細胞の拍動周期の計測は可能であることから、この拍動周期が安定状態から不整脈などの不安定状態になることはシステムに配置されている光学計測装置のみで計測し、その結果から必要に応じて、可動電極を用いて特定の細胞の電気的特性のデータを取得するものであり、この場合には、システム上に事前に配置している微小電極数の制約を受けず、光学計測できる範囲で、より自由に大きな細胞ネットワークを構築することができる。

[0033] 図８に、細胞の電気信号の発生の一例について模式図で示した。最初に、細胞膜にあるナトリウムイオンチャンネルからのナトリウムイオンの細胞内への流入が発生し細胞電位が急激に下がり、次に、少しの遅延を経て、カルシウムイオンの流入による細胞電位の低下が起き、そして、次のステップとして、カリウムイオンの細胞外への排出が起きることで細胞電位の上昇が起きる。細胞電位の変化は、心筋細胞膜に存在する応答特性の異なるさまざまなイオンチャンネルの特性の違いによって引き起こされる。それぞれのイオンチャンネルに起因して引き起こされる電位変化のピークの位置を、各イオンチャンネルの特性時間として解析すると、薬剤の影響によって各イオンチ

ャンネルがブロックされることで、その薬剤の特性に応じてブロックされたイオンチャンネルの種類に応じた電気信号の波形の変化を計測することができ、それによって薬剤のイオンチャンネルへの阻害効果を見積もることができる。薬剤の評価に特に重要なイオンチャンネルは、ＦａｓｔＮａ、ＳｌｏｗＮａ、Ｃａ、ＩＫｒ、ＩＫｓの４つのイオンチャンネルであり、これら4種類のイオンチャンネルのブロックの状態を計測することができる。

[0034] 図９（ａ）は、図８で示した細胞の電気信号について、実際にカリウムイオンチャンネルを選択的に阻害する試薬Ｅ－４０３１を様々な濃度で添加したときの変化を示したものである。細胞の電位を上げるＫイオンの細胞外への排出機能を担うＩＫｒイオンチャンネルを阻害することから、薬剤濃度が高まるにつれて正方向の細胞電位の変化が徐々に遅延することが分かる。図９（ａ）に示したのは、細胞の応答の特定の1拍動データであるが、実際には隣接する各拍動での応答のゆらぎ幅の大きさが、薬剤の影響を見積もる重要な指標となる。図９（ｂ）がその一例であり、ポアンカレプロットと呼ばれる隣接する拍動データの相関を比較する解析手法である。ここでは、Ｘ軸にｎ回目の拍動時の特定のイオンチャンネルの応答時間の位置を、Ｙ軸に（ｎ＋１）回目の拍動時の同じイオンチャンネルの応答時間の位置を置いたプロットを行う。すると、隣接する拍動同士での特性が同じ場合は、グラフ内の点線で描かれたＹ＝Ｘ上にプロットが並ぶこととなるが、隣接する拍動同士での応答に大きな揺らぎがある場合は、Ｙ＝Ｘから離れた位置までの大きなプロットの分布が観測されることとなる。実際に、この例では、薬剤を添加しないControlに対して、４０ｎＭの添加では応答時間の遅延があるが、隣接した拍動間での相同性は維持されている。他方、４００ｎＭまで薬剤の添加をすると応答時間の更なる遅延に加えて、隣接した拍動間での相同性も崩れ、不安定な拍動周期が発生することとなることが本プロットによって明らかにできる。この結果は、心毒性を示すＱＴ延長計測の結果とも一致しており、ポアンカレプロットによって1細胞レベルでの隣接した各拍動のゆらぎの増大の計測を指標にすることでＱＴ延長の発生を見積もることができる。この

現象は、特定のイオンチャンネルが薬剤によってブロックされたときに、そのブロックの程度が軽微な場合は、イオンの排出能力の低下という現象が見えるだけで細胞応答の不安定性はまだ発生しないのに対して、さらに、ブロックの程度が重度になると、機能しているイオンチャンネルの数が極端に減少して、そのために細胞のイオン排出能力について同じ細胞でありながら再現性の低い大きな揺らぎを持った結果が出てくることとなる。そして、このゆらぎの大きさがＱＴ延長の発生し易さの指標として用いることができるものである。

[0035] 図１０（ａ）は、１細胞レベルでの細胞配置技術を用いた心筋細胞の環状ネットワークによるリエントリー回路薬剤の一例を示す模式図である。心筋細胞のみで細胞の環状ネットワークを作成したものは、正常ネットワークモデルとなり、心肥大などの病理モデルの場合には、細胞ネットワークの中に線維芽細胞を組み込むことで実現される。そして、ネットワーク中に混在する線維芽細胞が心筋細胞ネットワークの伝達速度の遅延や減衰をもたらし、その結果、期外収縮の発生を予測することができる。図１０（ｂ）は、実際に微小電極上に心筋細胞を配置した一例を示す顕微鏡写真である。実際に、この写真に示したように、１細胞単位で微小電極上に配置された場合は、隣接した心筋細胞同士の信号伝達の遅延を計測することができる。そして、この伝達速度は拍動のときに発生する最初の電気信号の大きさに依存することから、Ｎａイオンチャンネルへの阻害効果として、この信号伝達の遅延データを用いることができる。

[0036] 図１１（ａ）は、一定の幅の細胞集団を用いて心筋細胞の環状ネットワークによるリエントリー回路の一例を示す模式図である。図１０に示した１細胞単位での環状細胞ネットワークでは、心筋細胞の拍動信号はその通過について一意的であり、図９に示したような細胞自体の拍動のゆらぎが無い限り、同一の特性を維持して細胞は拍動信号を隣接細胞間で伝達する。他方、この図１１で示したように、一定の幅で細胞を配置して環状ネットワークを形成した場合、細胞集団はその伝達について実線３５、破線３６、点線３７の

ように拍動ごとに異なる経路を取る自由度を持つこととなる。特に、図９で説明したように、薬剤の添加によって各心筋細胞の応答特性に大きなゆらぎが発生すると、容易に応答する細胞が環状ネットワークを刺激信号が周回するたびに、その都度異なることから、経路の違いがより顕著となる。これはスパイラル・リエントリーと呼ばれる心臓の致死に至る期外収縮の機構と同じメカニズムとなることから、このような幅を持った細胞集団ベースでの環状ネットワークを特に用いることでスパイラル・リエントリーの計測が可能となる。図１１（ｂ）は実際に微小電極上に細胞集団を配置した一例を示す顕微鏡写真であり、心筋細胞６０％程度に線維芽細胞４０％程度を混ぜた細胞集団を配置している。実際に、このような配置を行うと、隣接電極間での伝達速度の隣接拍動間でのゆらぎは大きくなり、特に薬剤の添加によって、そのゆらぎの増大が顕著となることから、隣接拍動間での伝達速度のゆらぎ幅の変化からスパイラル・リエントリーの発生を見積もることができる。図１１（ｃ）は、さらに実際に微小電極アレイ上に環状に細胞集団を配置した一例を示す顕微鏡写真である。実際のスパイラル・リエントリーの計測には、図７に示した高速蛍光計測カメラを用いることで、細胞集団ネットワーク中での各細胞のカルシウム発火を１細胞レベルで見積もることができ、その結果として細胞の信号伝達がどのような経路で進んでいるのか、各周回での経路の変化を実際に解析することができる。

[0037] 図１２（ａ）は環状電極を用いたリエントリー回路計測装置の一例を示す模式図である。この例では、１～３ｍｍの直径のリング状に形成した電極幅５０～１００ミクロンの環状電極３８を各９６穴ウエルプレート４２の底面に１つ配置し、電極表面にのみ４１のように細胞集団が環状に配置されるように、電極上の除く周囲の底面については、表面にアガロース等の細胞接着性の無い素材をコートしている。この細胞が接着しないようにコートされた領域に、同心円状に参照電極リング３９が配置されており、また、試薬の出入りが行える流路４０が配置されている。このような電極を用いることで簡易に心筋細胞の異常拍動を簡便に計測することができる。図１２（ｂ）は実

際に電極で計測した、正常拍動データと異常拍動データを示したグラフである。本実施例では、環状電極を用いたが、図７で示した光学計測システムを用いることで、本環状電極による効果と同じ、異常拍動を光学的に計測することができるシステムを構築することができる。その場合、電気的信号を計測する場合には、図７で示した移動電極を環状細胞ネットワークに接触させることで、電気信号も取得することができる。

[0038] 図１３（ａ）は１細胞の電位計測を行う微小電極２と細胞の配置の一例を示す模式図であり、直径１０ミクロンから５０ミクロンの微小電極２上に、計測の対象となる細胞を１細胞だけ配置して計測する手法を示したものである。本実施例でも、他の実施例と同様、電極上の細胞がその場所に保持されるように電極周囲には、細胞接着性を阻害するアガロースなどの素材がコートされている。図１３（ｂ）は実際に微小電極２で計測した孤立１細胞の電極上の写真とその拍動電気データを示したものであるが、孤立した一細胞の信号は不安定でありグラフに示したように大きなゆらぎを持って拍動している。他方、図１３（ｃ）で示したように、微小電極２上には、図１３（ｂ）と同様に１細胞が配置されているが、他の細胞と連結された細胞集団となることで、拍動信号グラフを見てもわかるように、拍動周期の安定性が実現される。実際の１細胞レベルでの拍動計測では、図９でも示したように、隣接した各拍動間のゆらぎの大きさが指標となることから、本実施例で示したような、細胞集団化による安定化を実現しながら、１細胞の拍動データが取得できるように、特定の計測したい１細胞のみ微小電極上に配置され、この特定細胞の安定性を維持するために他の心筋細胞を電極上には載らない形で配置されている構成を用いた計測システムが有用である。

[0039] 図１４は、本発明でカメラ受光素子を１細胞の電位計測に用いた実施例を説明する模式図である。通常、カメラ受光素子は、光電変換面で光信号を電気信号に変換して、この電気信号を計測に用いるものであることから、この光電面を除去して、電気信号アレイ部分を用いることで、２次元での電気信号を取得することが可能となる。これによって、１細胞レベルでのサイズの

電極アレイを用いることができるため、例えば細胞集団の中の各細胞の電気信号を同時に計測することが必要な、図１１で示した一定の幅を持つ細胞集団ネットワーク中での信号伝達経路の変化というスパイラル・リエントリーの発生を計測することが可能となる。実際の計測では、画素の計測インターバルは１万分の１秒程度必要であり、１万分の１秒のシャッタースピードの高速カメラのカメラ受光素子を利用する必要がある。この場合、取得された細胞の信号データは、既存のカメラで用いられている画像処理技術をそのまま適用することができ、画像処理用のFPGAを用いた実時間処理が可能となる。また、その実時間処理によって得られたデータに基づいて刺激電極へのフィードバック刺激を行うことも可能となる。

[0040] 図１５は、本発明の細胞計測システムで複数試料を計測できる機構の一例を説明する模式図である。この実施例のシステムは、分析モジュール、多段インキュベーター、電気解析モジュールとオンラインネットワークで接続されたオンライン解析モジュールとからなる。ここで、分析モジュールには、細胞形状の変化を計測する位相差顕微鏡あるいは微分干渉顕微鏡、そして蛍光顕微鏡とカメラ撮影解析による光学的計測、アガロースを顕微鏡システムを利用してミクロンのスケールで局所溶解できるアガロース加工技術からなっている。多段インキュベーターには、複数の細胞培養槽が配置されており、また、細胞培養槽の中には微小電極チップが配置されており、各細胞の電気信号の計測、電気的刺激がインキュベーター中で並列で連続処理できるようになっている。得られた電気信号については、電気解析モジュールで実時間計測を行い、そのデータはオンラインアクセスが可能なストレージに、光学計測のデータと電気計測データの結果が同じ時間スタンプを持った形で記録されており、この記録データにオンラインで解析モジュールが適宜アクセスすることで解析することができる。

[0041] 図１６は、本発明の細胞計測システムで計測できる心臓情報を説明する模式図である。微小電極上での１細胞の電気信号計測によって、Na、Ca、IKｒ、IKｓなどのイオンチャンネルの信号データを計測することができ、隣接す

る心筋細胞間での信号伝達の速度変化の計測からNaイオンチャンネルの阻害を計測することができる。また、１細胞の形状変化の光学計測によって不整脈の発生の計測および心臓の拍出量の見積もりができる。さらに、環状に細胞ネットワークを配置することによってリエントリーの発生を計測することができ、さらに線維芽細胞を細胞配置に加えることで心肥大などの病理心臓モデルとしての計測がすることができる。

[0042] 図１７は、本発明の細胞計測システムにおいて、自律拍動している心筋細胞から取得される細胞のフィールド・ポテンシャル（FP）信号波形の薬剤添加に対する変化の一例を例示するグラフである。細胞のフィールド・ポテンシャル信号波形は、図８に示したように細胞に流入するイオン、流出するイオンによって発生する細胞電位の変化を示すものであり、細胞電位の微分値、すなわち単位時間当たりのイオン電流の流れの総和であり、この場合、脱分極に至るプロセスでのナトリウムやカルシウムなどの内向きイオン電流が負に、それに続く再分極プロセスでのカリウムなどの外向きイオン電流が正に取られている。通常、この細胞のFP信号波形については、この図17に示すように隣接する各拍動についての相互の違いに着目するより、複数の隣接する波形を平均化してノイズ成分や隣接した各波形の違いなどの影響を排除した平均値としての１つのFP波形を抽出し、その平均値を反映した１波形の詳細解析によって各イオンチャンネルの状態を見積もるために用いている。しかし、本発明では、隣接するFP信号波形の平均値を取得するのではなく、隣接するFP信号波形の違いのうちイオンチャンネルの応答のゆらぎに依存する部分を比較抽出して、このゆらぎの大きさに基づいてイオンチャンネルのブロックされた量を定量的に推定するものである。これについては、一般にゆらぎの大きさが要素ｎの平方根の逆数[１／（ｎ）$^{1/2}$]で示されることから、その意味を理解することができる。すなわち、細胞表面にあるイオンチャンネルの数が、例えば10^4個機能している場合には、イオンチャンネルの総和としての機能のゆらぎの大きさは１％[１／(10^4)$^{1/2}$]となるが、薬剤によってブロックされることで10^2個まで機能しているイオンチャンネルが減少すれば、

その機能のゆらぎの大きさは１０％[１／(10²)^1/2]と急激に増加し、隣接するFP波形は大きな変化を発生させることとなる。すなわち、隣接するFP波形の変化を比較してゆらぎの大きさを見積もることができれば、このゆらぎの大きさからブロックされたイオンチャンネルの総量を見積もることができるのである。

[0043] この隣接する波形の変化について、特にカリウムイオンの放出によって発生する外向きイオン電流のピークの場所に着目し、例えばナトリウムイオンの細胞内への流入時間を基準（ゼロ）とし、その時間からカリウムイオンの放出のピークまでの時間をフィールド・ポテンシャル・デュレーション（ＦＰＤ）として定義すると、このＦＰＤの長さの変化は、ナトリウム、カルシュウム等のイオンの出入りに続くカリウムイオンの流入のピークの値となるため、細胞が持っているさまざまなイオンチャンネルが薬剤によってブロックされたことで発生するイオンの出入りの変化の総和としての変化量の指標として着目することができ、また、このＦＰＤの位置のゆらぎは、すべての関係する細胞のイオンチャンネルの隣接するＦＰ波形のゆらぎの総和を反映したものとなる。実際に、図１７のＦＰＤの位置（赤矢頭の位置）を確認すると、カリウムイオンチャンネル阻害剤であるＥ４０３１の添加によってＦＰＤは添加前には425-450msの間であったものが、10nMの添加で642-645ms、100nMの添加で663-694ms、1μMの添加で746-785msというように阻害剤の添加によって単調にＦＰＤの値は増加してゆき、また、隣接するＦＰＤは同じ値ではなく、ゆらぎを反映した異なる値を取ることとなる。

[0044] 図１８は、実際にカリウムイオンチャンネルを特異的に阻害する機能を持つ薬剤Ｅ４０３１によって細胞のカリウムイオンチャンネルが阻害されたときのＦＰＤの延長についてＥ４０３１濃度依存の実験結果の一例を示したものである。ここでは、カリウムイオンチャンネルの阻害によってイオンの流出が遅延し、ＦＰＤが濃度依存的に延長することが推測できる。この実験結果について、同様に上記ゆらぎ計測をする場合を考える。

[0045] 図１９は一般に心電図の拍動ゆらぎを計測するポワンカレプロッティング

を、ＦＰ波形におけるＦＰＤの値の評価に用いてＦＰＤのゆらぎに着目した場合の、隣接した拍動のＦＰＤがどの程度、相同状態からずれたかを見積もる手法の着目点のうち、隣接する拍動の揺らぎの大きさ（短期変動：STV:Short-term variability）の見積もり方を説明するものである。図１９（ａ）において、Ｘ＝Ｙとなる対角線は、隣接した拍動FPD_nとFPD_{n+1}がまったく同じＦＰＤの大きさを持つ場合に位置するものとなり、２つのＦＰＤの差（$FPD_{n+1}-FPD_n$）の大きさの対角線からの鉛直距離が基準化された隣接拍動自体のゆらぎの大きさとなり、特にｋ個のサンプリング数に対しては、図１９(ｂ)に示す（式１）のような数式で評価することができる。

[0046] 他方、図２０はポワンカレプロッティングを用いてＦＰＤのゆらぎに着目した場合の、隣接した拍動のＦＰＤがどの程度、相同状態からずれたかを見積もる手法の着目点のうち、隣接した各拍動が拍動の平均値（すべてのサンプルの総和でイオンチャンネルの応答の理想値に相当）からどれだけずれているかという観点での拍動の揺らぎの大きさ（長期変動：LTV:Long-term variability）の見積もり方を説明するものである。図２０（ａ）において、Ｘ＝Ｙとなる対角線上に配置されたＦＰＤの平均値FPD_{mean}と隣接した拍動FPD_nとFPD_{n+1}それぞれとの距離の２つのＦＰＤの差［（$FPD_{n+1}-FPD_{mean}$）＋（FPD_n-FPD_{mean}）］の大きさの対角線からの鉛直距離が基準化されたものでＦＰＤの平均値からのゆらぎの大きさとなり、特にｋ個のサンプリング数に対しては図２０（ｂ）の（式２）のような数式で評価することができる。これはＸ＝－Ｙの対称性からのずれを示しており、この大きさによって拍動が単なる平均値まわりのゆらぎを行っているのか、あるいは、履歴を持っているものかを見出す指標とすることができる。

[0047] 図２１には、実際にＥ４０３１を段階的に添加したときの心筋細胞の応答の一例をポワンカレプロッティングで示したＦＰＤのゆらぎと、そのゆらぎを定量的にＳＴＶとしてまとめたものである。イオンチャンネルがＥ４０３１の添加に応じてブロックされてゆくことがＦＰＤの時間の長さの延長によって推定されるとともに、特に高濃度の添加によって急激にＳＴＶの値が増

加することがわかる。

[0048] 図２２は、Ｘ軸に従来のＱＴ延長計測に当たるＦＰＤの延長が心筋細胞を用いて観察された割合（％）を、Ｙ軸にはＳＴＶの増加が観察された割合（％）を取り、心毒性があることが知られている薬剤、心毒性が無いことが知られている薬剤について評価を行ったものである。従来の薬剤の毒性検査については、Ｘ軸にあるＦＰＤのデータの結果のみによって評価していたが、Ｙ軸にあるＳＴＶの結果を加えて評価を行うと、図からもわかるように２次元のグラフ上のマッピングの中で、心毒性が高い高リスク(High risk)、リスクが低い（Low risk）、リスクなし（No risk）の３つの領域の中に、既知の文献結果と同じ分布をすることがわかる。このことから従来のＦＰＤに加えてＳＴＶを加えることで薬剤の心毒性の可能性についてより的確にかつ容易に推測を行うことが可能となることがわかる。

[0049] 図２３は薬剤の添加に対するＦＰＤについてのＳＴＶの応答の違いを示したものである。図２３（ａ）では、その一例として環状に心筋細胞ネットワークを構築した場合の局所のＦＰＤのポワンカレプロッティング（Ａ，Ｂ）、ならびに2次元に拡がった心筋シートを構築してその局所のポワンカレプロッティング（Ｃ，Ｄ）を計測した結果を（ｂ）に示す。この例ではペーメーカー領域ＰＭからＢ，Ｄは近傍、Ａ，Ｃは遠方に位置している。（ｂ）において薬剤添加前には、ポワンカレプロッティングのＸ＝Ｙの対角線上に分布していたＦＰＤは、低容量の心毒性薬剤添加によって、環状モデル（Ａ，Ｂ）ではともに大きなゆらぎが発生しＳＴＶの増加が観察されるが、2次元シートモデル（Ｃ，Ｄ）ではほとんどゆらぎが発生しない。また中容量の薬剤添加に対しては、環状モデル（Ａ，Ｂ）では拍動が細動状態あるいは停止状態に移行するが、2次元シートモデル（Ｃ，Ｄ）ではＰＭから遠方の領域ＣでＳＴＶの増加が見られるが、近傍の領域Ｄでは、まだＳＴＶの増加量は領域Ｃより低いことが観察される。この例でもわかるようにＦＰＤのＳＴＶ計測による薬物の心毒性の予測に対しては2次元シート状の細胞集団（ネットワーク）より、ペースメーカー領域から線状に配置された細胞集団（ネットワーク

）が、より正確に薬物の影響を反映させることがわかる。

[0050] 図２４は薬剤の添加に対するＰＭ領域からの拍動刺激の伝達速度（Ｖ）についてのＳＴＶの応答の違いを示したものである。これは、心毒性としておこるトルサードポワンツ（ＴｄＰ）は心筋細胞組織での伝達異常であることから、実際にＰＭ領域からの伝達速度がどの程度のゆらぎとして発生するかを確認することで薬物毒性を見積もる手法である。この場合にはＳＴＶの定義については図２４（ｃ）の（式３）に示したように、ＦＰＤに変わって、ＰＭ領域からの伝達時間Ｔ（あるいはＰＭからの距離をこの伝達時間で割った、その観測点での見かけ上の伝達速度Ｖ）を用いて計測することとなる。ＬＴＶについても定義はＳＴＶと同様にＦＰＤを、ＴあるいはＶに変更したものとなる。図２４（ａ）では、その一例として環状に心筋細胞ネットワークを構築した場合の局所の伝達時間Ｔのポワンカレプロッティング（Ａ，Ｂ）、ならびに2次元に拡がった心筋シートを構築してその局所のポワンカレプロッティング（Ｃ，Ｄ）を計測した結果を図２４（ｂ）に示す。この例でも図２３と同じくペーメーカー領域ＰＭからＢ，Ｄは近傍、Ａ，Ｃは遠方に位置している。図２４（ｂ）において薬剤添加前には、ポワンカレプロッティングのＸ＝Ｙの対角線上に分布していたＦＰＤは、低容量の心毒性薬剤添加によって、環状モデル（Ａ，Ｂ）ではともに大きなゆらぎが発生し伝達時間の大きなゆらぎを示すＳＴＶの増加が観察されるが、2次元シートモデル（Ｃ，Ｄ）ではほとんどゆらぎが発生しない。また中容量の薬剤添加に対しては、環状モデル（Ａ，Ｂ）では拍動が細動状態あるいは停止状態に移行するが、2次元シートモデル（Ｃ，Ｄ）ではＰＭから遠方の領域ＣでＳＴＶの増加が見られるが、近傍の領域Ｄでは、まだＳＴＶの増加量は領域Ｃより低いことが観察される。この例でもわかるように伝達時間Ｔ（あるいは各局所での見かけの伝達速度Ｖ）のＳＴＶ計測による薬物の心毒性の予測に対しては2次元シート状の細胞集団（ネットワーク）より、ペースメーカー領域から線状に配置された細胞集団（ネットワーク）が、より正確に薬物の影響を反映させることがわかると同時に、より効果的に空間配置依存的なゆらぎの発生を計

測できることがわかる。

[0051] 図２５は、本発明の心筋細胞ネットワークで各心筋細胞から電気的FP波形を取得するときの、そのFP波形と従来のin vitro計測技術（例えばパッチクランプ法）、従来のin vivo計測技術（例えば心電図）との関係を模式的に図示したものである。本発明の細胞のFP計測で得られた波形は、細胞から出入りする単位時間当たりのイオン流の大きさを示すものであり、細胞の電位変化情報（電気的にはイオン電流）となり、これは、従来の細胞ベースでのin vitro計測で得られる細胞電位とは、図２５に描いているように微分・積分の関係となる。次に、各細胞（あるいは細胞ネットワークの局所）から得られた１電極から計測したFP波形を、細胞ネットワークの複数の領域に配置した複数の電極から得られた各々のFP波形を重ね合わせることで細胞ネットワークの合成FP波形を得ることができるが、これは心臓から得られる電位変化信号波形である心電図のうち心室組織部分の応答にあたるQT領域の心電図データと相同性を持っているものである。

[0052] 図２６は、上記、図２５で説明を行った従来の技術で計測した情報と、本発明の装置で得られたＦＰデータとの相関関係を見積もるための装置システムの構成を模式的に示したものである。1細胞あるいは細胞ネットワーク上の局所のＦＰを計測できるように配置された複数の微小電極から１電極単位で取得したFPデータを集積し、その各ＦＰデータを微分して細胞電位を見積もることができる機能を有する演算回路、あるいは、各電極データを重ね合わせることによって、心電図の心室部分の心電図波形（Ｑ－Ｔ部分）との比較ができる演算回路を有するものである。特に、重ね合わせ回路を用いて単一の電極のＦＰデータを解析するだけでなく、例えば直列に配置された細胞ネットワーク上に等間隔で配置された複数の微小電極アレイのデータを合成することは、各電極上の細胞のＦＰの結果のみならず細胞間を伝達する状態を反映したデータを表示することとなり、特に、心筋細胞間の伝達異常である不整脈を見積もる上では、伝達の異常が合成ＦＰ波形に反映されることから、この重ね合わせ回路の結果を期外収縮発生の直接予測機構に情報を移動さ

せて、この結果から心電図解析と同様な予測が可能である。他方の微分回路から得られる細胞電位データについても細胞電位依存的に活性化状態が異なるイオンチャンネルの状態を見積もるために用いられる。

[0053] 図２７および図２８は、実際に上記図２６で述べた演算回路によって各電極からのＦＰデータを重ね合わせた例を示したものである。図２７では、図２７（Ａ）に示したように心筋細胞ネットワークが環状に配置されており、そのネットワークに沿って一定間隔で微小電極が配置されている。ペースメーカー（ＰＭ）領域が電極Ｒ１の位置にある環状心筋細胞ネットワークにおいて拍動シグナルがＲ２→Ｒ８、あるいはＬ１→Ｌ８に伝達するところが図２７（Ｂ）の各電極のＦＰ波形で見ることができる。そしてこれを重ね合わせた波形が下Sの波形となる。図２７（Ｃ）は、実際に長期で計測して合成した結果である。これが実際の心電図のＱＴ領域の波形を推定するために必要な、ＦＰの伝達情報が含まれた合成ＦＰ波形となる。この図からもわかるようにＰＭ領域から正常に拍動シグナルが伝達する場合には、図２７（Ｃ）からもわかるように合成波形もなめらかな波形となる。他方、図２８は図２８（Ｂ）からもわかるようにＰＭ領域からの拍動シグナルが規則正しく伝達しなくなる不整脈状態になると、合成ＦＰとなるＳも非常に乱れた波形となり、心電図に相当する図２８（Ｃ）でも不整脈と同様な形状の波形が合成ＦＰ波形としてみることができる。ここで特記すべきことは、図２８（Ｂ）の各微小電極の１電極データのみから不整脈を予測する場合には、観測する電極によっては（例えばＬ５）顕著な不整脈の発生を予測することは難しく、図２８（Ｂ）Ｓあるいは図２８（Ｃ）に見られるように合成ＦＰ波形とすることでより的確に予測することが可能となることがわかる。

[0054] 図２９は、心筋細胞の拍動周期に対するＦＰＤの大きさの関係を示したものである。黒丸は、いろいろな自律拍動を持つ心筋細胞でのＦＰＤを本発明の装置システムで計測した結果である。この結果からもわかるように、細胞はその拍動周期に依存してＦＰＤの値を変化させることがわかる。これは自律拍動を用いた心筋細胞による計測を行った場合に、薬剤によって細胞の拍

動周期が変化したり拍動停止したり、あるいは不安定化したときに、この副作用によって本来のイオンチャンネルのブロックではない原因によってＦＰＤが変化する可能性があることを示唆している。また、赤×印は強制拍動によって細胞の拍動周期を強制的に変化させたときのＦＰＤの値を示しており、外部からの連続刺激によって一定時間以上、一定の拍動周期を維持すれば、安定したＦＰＤとなることがわかる。

[0055] 図３０は、実際に自律拍動をしている心筋細胞に本発明のシステムを用いて外部から強制拍動刺激を与えたときの心筋細胞のＦＰＤの時間変化の一例を示したものである。この例でもわかるように、自律拍動インターバルが4秒程度であった細胞が１Ｈｚの強制拍動刺激を与えると直後からＦＰＤの値が大きく変化し、刺激開始後およそ３０秒ほどで550msの位置で安定化することがわかる。また強制拍動刺激終了後においても異なる自律拍動周期を持ち、また、ＦＰＤが徐々に長くなってゆくことも見ることができる。これらの結果からもわかるように、強制拍動刺激を与え始めてからＦＰＤが安定化するまでの刺激開始後30秒後以降に、薬物毒性検査をすることが望ましい。

[0056] 図３１は、実際に本発明のシステムを用いて外部から強制拍動刺激を与えてＦＰＤ、あるいは伝達時間Ｔ、あるいは伝達速度Ｖを計測する場合の細胞配置の例を示すものである。図３１（ａ）は、少なくとも２つの微小電極を覆うように配置された細胞集団を用いた刺激計測の例である。刺激電極から例えば毎分６０拍の一定のインターバルの強制刺激信号を与えながら隣接する計測電極で細胞のＦＰＤ、あるいは刺激電極の刺激時間から計測電極上の細胞までの拍動信号の伝達時間Ｔ，あるいは計測電極上の細胞までの伝達速度Ｖを計測するものである。図３１（ｂ）は、直線状に配置された心筋細胞ネットワークの端点に配置した刺激微小電極によって強制刺激拍動を与え、この伝達について、一定の間隔で心筋細胞ネットワークに沿って配置された微小電極アレイによって、各電極上の心筋細胞のＦＰＤ，Ｔ，Ｖを計測し、刺激電極の刺激信号に対する各電極のデータのみならず、各記録電極のＦＰの合成ＦＰによる不整脈発生の予測、各電極間でのＴとＶの関係などについ

て見積もることができる。ただし、ここで示したのはあくまでも細胞配置の一例であり、上記、図２７で示した環状細胞ネットワークのＰＭ領域に強制拍動を与えて同様の計測をすることもできるし、あるいは、刺激電極上の細胞をこの刺激電極を計測電極として用いることで、最小細胞数でのＦＰＤの計測をおこなうことも可能である。

[0057] またここまでのすべての実施例について、心筋細胞ネットワークについては一部心筋のみについて言及したが、繊維芽細胞を生体組織と同様な性質となるように添加することが含まれるものである。

[0058] 図３２は、微小電極２上に配置された細胞のＦＰ計測を行うために、電極２の電位を一定に保つ電位クランプ型のフィードバック制御機構を用いることができる。ここでは、細胞のＦＰは、従来の電極からの信号を増幅して計測するのではなく、電極２の電位を保つために外部の電源から供給される電流をモニターして、この結果を実時間で解析することで見積もるものである。これによって従来のたときの心筋細胞のＦＰＤの時間変化の一例を示したものである。ここで一定に保つ電位としては、通常はゼロを選ぶが、脱分極電位を変化させるなどの細胞状態を変化される場合には、電位を異なるものに調整することも可能である。

[0059] 図３３は、実際にヒトＥＳ細胞から心筋細胞に分化させた細胞集団の一部領域に上記記載の本発明のシステムを用いて強制拍動刺激を与えた時の、細胞集団の拍動周期の変化を計測した結果の一例のグラフである。このグラフからもわかるように、正常な心筋細胞集団では、この例のようにたとえば０．６Ｈｚから１．８Ｈｚの強制刺激を与えた時に、この範囲のすべてにおいて線形に、強制刺激に応答して拍動が追従することがわかる。

[0060] 図３４（ａ）は、実際に強制拍動刺激を与えたところで細胞集団の拍動がその強制拍動刺激のインターバルと同じ周期になることから、この強制拍動刺激状態での心筋細胞集団のＦＰの波形の変化とＦＰＤの長さの変化を示したものである。グラフからもわかるように、強制拍動刺激インターバルを早くすることでＦＰ波形が変化し、ＦＰＤの長さが短くなることがわかる。そ

こで図３４（ｂ）に示したように、このＦＰＤの変化をグラフで示すと、この短縮が、強制拍動インターバルの周期（ＲＲ）に依存することから、この補正

関係について、既知のヒト心臓においてのＱＴインターバルの長さと心拍との関係の研究（Patrick Davey, How to correct the QT interval for the effects of heart rate in clinical studies. Journal of Pharmacological and Toxicological Methods 48 (2002) 3 - 9）からFredericiaの補正、すなわち拍動の速さの違いによってＱＴの長さが変化してしまい相対比較できないことから、拍動周期が毎分６０拍の心筋拍動時のＱＴの長さ（QT_C）に補正するためにQT_C＝ＱＴ／（ＲＲ）$^{1/3}$として換算するもの、あるいは、Bazettが提唱したQT_C＝ＱＴ／（ＲＲ）$^{1/2}$として換算するものに一致するかどうかが重要となる。これは、上に説明したようにin vivoでのＱＴの長さは、本システムで計測する細胞ネットワーク全体で計測したＦＰＤの長さの重ね合わせに相当し、それは、すなわち各細胞のＦＰＤ自体が、上記、FredericiaあるいはBazettの補正の範疇に入るべきであることを示唆しているが、実際に、図３４（ｂ）の結果は、QT_C＝ＱＴ／（ＲＲ）$^{1/2.5}$となっており、FredericiaあるいはBazettの補正の間にあることがわかる。また、図３５は図３３および図３４のグラフに示したデータを表にしたものである。

[0061] これらの結果は、実際にスクリーニングや再生医療で使用する心筋細胞の品質の確認に、心筋細胞に対して強制拍動刺激を与えたときの心筋細胞の応答を計測することで対応できることを示している。すなわち

１）心筋細胞あるいは心筋細胞集団に対して強制拍動刺激を与え、細胞あるいは細胞集団がその強制拍動刺激に応答して強制拍動刺激と同じインターバルで応答するかどうかを確認し、かつ、その応答がどの強制拍動刺激の周波数の範囲で対応するかどうかを計測し、拍動が追従できることを確認することで健全な心筋細胞である十分条件の一つを満たしていると判断すること、あるいはさらに具体的に、この応答の追従がたとえば少なくても１．８Ｈｚまで追従することで健全な心筋細胞である十分条件の一つを満たしていると

判断すること。

２）上記、強制拍動刺激インターバル（ＲＲ）に対して細胞の拍動の追従が確認できた周波数の範疇で、強制拍動刺激に対するＦＰＤの変化が、ＦＰＤ／（ＲＲ）$^{1/3}$からＦＰＤ／（ＲＲ）$^{1/2}$の間にあることを確認することで健全な心筋細胞である十分条件の一つを満たしていると判断すること。

[0062] 上記手順を用いることで、心筋細胞の品質の管理が可能となる。健全な心筋細胞とは、安定した拍動を行うことが可能な細胞である。ここで評価対象となる細胞集団については、分化誘導を行った細胞集団をそのまま用いても良いし、あるいは、分化誘導を行った心筋細胞を分散し１細胞単位で計測評価しても良いし、あるいは、これら分散した心筋細胞を再度細胞集団化して計測しても良いし、あるいは、これら分散した心筋細胞をヒト心臓由来の線維芽細胞と混合して新たな細胞集団として計測評価しても良い。

これらの心筋細胞は、心筋毒性検査に使用することができる。

[0063] 図３６は、本発明の一実施形態に係る心筋毒性検査装置（または心筋毒性検査装置システム）の例示的な全体構成を示すブロック図である。本発明の心筋毒性検査装置３０００は、基本的には、測定部３００１および制御・解析部３００２から構成される。測定部３００１と制御・解析部３００２（例：パソコン）との間に、測定部３００１からの電気信号を増幅する増幅器３００４、増幅器３００４からのアナログ信号をデジタル信号に変換するアナログデジタル変換器（ＡＤＣ）３００３が介在する。インターフェース用パソコン３００２は、ＡＤＣからの信号を受けて記録・解析・表示等を行う。測定部３００１は、基本的には、複数の微小電極を含む多電極アレイを備えた培養容器、それを載置するｘ－ｙステージ、光学顕微鏡、レーザー光源、光学カメラ等の光学系を備える。複数の微小電極を含む多電極アレイを備えた培養容器は、微細加工技術により作製することもできるが、市販のもの（例：MED-P515A、アルファメッドサイエンティフィック社、日本）を使用してもよい。

[0064] 図３７Ａは、本発明の心筋毒性検査装置システム３０００のコアである測

定部３００１の拡大図である。図３７Ｂは、本発明のシステムにおいて使用される多電極アレイを備えた多電極チップ（または培養容器）の拡大図である。図３７Ｃは、その電極部分をさらに拡大した図である。複数の微小電極がアレイ状に配置され、それぞれの微小電極に引き出し線（またはリード線）が繋がっており、さらにその上にヒトＥＳ細胞由来の心筋クラスターが載置されているのが確認できる。

[0065] 図３８は、多電極アレイチップ（図３８ａ，ｂ，ｄ）を用いて心筋細胞（図３８ｂ）に対する薬物の毒性評価を行う際の、チップの電極と測定ウインドウとの対応関係（図３８ａ～ｃ）および分析に用いるデータの表示例（図３８ｅ）を示す図である。

[0066] 以下、本発明の心筋毒性検査装置を用いて薬物の心筋毒性検査を行う一例について説明する。

材料と方法

１．細胞培養

ヒトES細胞由来心筋クラスターはセラーティス社から購入した。生状態で受け取り、ガラスキャピラリー（Vitrolife社）を使って回収し、DMEMに20%非動化FBS、1%非必須アミノ酸、1%ペニシリンストレプトマイシン、0.2%ベータメルカプトエタノールを加えた培地中で培養した（全てInvitrogen社より購入）。

[0067] ２．多電極チップの用意(Day 0-3)

次に、回収したクラスターを多電極チップ（アルファメッドサイエンティフィック社）に配置した。配置した日をDay 0とした。Day3に200 mV以上の細胞外電位（FP)の1stピークが得られたクラスターをデータ取得に使用した。データ取得には多電極細胞電位計測システム（日京テクノス社）を使用した。

[0068] ３．薬剤適用(Day 4以降)

薬剤適用前に対象データ（’Before’と呼ぶ）を10分間取得した。薬剤は脱イオン化蒸留水(DDW)、またはDMSOに溶解し、PBSで希釈して適用した。最

終濃度はE-4031は0.1 μM 及び 1μM、DL-sotalolは0.1 mM 及び 1 mM、Diltiazemは0.1 mM 及び 1 mMで適用した。取得したデータから細胞外電位持続時間 (FPD)とFPDのSTV (short term variability)を算出した。

[0069] ４．データ解析

FPの下向きの急峻なピークはNa+流入に相当する。上向きの緩やかなピークはK+の流出に相当する。Na+とK+ピーク間の時間をFPDとした。さらに、FPDの拍動間のバラツキをFPDのSTVとして評価した。STVは以下の式で求めた。

$$STV = \frac{\sum \frac{|FPD_n - FPD_{n+1}|}{N \times \sqrt{2}}}{N = \text{Total number of FPD}}$$

FPD間のばらつきの増加はSTVの値の増加として評価できる。拍動レートのばらつきによるFPDへの影響を抑えるため、下のFredericiaの補正式を用いて、FPDとFPDのSTVを補正し、cFPD、cSTVを得た。

$$FPDc = \frac{FPD}{\sqrt[3]{RR}}$$

(RR は FPの直前のNa+ピーク間の時間)

[0070] ５．２次元プロットの作成手順

薬物の心毒性リスクを評価するため、QT延長やAPDの延長、及びこれらのSTVが単独で利用されている。FPD延長とSTV増加を統合して評価するため、我々は「2次元プロット」を開発した。X軸はFPDがBeforeより10%以上延長したクラスターの頻度(FPDスコア)を表す。Y軸はSTVがBeforeより100%以上増加したクラスターの頻度（STVスコア）を表す。それぞれのクラスターで適用２濃度のうち、いずれかで上記の増加が認められた場合、「延長した（FPD）」「増加した（STV）」とした。このプロットでは、薬物の心毒性の強度に依存した領域に薬物がプロットされる。

[0071] ６．結果

2次元プロット

図３９は評価した薬物の2次元プロットである。E-4031とDL-sotalolは図の右上にプロットされた。Diltiazemは左上に、DDWは右下にプロットされた。E-4031及びDL-sotalolはIKr阻害剤で、APDとSTVを増加させることがin vivo、in vitro両方で知られている。E-4031は心毒性のリスクが高い理由で開発が中断した薬物であり、心毒性が高い薬物である。DL-sotalolは抗不整脈薬だが、致死性不整脈のリスクが無視できないことから心毒性は高いと考えられる。DiltiazemはCa2+チャネル阻害剤で、APDはin vivoで減少することが知られており、心毒性リスクは低いと考えられている。DDWは心毒性無し、と考えられた。従って、我々の得たプロットでは、図中右上は「心毒性が高い」領域であり、左上および右下は「心毒性が低い」領域と判断した。未知の薬物を本評価法で評価したときに２次元プロットのどの領域にプロットされたかにより、心毒性の高低を評価できる。

[0072] 図４０（ａ）は、細胞信号のノイズを電子回路的に低減するために、と細胞１０が載っている微小電極２の近傍に細胞が載っていないむき出しの比較電極２cの間の電位の差分値を出力する回路を模式的に示したものである。実際に、この回路を図４０（b）に示すように、増幅回路の初段に組み込むことで、図４０（c）に示したようにノイズが特定の周波数に依存せず低減されることが分かる。このとき、比較電極２cの微小電極に対する位置はより近傍であることが望ましく、たとえば５０μmの距離に配置されているものは十分な機能を果たすが１ｍｍの距離までノイズ低減の機能をもたらすことができる。

[0073] 図４１は、本発明の心毒性評価の総合的評価法の例を模式的に示した図である。特定の濃度の薬剤を添加した後の心筋細胞の細胞電位計測の結果から得られたFPDの値について、まずFPD延長の程度の集計結果をX軸上の値とし、さらに上述のFPDの時間的ゆらぎの大きさをポワンカレプロッティングからSTVとしてY軸上の値とし、この結果をプロットする。図４１（b）は、様々な薬剤についてその結果X-Y図にプロットしたものの一例である。図からわかるように、FPDの延長とゆらぎ（STV）の増大が小さい領域にある薬剤は、QT延長

はあるが心毒性は無いと判断でき、FPDの延長とゆらぎ（STV）の増大が同時に起こる場合（X-Y図面で右上）にTdPなどの心毒性があることを予測することができる。

[0074] 図４２は、実際に心毒性を計測するためのシステムの構成の一例を示した模式図である。この実施例のシステムは、送液部、細胞培養計測部、細胞解析・刺激部からなっている。

[0075] 送液部は、細胞培養計測部の中で培養している各細胞培養チャンバーに培養溶液を連続して送液するシリンジポンプ系あるいはペリスタポンプ系あるいはHPLCポンプ系によって送液することができる。また、送液部の配管の外周には温度調整用の抵抗加熱用配線を巻き付け管内の液温を微小K型熱電対やサーミスタ等の熱検知機構で連続モニターすることで、導入する液温を制御する抵抗加熱の程度を調整して、つねに一定の温度での溶液が導入されるようになっている。また、この送液部には、試験する薬剤を添加するために合流配管や切り替え配管などの機構が配管中に配置されており、これによって希望する濃度の薬剤を各細胞培養チャンバーに導入することができる。また、導入される薬液の濃度を定量的に確認するために、液の導入管の一部が光学的に透明であり波長２８０nmから８００nmの範囲で吸光分光計測によって、定量的な評価が可能な機構が付加されていることが望ましい。また、同様に廃液についても廃液管の一部が光学的に透明であり波長２８０nmから８００nmの範囲で吸光分光計測によって、定量的な評価が可能な機構が付加されていることが望ましい。また薬液の制御温度としては、人体の正常温度に近い温度が通常望ましく、その観点からセ氏３０度から４５度の範囲での温度制御ができることが望ましい。

[0076] 図４３は、本発明の心毒性を計測するシステム中の細胞培養計測チャンバーの構成の一例を示す模式図と写真である。複数の細胞電位計測電極が配置された多電極基板４２０１（図４４を参照）は、培養液の導入機構・排出機構が配置された細胞培養容器４２０２が接着しており、同時に８検体の計測が可能な細胞培養計測プレートとなっている。図４４に細胞培養計測プレー

トの断面を模式的に示しているように、この細胞培養容器４２０２において、溶液の導入口は、多電極基板４２０１に一番近い底面に扇状に広がる形状で配置されており、他方、液の排出機構は、上部の液面の高さ４２０４を決める位置に液面の界面の方向と同じ向きに扇状に展開している。

[0077] 図４５は、多電極基板に配置されている電極配置の電極配線の構成を模式的に説明する図である。本発明では、細胞の形状を観察するために、電極にITOなどの透明電極を用いているが、透明電極の特性として通常の金属電極に比べて高い抵抗値を持っているため、特に多電極基板がプレートなどの大きなものとなると、配線が長くなりインピーダンスが非常に大きくなる。これを回避するために、透明電極上に金属レイヤーを透明電極と同じ配線で配置すれば、金属電極の導電性によって抵抗値を下げることができる。実際には、細胞を培養する領域では、光学的観察をすることからガラス基板４３０１上の透明電極４３０２を用いた配線を配置しており、細胞観察をしない領域では透明電極に重なるようにその上に金属レイヤー４３０３が配置されており、その上面が絶縁膜で被覆されている。ここで用いる金属電極素材としては、たとえば金、プラチナ、チタン、銅、アルミニムなどを用いれば良い。

[0078] 図４６は、多電極基板上の電極配置の例を示した模式図である。まず、図４６（ａ）では、細胞培養領域４４０４に直列に配置した心筋細胞ネットワークの一端を局所刺激する刺激電極４４０１と、この刺激電極によって刺激された心筋細胞の興奮伝導を計測する計測電極４４０２と、ノイズ除去のための参照電極４４０３が配置されている。各計測電極４４０２から得られた心筋細胞ネットワークの複数の局所応答の結果を計測することができるとともに、伝達速度のゆらぎを各計測電極間の伝達毒度の比較解析をすることで求めることができる。図４６（b）では、計測電極が一直線に繋がっているものであり、この構成にすることで、心電図のST領域（心室領域）の心電図波形と同様な波形を計測することができる。図４６（c）では、上記、図４６（b）の一部を切り離して、局所の心筋細胞のFP波形を取得することを容易にしたものである。図４６（d）は、リング状電極の上にリング状に細胞を配置し

て計測するための電極配置の一例である。これらは図１１、図１２に示したようにリング状に配置した心筋細胞ネットワークを計測するものであるが、リング状の計測電極の一部を欠損させ、その部位に局所強制刺激を与えるための刺激電極を配置し、また、ノイズ除去のための参照電極を配置している。さらに図４６（ｅ）では、計測電極も分割して、局所心筋細胞の応答を計測することが可能な配置となっている。

産業上の利用可能性

[0079] 本発明によれば、iPS細胞等の幹細胞から分化させた心筋細胞について、その心筋細胞が創薬スクリーニングあるいは再生医療のために使うことができる健全な心筋細胞であるかどうかを評価できる。

符号の説明

[0080] １…透明基板、２…微小電極、２ｃ…比較電極、２’…微小電極２の引き出し線、３_{1}，３_{2}，３_{3}及び３_{4}…アガロースゲルによる壁、４_{1}，４_{2}，４_{3}及び４_{4}…間隙、７…周辺を取り巻く壁、８_{1}，８_{2}，８_{3}…パイプ、ＰＣ…パソコン、Ｍｓ…パソコンの操作信号、１０，１０_{1}，１０_{2}，１０_{3}，－－－，１０_{n}…心筋細胞あるいは線維芽細胞、１５…光学観察装置の透明ステージ、１６…Ｘ－Ｙ駆動装置、１８…Ｚ駆動装置、$ＣＨ_{1}$、$ＣＨ_{2}$、$ＣＨ_{3}$および$ＣＨ_{n}$…細胞保持部、ＣＣＣ…細胞連絡チャネル、１０_{G}…細胞集団、１１_{a}…障壁、１１_{b}…開口、１９、１９１、１９２、１９３…ダイクロイックミラー、２０、２０１…バンドパスフィルタ、２１、２１１…カメラ、２２…光源、２１…蛍光光源、２３、２３１…バンドパスフィルタ、２４、２４１…シャッター、２５…コンデンサレンズ、２６…対物レンズ、２７…可動電極、２８…接地電極、２９、２９１…スイッチング回路、３０、３０１…電気信号計測回路、３１、３１１…電気刺激回路、３２…心筋細胞、３３…線維芽細胞、３４…細胞配置用ピペット、３５…N周回目の伝達経路、３６…（N＋１）周回目の伝達経路、３７…（N＋２）周回目の伝達経路、３８…測定電極、３９…参照電極、４０…送液系、４１…環状に配置した細胞集団、４２…９６穴ウエルプレート、４３…カメラ受光素子、４４…細胞、４５…細胞刺激

電極、１００…心筋毒性検査装置、3000…心筋毒性検査装置、3001…測定部、3002…インターフェース用パソコン、3003…アナログデジタル変換器、3004…増幅器、4201…多電極基板、4202…細胞培養容器、4203…溶液の流れ、4204…液面の高さ、4301…ガラス基板、4302…透明電極、4303…金属レイヤー、4304…絶縁膜、4401…刺激電極、4402…計測電極、4403…参照電極、4404…細胞培養領域。

請求の範囲

［請求項1］ 基板、

該基板上に配置した複数個の安定した拍動を行う評価対象の心筋細胞あるいは該心筋細胞を含む細胞集団、

前記基板上に前記細胞集団の周辺を囲むように形成された細胞培養液を満たすための壁、

前記細胞培養液を前記壁で囲われた領域内に供給しおよび／または排出する培養液供給／排出チャネル、

前記細胞集団の一つの細胞あるいは細胞集団の局所部分を載置している微小電極、

前記壁で囲われた細胞培養液を満たすための領域内に設けられた比較電極、

前記微小電極のそれぞれに接続された引き出し線と前記比較電極に接続された引き出し線とを用いて前記微小電極に載置されている細胞電位を計測する電位計測手段、ならびに

前記微小電極へ送る電気刺激を制御し、前記電位計測手段で計測した電位データを記録する、制御／記録手段

を備えた細胞品質評価装置であって、

前記微小電極へ送る前記電気刺激のインターバルを段階的に変化させて、

１）前記心筋細胞あるいは前記細胞集団が前記電気刺激のインターバルによる強制拍動刺激に応答して前記強制拍動刺激と同じインターバルで応答するかどうかを確認し、かつ、その応答がどの強制拍動刺激の周波数の範囲で対応するかどうかを計測し、拍動が追従できることを確認することで健全な心筋細胞である十分条件の一つを満たしていると判断すること、および

２）前記電気刺激による強制拍動刺激インターバル（ＲＲ）に対して細胞の拍動の追従が確認できた周波数の範疇で、強制拍動刺激に対す

るＦＰＤの変化が、ＦＰＤ／（ＲＲ）1/3からＦＰＤ／（ＲＲ）1/2の間にあることを確認することで健全な心筋細胞である十分条件の一つを満たしていると判断すること
を可能にする、細胞品質評価装置。

[請求項2] 基板、

該基板上に配置した複数個の安定した拍動を行う評価対象の心筋細胞あるいは該心筋細胞を含む細胞集団、

前記基板上に前記細胞集団の周辺を囲むように形成された細胞培養液を満たすための壁、

前記細胞培養液を前記壁で囲われた領域内に供給しおよび／または排出する培養液供給／排出チャネル、

前記細胞集団の一つの細胞あるいは細胞集団の局所部分を載置している微小電極、

前記壁で囲われた細胞培養液を満たすための領域内に設けられた比較電極、

前記微小電極のそれぞれに接続された引き出し線と前記比較電極に接続された引き出し線とを用いて前記微小電極に載置されている細胞電位を計測する電位計測手段、ならびに

前記微小電極へ送る電気刺激を制御し、前記電位計測手段で計測した電位データを記録する、制御／記録手段
を備えた細胞品質評価装置を用いて、

前記微小電極へ送る前記電気刺激のインターバルを段階的に変化させて、

１）前記心筋細胞あるいは前記細胞集団が前記電気刺激のインターバルによる強制拍動刺激に応答して前記強制拍動刺激と同じインターバルで応答するかどうかを確認し、かつ、その応答がどの強制拍動刺激の周波数の範囲で対応するかどうかを計測し、拍動が追従できることを確認することで健全な心筋細胞である十分条件の一つを満たしてい

ると判断すること、および

２）前記電気刺激による強制拍動刺激インターバル（ＲＲ）に対して細胞の拍動の追従が確認できた周波数の範疇で、前記強制拍動刺激に対するフィールド・ポテンシャル・デュレーション（ＦＰＤ）の変化が、ＦＰＤ／（ＲＲ）$^{1/3}$からＦＰＤ／（ＲＲ）$^{1/2}$の間にあることを確認することで健全な心筋細胞である十分条件の一つを満たしていると判断すること

を含む、細胞品質評価方法。

[請求項3] 心筋拍動のフィールド・ポテンシャル波形のゆらぎの大きさを、隣接する拍動信号の比較によって計測するように構成されている、心筋毒性検査装置。

[請求項4] フィールド・ポテンシャル波形のうちカリウムイオンの細胞からの流出のピーク位置のナトリウムイオン流出ピークからの経過時間（フィールドポテンシャルデュレーション：ＦＰＤ）のゆらぎの大きさを、隣接する拍動信号の比較によって計測するように構成されている、請求項３に記載の心筋毒性検査装置。

[請求項5] ＦＰＤおよび該ＦＰＤのゆらぎの大きさを算出し、該算出したＦＰＤおよび該ＦＰＤのゆらぎの大きさの組み合わせを指標とするように構成されている、請求項３または４に記載の心筋毒性検査装置。

[請求項6] 前記ＦＰＤのゆらぎの大きさが該ＦＰＤの短期変動（ＳＴＶ）である、請求項３～５のいずれか一項記載の心筋毒性検査装置。

[請求項7] 透明基板、

前記透明基板上に形成された壁で囲まれた領域であって、細胞培養液で満たされている領域、

前記細胞培養液で満たされている領域内に配置された複数個の安定した拍動を行う心筋細胞を含む細胞集団、

前記細胞培養液で満たされている領域内に設けられた、前記細胞集団の一つの心筋細胞あるいは前記細胞集団の局所部分を載置している

微小電極、

前記細胞培養液で満たされている領域内に設けられた比較電極、

前記微小電極のそれぞれに接続された引き出し線と前記比較電極に接続された引き出し線とを用いて前記微小電極に載置されている前記心筋細胞の電位を計測する電位計測手段、

前記電位計測手段で計測した被検薬物加入前後における前記心筋細胞の電位データを記録する記録手段

を備える、請求項３～６のいずれか一項記載の心筋毒性検査装置。

[請求項8] 前記安定した拍動を行う心筋細胞を含む細胞集団が、拍動を伝えることが可能な複数個の心筋細胞および線維芽細胞を含む細胞ネットワークである、請求項３～７のいずれか一項記載の心筋毒性検査装置。

[請求項9] 透明基板、

該透明基板上に配置した複数個の安定した拍動を行う心筋細胞を含む細胞集団からなるペースメーカー領域、

該透明基板上に配置され、該細胞集団と連携して前記細胞集団の拍動を伝える複数個の直列配列された心筋細胞および線維芽細胞を含む細胞ネットワーク、

前記透明基板上に前記細胞集団および前記細胞ネットワークの周辺を囲むように形成された細胞培養液を満たすための壁、

前記細胞培養液を前記壁で囲われた領域内に供給しおよび／または排出する培養液供給／排出チャネル、

前記細胞に作用する薬剤を前記細胞培養液に加えるための薬剤供給チャネル、

前記細胞集団の一つの細胞あるいは細胞集団の局所部分を載置している微小電極、

前記細胞ネットワークの個々の細胞をそれぞれ載置している微小電極、

前記壁で囲われた細胞培養液を満たすための領域内に設けられた比

較電極、

前記微小電極のそれぞれに接続された引き出し線と前記比較電極に接続された引き出し線とを用いて前記微小電極に載置されている細胞電位を計測する電位計測手段、ならびに

前記微小電極へ送る電気刺激を制御し、前記電位計測手段で計測した電位データを記録する、制御／記録手段

を備える、請求項３～８のいずれか一項に記載の心筋毒性検査装置。

[請求項10] 前記安定した拍動を行う心筋細胞を含む細胞を載置するための少なくとも１つの微小電極から該微小電極上に載置される細胞に一定のインターバルで強制拍動刺激を与えることができるように構成されている、請求項３～９のいずれか一項記載の心筋毒性検査装置。

[請求項11] 前記直列配列された細胞ネットワークの端点に局所に刺激を与えるための電極が配置されている、請求項８～１０のいずれか一項記載の心毒性検査装置。

[請求項12] 前記細胞ネットワークが、リング状に配置されて形成されており、

該リングの一部を欠損させて、その点に局所に刺激を与える電極が配置されている、請求項８～１１のいずれか一項記載の心毒性検査装置。

[請求項13] 前記細胞が載置された各微小電極から取得される心筋拍動のフィールド・ポテンシャル波形のゆらぎの大きさを、前記細胞ネットワークの前記細胞の拍動が発生する領域から観察電極までの伝達時間あるいは伝達速度のゆらぎを隣接する拍動の比較によって計測するように構成されている、請求項８～１２のいずれか一項記載の心筋毒性検査装置。

[請求項14] 前記心筋細胞を適切な温度に維持する温度制御機構を有する請求項１～１３のいずれか一項記載の心筋毒性検査装置。

[請求項15] 前記微小電極が、複数の微小電極からなる多電極アレイを構成している、請求項１～１４のいずれか一項記載の心筋毒性検査装置。

[請求項16] 心筋拍動のフィールド・ポテンシャル波形のゆらぎの大きさを、隣接する拍動信号の比較によって計測することを特徴とする心筋毒性検査方法。

[請求項17] 複数個の安定した拍動を行う心筋細胞を含む細胞集団を準備する工程、

培養容器内の底面に多電極アレイが配された該培養容器中で前記細胞集団を培養する工程、

前記培養している前記細胞集団へ被検薬物を添加し、前記多電極アレイを用いて前記培養細胞集団の細胞電位を測定する工程であって、前記薬物の添加の前後において、前記培養細胞集団の細胞電位を測定し、該細胞電位のデータを取得する工程、ならびに

前記取得した細胞電位のデータから、フィールド・ポテンシャル（ＦＰ）波形のうちナトリウムイオンの細胞への流入ピークからカリウムイオンの細胞からの流出ピークまでの経過時間（細胞外電位持続時間）（ＦＰＤ）および該ＦＰＤのゆらぎの大きさを算出し、該算出したＦＰＤおよび該ＦＰＤのゆらぎの大きさの組み合わせを指標として用いて前記薬物の心筋毒性を評価する工程、

を含む、請求項１６に記載の薬物の心筋毒性検査方法。

[請求項18] 請求項８～１５のいずれか一項記載の心筋毒性検査装置を使用して、前記細胞に作用する薬剤が前記細胞培養液に加えられたときに、前記心筋細胞集団の発生する拍動が前記細胞ネットワーク領域の前記細胞を伝播する速度を遅延させるか否かを評価して前記細胞に作用する薬剤の心筋細胞に対する毒性を検査する請求項１６または１７に記載の心筋毒性検査方法。

[請求項19] 請求項８～１５のいずれか一項記載の心筋毒性検査装置を用いて前記細胞に作用する薬剤が前記細胞培養液に加えられたときに、前記心筋細胞集団の発生する拍動が前記細胞ネットワーク領域の前記細胞を伝播する速度を遅延させるか否かを評価して前記細胞に作用する薬剤

の心筋細胞に対する毒性を検査する方法であって、下記のうちの少なくとも１つの工程を含む請求項１６～１８のいずれか一項記載の方法：

(i)前記細胞ネットワークの前記細胞を載置するための少なくとも１つの微小電極から該微小電極上に載置される細胞に一定のインターバルで強制拍動刺激を与える工程；

(ii)前記細胞が載置された各微小電極から取得される心筋拍動のフィールド・ポテンシャル波形のゆらぎの大きさを、隣接する拍動信号の比較によって計測する工程；

(iii)フィールド・ポテンシャル波形のうちカリウムイオンの細胞からの流出のピーク位置のナトリウムイオン流出ピークからの経過時間（フィールドポテンシャルデュレーション）のゆらぎの大きさを、隣接する拍動信号の比較によって計測する工程；

(iv)前記細胞が載置された各微小電極から取得される心筋拍動のフィールド・ポテンシャル波形のゆらぎの大きさを、前記細胞ネットワークの前記細胞の拍動が発生する領域から観察電極までの伝達時間あるいは伝達速度のゆらぎを隣接する拍動の比較によって計測する工程；

(v)前記細胞が載った各微小電極から取得される心筋拍動のフィールド・ポテンシャル波形の各々について微分することによって細胞電位を計測する工程；

(vi)前記細胞が載置された各電極から取得される心筋拍動のフィールド・ポテンシャル波形を重ね合わせて合成フィールド・ポテンシャル波形を合成し、心電図データと比較可能な細胞集団全体としての拍動伝達の乱れ具合を計測する工程；

(vii)前記細胞の配置された微小電極の電位を一定に保つフィードバック電位制御機構を用いて、該電位制御機構が供給する電流量を解析する工程；および

(viii)前記細胞への周期的強制拍動刺激開始後、３０秒以上経過してから計測を開始する工程。

［請求項20］ 前記ＦＰＤのゆらぎの大きさが該ＦＰＤの短期変動（ＳＴＶ）である、請求項１６～１９のいずれか一項記載の方法。