

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43bis.1)

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Date of mailing
(day/month/year)

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Applicant's or agent's file reference
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FOR FURTHER ACTION

See paragraph 2 below

International application No.

PCT/US 23/62398

International filing date (day/month/year)

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ADD. A61L 27/60, A61P 17/00

Applicant **REGENX SCIENCE, INC.**

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☒ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1bis(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA/US
Mail Stop PCT, Attn: ISA/US
Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450
Facsimile No. 571-273-8300

Date of completion of this opinion

20 April 2023 (20.04.2023)

Authorized officer

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Form PCT/ISA/237 (cover sheet) (revised July 2022)

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Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:
☒ the international application in the language in which it was filed.
☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).
2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(b)).
3. ☐ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing:
 - a. ☐ forming part of the international application as filed.
 - b. ☐ furnished subsequent to the international filing date for the purposes of international search (Rule 13ter.1(a)),
☐ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.
4. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this opinion has been established to the extent that a meaningful opinion could be formed without a WIPO Standard ST.26 compliant sequence listing.
5. Additional comments:

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Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 33-35

because:

☐ the said international application, or the said claims Nos. _____ relate to the following subject matter which does not require an international search (*specify*):

☒ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. 33-35 are so unclear that no meaningful opinion could be formed (*specify*):

Claims 33-35 are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

☐ the claims, or said claims Nos. _____ are so inadequately supported by the description that no meaningful opinion could be formed (*specify*):

☒ no international search report has been established for said claims Nos. 33-35

☐ a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit:

☐ furnish a sequence listing in the form of an Annex C/ST.25 text file, and such listing was not available to the International Searching Authority in the form and manner acceptable to it; or the sequence listing furnished did not comply with the standard provided for in Annex C of the Administrative Instructions.

☐ furnish a sequence listing on paper or in the form of an image file complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in the form and manner acceptable to it; or the sequence listing furnished did not comply with the standard provided for in Annex C of the Administrative Instructions.

☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rule 13ter.1(a) or (b).

☐ See Supplemental Box for further details.

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Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees the applicant has, within the applicable time limit:
- ☐ paid additional fees.
 - ☐ paid additional fees under protest and, where applicable, the protest fee.
 - ☐ paid additional fees under protest but the applicable protest fee was not paid.
 - ☒ not paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is

- ☐ complied with.
- ☒ not complied with for the following reasons:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I: Claims 1-14 directed to an isolated ECM.

Group II: Claims 15-32 directed to a method of decellularizing amphibian ECM.

The inventions listed as Groups I-II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

SPECIAL TECHNICAL FEATURES

The invention of Group I includes the special technical feature of an isolated amphibian ECM, wherein: only 1% to 10% of the total DNA present in a corresponding native ECM sample, residual DNA fragments of less than 300 bases (bps), and/or greater than 70% of its cells removed, not required by the claims of Group II.

The invention of Group II includes the special technical feature of a method comprising the steps of harvesting an amphibian biological sample, washing the sample with a detergent solution, and treating the sample with one or more proteases and one or more nucleases, not required by the claims of Group I.

COMMON TECHNICAL FEATURES

Groups I-II share the common technical feature of an isolated, decellularized amphibian ECM. However, this shared technical feature does not represent a contribution over prior art as being anticipated by US 2020/0188557 A1 to ISE Professional Testing & Consulting Services, Inc., which discloses of an isolated, decellularized amphibian ECM (para [0036]: "According to one aspect of the invention, a product is provided. The product is prepared by isolating Urodele ECM from a Urodele, decellularizing the ECM, and sterilizing the decellularized ECM"; para [0014]: "Urodele' here denotes a salamander of the order Urodela, also known as the order Caudata, in the class Amphibia").

As the common technical features were known in the art at the time of the invention, these cannot be considered special technical features that would otherwise unify the groups.

Therefore, Groups I-II lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.

Item 4 (cont'd):

Claims 33-35 have been found to be unsearchable because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Notes to Application:

The term "ECM" used throughout the claims and specification has been interpreted to mean "extracellular matrix" (see instant specification [0004]).

4. Consequently, this opinion has been established in respect of the following parts of the international application:

- ☐ all parts.
- ☒ the parts relating to claims Nos. 1-14

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Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-14	YES
	Claims	None	NO
Inventive step (IS)	Claims	None	YES
	Claims	1-14	NO
Industrial applicability (IA)	Claims	1-14	YES
	Claims	None	NO

2. Citations and explanations:

Claims 1 and 5-14 lack inventive step under PCT Article 33(3) as being obvious over US 2020/0188557 A1 ISE Professional Testing & Consulting Services, Inc. (hereinafter "ISE").

Regarding claim 1, ISE discloses of an isolated amphibian ECM, wherein the ECM is decellularized and comprises an intact native ECM structure (para [0036]: "According to one aspect of the invention, a product is provided. The product is prepared by isolating Urodele ECM from a Urodele, decellularizing the ECM, and sterilizing the decellularized ECM"), which is substantially free of cells (para [0049]: "Decellularized ECM" means extra cellular matrix sufficiently free of cellular components to eliminate or reduce antigenicity of the extra cellular matrix to an extent where the matrix would be considered non-toxic as a xenograft"). ISE does not disclose that only 1% to 10% of the total DNA present in a corresponding native ECM sample; residual DNA fragments of less than 300 bases (bps); and/or greater than 70% of its cells removed. However, it would have been obvious to one of ordinary skill in the art that the ECM disclosed by ISE has greater than 70% of its cells removed, since ISE discloses that the ECM is "substantially free" of cells to reduce the negative or toxic side effects of the cells, as discussed above.

Regarding claim 5, ISE discloses of the ECM of claim 1, which is substantially free of cells, as discussed above. However, ISE does not disclose that the ECM has 80% to 90% of its cells removed. However, it would have been obvious to one of ordinary skill in the art that the ECM disclosed by ISE has 80% to 90% of its cells removed, since ISE discloses that the ECM is "substantially free" of cells to reduce the negative or toxic side effects of the cells, as discussed above.

Regarding claim 6, ISE further discloses of the ECM of claim 1, wherein the ECM is in the form of dry powder, a paste, a solution, an extract, a cream, a lotion, dispersion, or an emulsion (para [0028]: "In some embodiments, the decellularized ECM is a dry powder").

Regarding claim 7, ISE further discloses of the ECM of claim 1, is obtained from an amphibian, particularly Urodele (para [0014]: "The biomaterial of the present invention is obtained from tissue of a urodele. 'Urodele' here denotes a salamander of the order Urodela, also known as the order Caudata, in the class Amphibia"). ISE does not disclose that the ECM is obtained from a young amphibian. However, it would have been obvious to one of ordinary skill in the art through routine experimentation to select a particular source of the ECM, such as a young amphibian, in order to produce an ECM which is suitable for a particular use, e.g., cell growth scaffold, pharmaceutical formulations, etc. (see para [0086]-para [0087], para [0093]- para [0095]).

Regarding claim 8, ISE further discloses of the ECM of claim 1, wherein the ECM is obtained from a neotenic Urodele (para [0014]: "The biomaterial of the present invention is obtained from tissue of a urodele. 'Urodele' here denotes a salamander of the order Urodela, also known as the order Caudata, in the class Amphibia"; para [0015]: "Some families of urodeles are 'neotenic,' which means that individuals with such families can exhibit juvenile features, such as gills and fins, even after reaching sexually maturity").

Regarding claim 9, ISE discloses of a composition, wherein the composition comprises the ECM of any one of claims 1-8 and a carrier (para [0096]: "Pharmaceutical formulations [of the ECM] may additionally comprise a pharmaceutically acceptable excipient, which, as used herein, includes, but is not limited to, any and all solvents, dispersion media, diluents, or other liquid vehicles, dispersion or suspension aids, surface active agents, isotonic agents, thickening or emulsifying agents, preservatives, and the like...").

Regarding claim 10, ISE further discloses of the composition of claim 9, wherein the composition is a pharmaceutical or cosmetic composition, and optionally wherein the pharmaceutical composition comprises a pharmaceutically acceptable carrier, and wherein the cosmetic composition comprises a cosmetically acceptable carrier (para [0096]: "Pharmaceutical formulations [of the ECM] may additionally comprise a pharmaceutically acceptable excipient, which, as used herein, includes, but is not limited to, any and all solvents, dispersion media, diluents, or other liquid vehicles, dispersion or suspension aids, surface active agents, isotonic agents, thickening or emulsifying agents, preservatives, and the like...").

---Continued in Supplemental Box---

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Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

The term "ECM" used throughout the claims and specification has been interpreted to mean "extracellular matrix" (see instant specification [0004]).

Claim 1 is unclear and confusing. Specifically, it is unclear from the indentation, the semicolons, and the placement of "and/or" whether claim 1 reads as: the ECM comprises (1) an intact native ECM structure, (2) only 1% to 10% of the total DNA present in a corresponding native ECM sample, (3) residual DNA fragments of less than 300 bases (bps), and/or (4) greater than 70% of its cells removed, or, the ECM comprises an intact native ECM structure, and (1) only 1% to 10% of the total DNA present in a corresponding native ECM sample, (2) residual DNA fragments of less than 300 bases (bps), and/or (3) greater than 70% of its cells removed. Instant specification [0097] contains very similar language, however, [0097] states that "the ECM is decellularized, includes an intact native ECM structure, and is characterized by having: less than 1% to 10% of the total DNA present in a corresponding native ECM sample; residual DNA fragments of less than 300 bases (bps); and/or greater than 70% of its cells removed." Therefore, claim 1 has been interpreted to be directed to an isolated amphibian ECM, wherein the ECM is decellularized and comprises an intact native ECM structure; and wherein only 1% to 10% of the total DNA present in a corresponding native ECM sample, residual DNA fragments of less than 300 bases (bps), and/or greater than 70% of its cells removed.

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Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of:

Box V.2 Citations and Explanations

Regarding claim 11, ISE further discloses of the composition of claim 10, wherein the composition further comprises one or more agents, and optionally, wherein the one or more agents are xenogenic to the isolated ECM (para [0095]: "When other active agents are included, relative amounts of agents combined with the ECM of the invention will be known to those of ordinary skill in the art..."; para [0028]: "In any of the embodiments, the decellularized ECM can be infused with, coated with, combined with or attached, covalently or non-covalently, to an agent xenogenic to a Urodele").

Regarding claim 12, ISE further discloses of the composition of claim 10, wherein the composition further comprises one or more agents comprising peptides, proteins, drugs, nutrients, retinoids, emollients, steroids, carbohydrates, glycoproteins, polymers, or a combination thereof (para [0028]: "In any of the embodiments, the agent can be any one or any combination of a growth factor, a cytokine, a chemokine, a protein, a carbohydrate, a sugar, a steroid, an antimicrobial agent, a synthetic polymer, an adhesive, a drug...").

Regarding claim 13, ISE further discloses of the composition of claim 10, wherein the composition further comprises one or more growth factors, cytokines, chemokines, or a combination thereof (para [0028]: "In any of the embodiments, the agent can be any one or any combination of a growth factor, a cytokine, a chemokine...").

Regarding claim 14, ISE further discloses of the composition of claim 9, wherein the composition further comprises one or more polymers, and optionally wherein the one or more polymers are synthetic or natural polymers or copolymers (para [0028]: "In any of the embodiments, the agent can be any one or any combination of a growth factor, a cytokine, a chemokine, a protein, a carbohydrate, a sugar, a steroid, an antimicrobial agent, a synthetic polymer...").

Claims 2-4 lack inventive step under PCT Article 33(3) as being obvious over ISE in view of the article entitled "Quantification of DNA in Biologic Scaffold Materials" by Gilbert et al. (hereinafter "Gilbert").

Regarding claim 2, ISE discloses of the ECM of claim 1, as discussed above, however, ISE does not disclose what the amount of DNA present in the corresponding native ECM sample is. Gilbert discloses of the DNA content and fragment length in certain commercially available ECM materials (abstract: "The objective of the present study was to determine the DNA content and fragment length in both laboratory produced and commercially available ECM scaffold materials"), with all ECM materials containing less than 1% DNA (page 4 para 2: "This study shows that most of the commercially available naturally occurring ECM devices tested contain at least trace amounts of DNA... [t]he extent of DNA removal did vary considerably, with Restore showing prominent histologic staining for nuclear material and 0.1% DNA compared to 0.01% DNA for most other devices"; see fig. 1). Therefore, it would have been obvious to one of ordinary skill in the art through routine experimentation to select a particular isolated amphibian ECM, or adjust the parameters of the decellularization process to produce a select isolated amphibian ECM, such that the ECM comprises between 2% to 10% of the total amount of DNA present in the corresponding native ECM sample, in order to ensure that the ECM has a reduced amount of DNA to prevent negative side effects, as disclosed by Gilbert (see page 4 para 3).

Regarding claim 3, ISE discloses of the ECM of claim 1, as discussed above, however, ISE does not disclose what the length of the DNA fragments are. Gilbert discloses of the DNA content and fragment length in certain commercially available ECM materials (abstract: "The objective of the present study was to determine the DNA content and fragment length in both laboratory produced and commercially available ECM scaffold materials"), with all ECM materials having DNA fragment lengths between 100 and 300 bp (page 4 para 6: "In most of the biologic scaffold materials that were investigated in the current study, the DNA that was present consisted of fragments less than 300 bp in length"; see fig. 2). Therefore, it would have been obvious to one of ordinary skill in the art through routine experimentation to select a particular isolated amphibian ECM, or adjust the parameters of the decellularization process to produce a select isolated amphibian ECM, such that the ECM has DNA fragments having lengths between 50 and 300 bps, based on the teachings of Gilbert, since Gilbert discloses that commercially available ECM materials have DNA fragments with this length, as discussed above.

Regarding claim 4, ISE and Gilbert disclose of the ECM of claim 3, and Gilbert further discloses wherein the ECM comprises residual DNA fragments of less than 250 bps in length (see page 3 para 4, ECM samples have lengths that range between 100 and 200 bps).

Claims 1-14 have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used in industry.