Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 3761–3764, June 1984
Cell Biology

Monitoring fibroblast behavior in tissue culture with an applied electric field

(cell locomotion/cytochalasin B)

I. GIAEVER AND C. R. KEESE

General Electric Research and Development Center, Schenectady, NY 12301

Contributed by I. Giaever, March 2, 1984

ABSTRACT **Mammalian fibroblasts have been cultured on evaporated gold electrodes subjected to an alternating electric field at 4000 Hz. The system consists of a large (≈ 2 cm^2) and a small ($\approx 3 \times 10^{-4}$ cm^2) electrode bathed in tissue culture medium. The applied electric field produces a voltage drop at the boundary between the solution and the small electrode of a few mV at a current density of a few mA/cm^2. The small population of cells that attach and spread on this electrode have a marked effect on the measured impedance and also cause it to fluctuate with time. The amplitude of these fluctuations is greatly reduced by cytochalasin B (10 μM), suggesting they are a consequence of cell movement.**

Vertebrate cell behavior in tissue culture is normally studied by periodic microscopic examinations of cell density and morphology. If a continuous record of behavior is required, it is generally obtained by using cinematographic arrangements. In this report, we describe a method in which fibroblasts can be monitored continuously using an electric field. Previously, related work on the electrical characteristics of continuous sheets of epithelial cells growing on permeable supports has been reported (1, 2). In other studies involving an elegant array of small electrodes, Thomas *et al.* (3) have monitored the bioelectrical activity of cultured embryonic chicken heart cells; however, these studies did not report the use of externally applied electric fields to detect cell spreading and motion. In the studies described below, fibroblasts at varying cell densities were cultured on evaporated gold electrodes and subjected to a small externally applied oscillating electric field. The total impedance of the system was observed to reflect changes in the morphology of the cells and the cell density on the electrode. Of more interest, however, were the small, relatively rapid fluctuations in impedance that were continually recorded from the spread fibroblasts. These fluctuations are believed to be primarily the result of cell movement on the electrode and were observed to have a different character for WI-38 cells and the transformed WI-38/VA-13 cells.

MATERIALS AND METHODS

Materials. Red Wax was from Cenco Scientific (Chicago). Cytochalasin B was from Sigma. Polystyrene dishes (60 mm) of tissue culture optical quality but without treatment to render the surface hydrophilic for cell attachment were gifts from Corning.

Cells. The fibroblast lines WI-38 and WI-38/VA-13 were obtained from the American Type Culture Collection. All culturing was under standard conditions of 37°C and 5% CO_2 in air with medium consisting of 90% Dulbecco's modified Eagle's medium with antibiotics and 10% fetal bovine serum.

Ultraviolet Irradiation. Irradiation of the dishes was accomplished with a Hanovia type SH high-pressure mercury arc lamp. Dishes were placed ≈ 4 cm from the lamp and irradiated for 5 min while enclosed in a small box (4).

RESULTS AND DISCUSSION

The preparation of 60-mm culture dishes containing gold electrodes required some care and is described in Fig. 1. Each finished dish contained one large (≈ 2 cm^2) and four small ($\approx 3 \times 10^{-4}$ cm^2) electrodes. To perform electrical measurements the dish was placed in an incubator, and about 6 ml of tissue culture medium was added over the electrodes. The large electrode and one of the small electrodes were connected to a phase-sensitive lock-in amplifier as shown in the circuit in Fig. 2. An AC signal of 0.1 V at 4000 Hz was supplied to the dish through a 100-KΩ resistor. Since the impedance of the dish with culture medium is ≈ 5 KΩ, the applied signal acts as a constant current source such that the measured voltage is proportional to the impedance. The measured impedance of the dish is dominated by the impedance at the boundary between the small electrode and the tissue culture fluid, whereas the total additional impedance associated with the electrodes themselves, the boundary between the large electrode and the tissue culture fluid, and the bulk of the tissue culture fluid is negligible in comparison.

The effect of cultured cells on the electrode's impedance is shown in Fig. 3*A*. Normal human fibroblast WI-38 cells were introduced as a cell suspension, and both the in-phase (resistive) and out-of-phase (capacitive reactance) components of the impedance were followed with time. As seen, following the introduction of the cells, the impedance increased slightly and began to fluctuate with time. We speculate that as the cells attach and spread on the small electrode, a fraction of the electrode's surface is blocked, causing the observed rise in impedance. The fluctuations in impedance are most likely due to the motion of the fibroblasts but may also be caused by cell-induced changes in the chemical environment close to the electrode. Both the reactance and the resistance showed the same behavior, but the effect was more pronounced in the latter. Fixing the cells with 10% formalin caused the fluctuations to cease (Fig. 3*A*), demonstrating that their origin is unquestioningly due to the living cells.

We could find no evidence for any effect of either the gold substrate or the applied electric field on the fibroblasts. Cell attachment, spreading, and growth appeared normal on the electrode at all times. Fig. 3 *B–E* shows cells at various times after inoculation on gold-coated polystyrene substrates.

From studies using 3T3-L1 cells, several generalizations can be made concerning the effects of electrode size and cell densities on changes in the total impedance of the system and the amplitude of the small fluctuations (data not shown). With larger electrodes, both the overall impedance and am-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviation: Me_2SO, dimethyl sulfoxide.

Axion Exhibit 1025
Axion v. Agilent

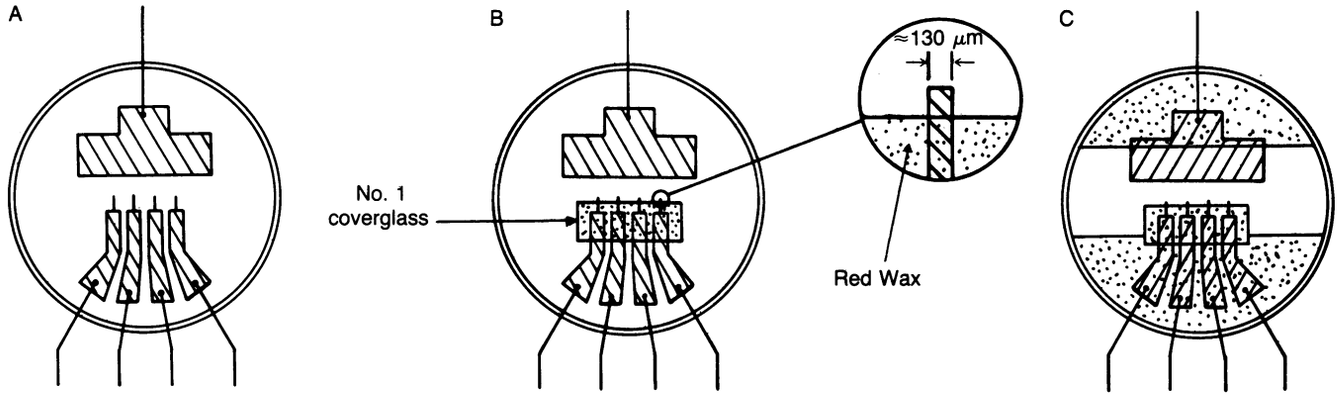

FIG. 1. (*A*) Gold was evaporated onto the bottom of 60-mm dishes through a mask in an evaporator at pressures of 10^{-4} Torr to form the electrodes. If ordinary polystyrene tissue culture dishes were used, the gold electrodes when exposed to culture medium became blemished with time. The apparent reason is that water found its way through pinholes in the gold, penetrated between the gold film and the surface of the hydrophilic dish, and caused the gold film to loosen. This problem could be largely avoided by using hydrophobic polystyrene dishes (see *Materials and Methods*). The dishes were later irradiated with an intense ultraviolet source to sterilize them and to make the plastic surface hydrophilic for better cell attachment. Varnish-insulated copper wires were soldered directly to the gold electrodes using pure indium. (*B*) A no. 1 coverglass was cut into an 8 × 22 mm rectangle and placed in the dish in such a manner that the tips of the four small electrodes reached just beyond the edge of the glass slide. The plastic dishes were gently heated and a small amount of molten Red Wax was made to run between the glass slide and the plastic dish by capillary action. (*C*) Finally, the soldered gold–copper contacts were covered with a generous amount of molten Red Wax. The completed dish contained one large and four small exposed gold electrodes.

plitude of the fluctuations decreased as expected due to the reduced contribution of the small electrode to the total impedance of the system. If electrode size is constant and one varies the initial cell density, as determined by the inoculum, the time profile of the overall impedance is different. At very high cell densities, sufficient to produce a fully confluent layer upon initial spreading, there is a pronounced rise in impedance by as much as a factor of 5. For 3T3-L1 cells this rise peaks ≈2 hr after inoculation and then falls during the next 2 or 3 hr to a level ≈2/3 the peak value. Following this, there is a gradual increase in total impedance as cell density rises. Eventually, following 2 or 3 days in culture, the magnitude of the impedance becomes relatively constant with the fluctuations somewhat reduced in amplitude but still present. Following ≈2 weeks in culture without medium changes, the impedance ultimately falls, and the curve becomes flat as the cells round up and detach from the electrodes. When the cell inoculum is low, resulting in only partial substrate coverage, the initial rise in impedance is considerably reduced in magnitude. In this case, often the impedance does not decrease after 2 hr as with a high 3T3-L1 inoculum; instead there is an extended period, lasting days, during which the impedance rises as cell density increases. The amplitude of the fluctuations is observed to vary in an inverse manner with cell density. This is expected when one considers that these variations are most likely the result of cell movement. At high densities not only is there a reduction in locomotion of individual cells due to crowding but the averaging effect of a larger cell population on the electrode tends to reduce individual cell contributions and smooth the observed variations.

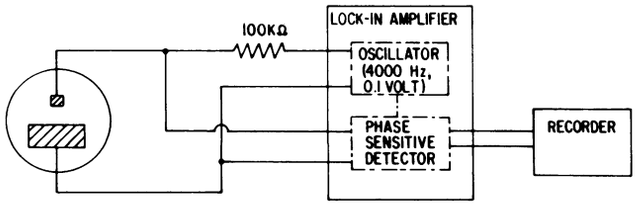

FIG. 2. The completed electrode system was connected to a PAR model HR-8 lock-in amplifier with a type A preamplifier as shown, and data were displayed using a chart recorder. A 10-sec time constant was used to reduce high-frequency noise in the output.

To further investigate the nature of the cell-induced changes in the impedance, we have employed the drug cytochalasin B that interferes with the polymerization of actin filaments and results in cell immobilization (5). Fig. 4 shows the effect of cytochalasin B and its solvent, dimethyl sulfoxide (Me_2SO), on the resistance (in-phase component of the impedance) when administered to the WI-38 cell line and its transformed counterpart WI-38/VA-13. These cells were run simultaneously by using two separate lock-in amplifiers in a dish that had been divided into two parts by a small glass partition sealed to the bottom of the dish with Red Wax. This configuration allowed us to inoculate the electrodes in each half of the dish with a different cell suspension. The design was such that the tissue culture fluid was common to both cell types, as it was made to rise above the partition. In Fig. 4, the initial cell density is ≈10 times that used in the experiment presented in Fig. 3. This cell density resulted in a large initial rise in the resistance that occurred as the cells attached and spread on the electrode. The rate of this large impedance change was considerably greater with WI-38 cells than with WI-38/VA-13 cells, reflecting a difference in the time course of electrode coverage. This in part is due to differences in the rate of spreading for each cell line. The addition of Me_2SO (0.5%, vol/vol) to the culture medium several hours after cell inoculation caused a small decrease in the resistance and a general attenuation of the fluctuations. These effects were more pronounced for the transformed cell line. Following about 2 hr in the presence of Me_2SO, these changes were observed to reverse as the cells apparently adapted to the presence of the solvent. Cytochalasin B (final concentration, 10 μM) was then administered to the cells, keeping the level of Me_2SO constant. The resistance immediately dropped, and the fluctuations to a large degree disappeared. These effects did not reverse until the drug was removed. Final removal of the Me_2SO had little effect on the cells (Fig. 4). By observing the cells visually when Me_2SO or cytochalasin B was added, a change in the cell morphology and degree of spreading could clearly be seen. Since this change was reflected in the electrical impedance, the time course of such events can easily be studied by our method.

It is somewhat apparent in Fig. 4 that the fluctuations from WI-38 and WI-38/VA-13 cells have a different character, as

Axion Exhibit 1025
Axion v. Agilent

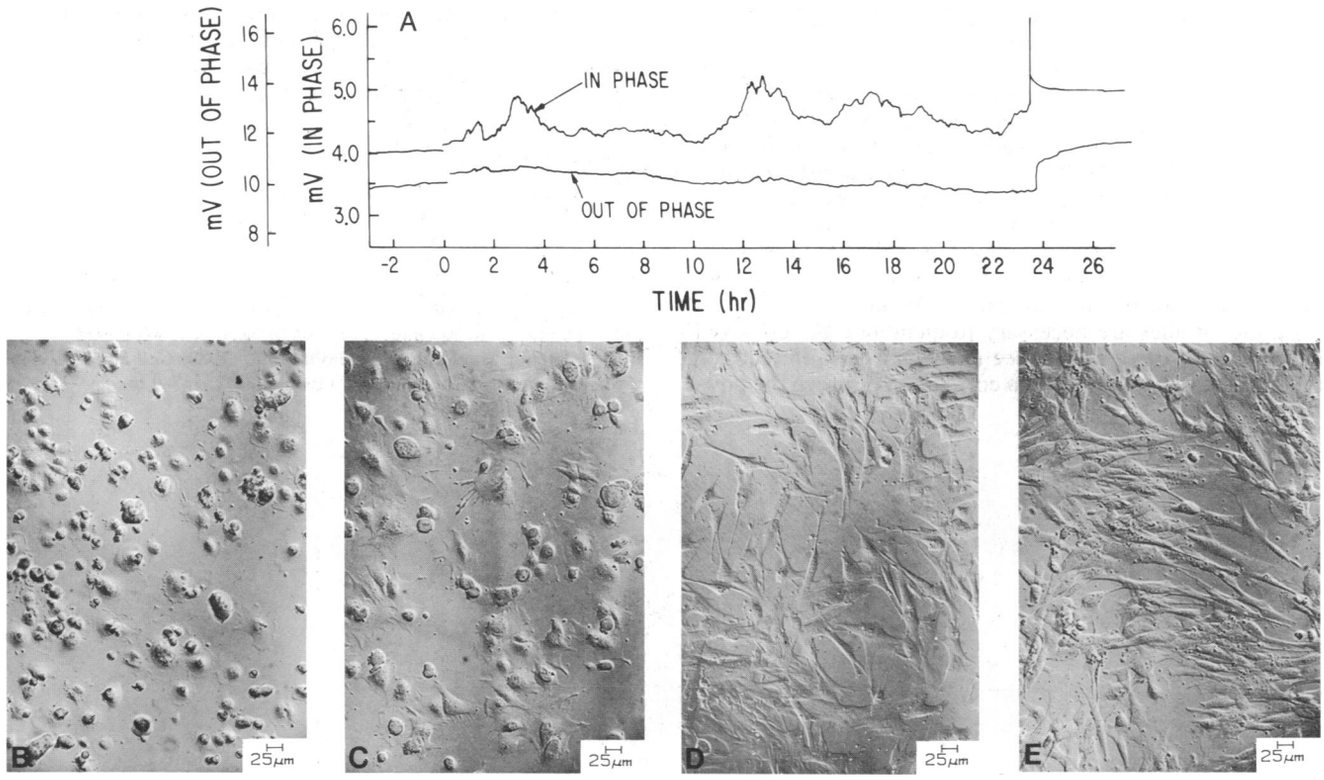

FIG. 3. (*A*) A suspension of WI-38 cells (passage 20) was added to the electrode system to give a final cell density on the available area of the dish of $\approx 1 \times 10^4$ cells per cm^2. Approximately 1 day following inoculation, formalin was added giving a final concentration of 10%. (*B–E*) Cells growing on evaporated gold substrates at various times after inoculation: 0.5 hr (*B*), 1.0 hr (*C*), 3.0 hr (*D*), and 23.5 hr (*E*).

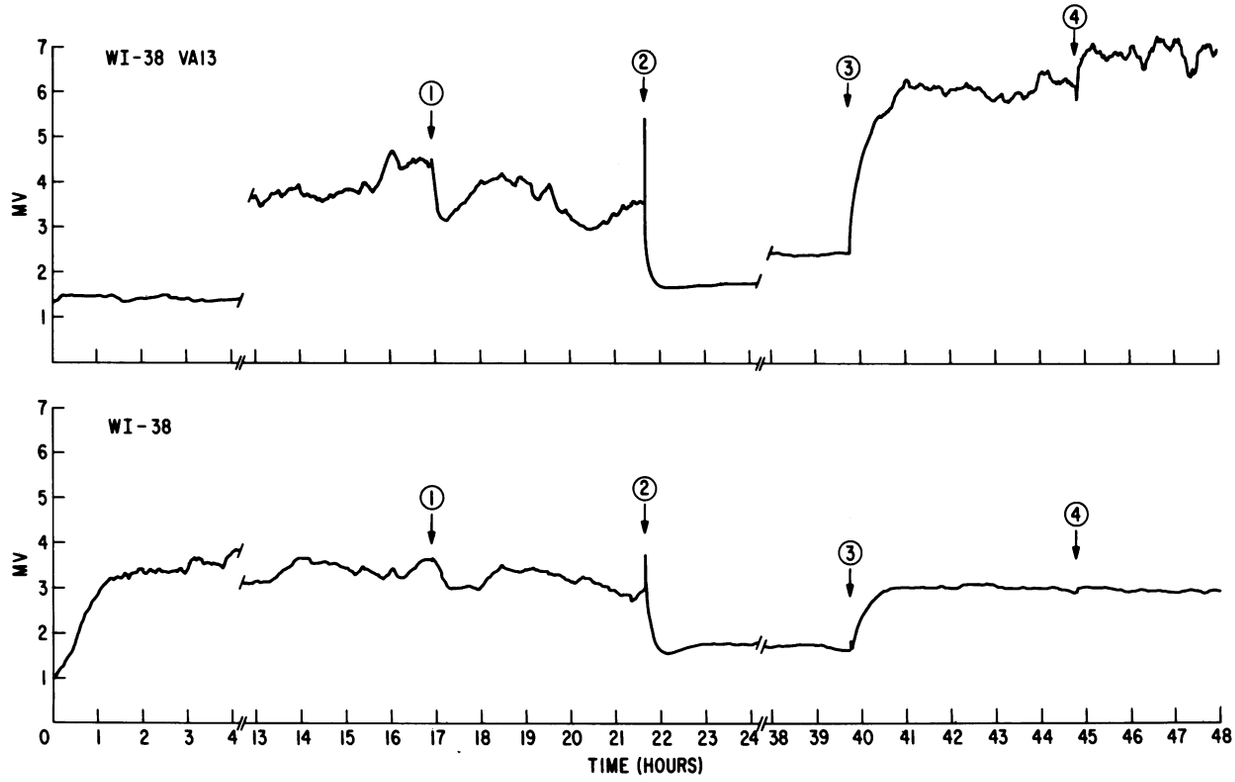

FIG. 4. A dish prepared as described in the legend to Fig. 1 was divided by a small glass partition such that it was possible to inoculate the separated electrodes with different cell lines. WI-38 cells (passage 30) and WI-38/VA-13 cells were used at concentrations resulting in final cell densities of $\approx 1 \times 10^5$ cells per cm^2. Conditions were as described in the legend to Fig. 2, except only the in-phase signal was followed. The volume of culture medium in the dish had slight effect on the measured potential and was maintained constant at 6 ml throughout the experiment. Me_2SO and cytochalasin B were added and withdrawn at the times indicated on the chart. Final concentrations were 0.5% (vol/vol) and 10 μM, respectively. Arrow 1, Me_2SO in; arrow 2, cytochalasin B in; arrow 3, cytochalasin B out; and arrow 4, Me_2SO out.

Axion Exhibit 1025
Axion v. Agilent

the WI-38 cells seem to produce fluctuations in the impedance of lower amplitude, particularly at the higher frequencies observed. This result is more obvious when the potential is amplified and is a consistent observation in several experiments with these two cell lines. When 3T3-L1 cells have been studied with this system, the impedance fluctuations are similar to the WI-38/VA-13 transformed cell line. Although 3T3-L1 cells are not considered to be fully transformed, on the other hand, they share some properties with the WI-38/VA-13 line in that they do not tend to form parallel cell arrays in confluent culture or exhibit a Hayflick limit (6) as is well documented for the WI-38 line.

Further studies are necessary to quantitate the observed differences in these impedance changes from different cell types to determine if there is a correlation between the power spectrum of the fluctuations and some aspects of the transformed state. The general utility of the system in monitoring various externally applied disturbances such as temperature and chemical additives must also be investigated.

This work was partially supported by the National Foundation for Cancer Research.

1. Misfledt, D. S., Hamamoto, S. T. & Pitelka, D. R. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 1212–1216.
2. Cereijido, M., Robbins, E. S., Dolan, W. J., Rotunno, C. A. & Sabatini, D. D. (1978) *J. Cell Biol.* **77**, 853–880.
3. Thomas, C. A., Jr., Springer, P. A., Loeb, G. E., Berwald-Netter, Y. & Okum, L. M. (1972) *Exp. Cell Res.* **74**, 61–66.
4. Panitz, J. A. & Giaever, I. (1980) *Surf. Sci.* **97**, 25–42.
5. Fletcher-MacLean, S. & Pollard, T. (1980) *Cell* **20**, 329–341.
6. Green, H. & Kehinde, O. (1974) *Cell* **1**, 113–116.

Axion Exhibit 1025
Axion v. Agilent