Adaptation of Plastic Surfaces for Tissue Culture by Glow Discharge¹

CHARLES F. AMSTEIN AND PAUL A. HARTMAN*

Department of Bacteriology, Iowa State University, Ames, Iowa 50010

Received for publication 4 April 1975

Plastic petri dishes and microtitration plates were electrically charged by a glow discharge unit installed in a vacuum evaporator. Charged and uncharged plates, as well as plates commercially treated for tissue culture, were inoculated with Vero and BHK-21 cell lines; secondary cultures of monkey kidney, chicken lung, canine kidney, and embryonic bovine kidney; and primary chicken embryo fibroblasts and chicken lung cells. All cell cultures grew normally on petri plates charged with the covers open. Growth rate and cell density compared favorably with growth on the commercial tissue culture plates; cell growth was somewhat less dense, however, on plates charged with the covers closed. Charged plates could be sterilized by ultraviolet light and ethylene oxide with no adverse effects on cell growth. Cells inoculated onto plates charged up to 7 months before inoculation grew as well as on freshly charged plates.

The use of disposable plastic materials for tissue culture has become popular, and in many laboratories plastic cell culture vessels have completely replaced glassware. Untreated plastic surfaces (usually made of polystyrene) are generally unsuitable for the culture of vertebrate cells, however, because they do not permit ready attachment and spreading of cells. Chemical methods, such as sulfuric acid-sodium carbonate rinses (4) and alcohol rinses (5), have been proposed to modify plastic surfaces so that cell attachment occurs. In this paper we report an alternative method for facilitating cell attachment; polystyrene surfaces were treated by the use of a high-voltage, alternating-current glow discharge.

MATERIALS AND METHODS

Apparatus. Materials were subjected to glow discharge in a Varian VE-30M vacuum evaporator equipped with a glow discharge unit (Fig. 1A). The glow discharge unit, manufactured primarily for use in electron microscopy, consisted of a transformer, variable from 0 to 4,500 V, which was connected to an electrode within a bell jar (Fig. 1B). Any grounded metal within the bell jar served as a second electrode to complete the circuit. In general, material to be subjected to glow discharge was placed in the bell jar, which was evacuated to a pressure of 0.03 to 0.05 torr. At a setting of about 30 on the transformer control (approximately 1,400 V), a bluish-glow discharge resulted.

Cell cultures. Cell suspensions were prepared from monolayers by treatment with trypsin. Cell densities of the suspensions were adjusted so that confluent growth would occur in 1 to 2 days after inoculation onto the test materials. The growth medium for all cell cultures was Eagle minimum essential medium containing Earle salts, 10% calf serum, 100 U of penicillin per ml, 100 µg of streptomycin per ml (BBL Culturstat 52048), and 50 µg of gentamicin reagent solution (Schering Diagnostics). After planting, the cell cultures in various test materials were incubated under 3 to 5% CO₂ at 35 C. Cell growth was observed daily by phase-contrast microscopy.

Effects of glow discharge treatment. Three materials were subjected to glow discharge to discover if the treatment was effective on different products. Growth of Vero cells on treated 60-mm diameter petri plates (Falcon 1007) was compared with growth on similar non-glow discharge-treated plates and on plates commercially treated for tissue culture (Falcon 3002). In addition, growth of Vero cells on glow discharge-treated, 100-mm diameter petri plates (Fisher 8-757-12) was compared with growth on untreated plates. Growth of Vero cells in glow discharge-treated microtitration plates (Linbro S-MVC-96) was compared with growth on untreated plates and on plates commercially treated for tissue culture (Linbro 13-FB-96-TC). Treated materials were subjected to glow discharge, with the covers off, for 5 min at a distance of 5 to 10 cm from the electrode.

The abilities of different cell lines to grow on plates treated by glow discharge was also studied. Growth on glow discharge-treated, 60-mm petri plates (Falcon 1007) was compared with growth on untreated plates and plates commercially treated for tissue culture (Falcon 3002). Eight types of cells

¹Journal paper no. J-8144 of the Iowa Agriculture and Home Economics Experiment Station, Ames Iowa, project 2018.

FIG. 1. Apparatus for glow discharge treatment: a, bell jar; b, transformer control (A). View of glow discharge treatment area: a, glow discharge electrode; b, petri plates in position for glow discharge treatment (B).

were used: primary chicken embryo fibroblast and chicken embryo lung cells; secondary canine kidney, monkey kidney, chicken embryo lung, and embryonic bovine kidney cells; and continuous lines of BHK-21 and Vero cells.

To study the effects of sterilization on plates treated by glow discharge, treated and untreated plates (Falcon 1007) were exposed with their covers open under two General Electric G15T8 germicidal lamps at a distance of about 1 m for 5 h. Growth of Vero cells on these plates was compared with growth

on untreated Falcon 1007 and Falcon 3002 tissue culture plates. The remainder of the glow discharge-treated plates, along with uncharged plates (Falcon 1007), were exposed to a mixture of 12% ethylene oxide and 88% carbon dioxide at 49 C for 16 h. Some of these plates were inoculated with Vero cells 24 h later; the remainder were inoculated 6 days after sterilization. Untreated Falcon 1007 and Falcon 3002 tissue culture plates were used as controls.

Other glow discharge parameters. Because the effects of glow discharge treatment might diminish

with time, Falcon 1007 plates were held for times ranging from 1 day to 7 months after treatment before the plates were inoculated. Furthermore, several plates were treated with the covers left on, for 5 min at a distance of 5 cm from the electrode. To determine the effect of distance from the electrode, plates were subjected to glow discharge with the covers open for 5 min at distances (from the edge of the electrode to the center of the plate) of 4, 7, 12, 17.5, 23, and 30 cm. Finally, to determine the effect of the length of time of glow discharge treatment, plates were treated with the covers open at a distance of 5 cm from the electrode for periods of 5, 10,

20, and 40 s and 1, 2, 5, and 10 min. Growth of Vero cells on all of the plates mentioned in this paragraph was compared with growth on untreated Falcon 1007 and Falcon 3002 tissue culture plates.

RESULTS

Effects of glow discharge treatment. Figure 2A shows Vero cells 24 h after inoculation on an untreated Falcon 1007 plate. As expected, only a few cells attached and spread out. On some areas of untreated plates a few cells attached and proliferated, but in no case did con-

FIG. 2. Vero cells 24 h after inoculation on an untreated Falcon 1007, 60-mm petri plate (A), a Falcon 1007 plate treated by glow discharge (B), and a Falcon 3002 tissue culture plate (C).

fluent growth occur. Figure 2B shows Vero cells, from the same inoculum, 24 h after inoculation on a similar plate that had been treated by glow discharge. The cells attached and grew normally. Cell growth was comparable to cells from the same inoculum grown on Falcon 3002 tissue culture plates (Fig. 2C). The same cell cultures after 4 days of incubation are shown in Fig. 3. The cells inoculated onto the untreated Falcon 1007 plate still had not attached appreciably (Fig. 3A). The cells grown on the Falcon 1007 plate treated by glow discharge (Fig. 3B),

and those grown on the Falcon 3002 plates (Fig. 3C), both showed equivalent aging. Vero cells inoculated onto untreated Fisher 8-757-12, 100-mm petri plates and Linbro S-MVC-96 microtitration plates also showed growth patterns similar to that shown in Fig. 2A. Cells inoculated onto similar plates that had been treated by glow discharge, and onto Linbro IS-FB-96-TC tissue culture microtitration plates, produced normal monolayers, similar to that shown in Fig. 2B.

Different types of cells could attach and grow

FIG. 3. Vero cells 4 days after inoculation on an untreated Falcon 1007, 60-mm petri plate (A), a Falcon 1007 plate treated by glow discharge (B), and a Falcon 3002 tissue culture plate (C).

on plates treated by glow discharge. Growth of BHK-21, primary chicken lung, secondary embryonic bovine kidney, and secondary monkey kidney cells on the plates that were subjected to glow discharge was comparable to growth on the Falcon 3002 tissue culture plates. In each case, cells inoculated onto untreated plates generally did not attach and grow. Figure 4 shows growth of secondary embryonic bovine kidney cells after 24 h on each type of plate. Primary chicken embryo fibroblast, secondary chicken

lung, and secondary canine kidney cells (Fig. 5), when inoculated onto plates treated by glow discharge, grew slower than those inoculated on the Falcon 3002 tissue culture plates. However, the cells seemed to attach and spread out without any difficulty.

Sterilization by ultraviolet radiation of plates that had been previously subjected to glow discharge did not affect the growth of Vero cells. Cell growth was equivalent to growth on plates that had been subjected to glow discharge but

FIG. 4. Secondary embryonic bovine kidney cells 24 h after inoculation on an untreated Falcon 1007, 60-mm petri plate (A), a Falcon 1007 plate treated by glow discharge (B), and a Falcon 3002 tissue culture plate (C).

FIG. 5. Secondary canine kidney cells 24 h after inoculation on an untreated Falcon 1007, 60-mm petri plate (A), a Falcon 1007 plate treated by glow discharge (B), and a Falcon 3002 tissue culture plate (C).

were not exposed to the germicidal lamps and to growth on the Falcon 3002 tissue culture plates. Sterilization by ethylene oxide of plates previously subjected to glow discharge did not affect the growth of Vero cells when the cells were inoculated 6 days after ethylene oxide treatment. Cell growth was equivalent to that on similar plates that had been subjected to glow discharge but were not exposed to ethylene oxide and to growth on the Falcon 3002 plates. However, cells inoculated onto plates

the day after ethylene oxide treatment did not attach and grow, probably because of residual traces of ethylene oxide.

Other glow discharge parameters. Vero cells grew into normal, confluent monolayers on plates that had been subjected to glow discharge from 1 day to 7 months before inoculation. Secondary monkey kidney cells inoculated onto similar plates that had been subjected to glow discharge 7 months earlier also produced normal, confluent monolayers. In both cases,

cells seemed to grow equally rapidly on the 7-month-old plates and on plates more recently subjected to glow discharge. As before, growth on the plates treated by glow discharge was equivalent to that on the Falcon 3002 tissue culture plates. Cells inoculated onto untreated Falcon 1007 plates at each time interval did not attach or grow.

Figure 6A shows Vero cells 24 h after inoculation of Falcon 1007 plates that were subjected to glow discharge with their covers left on. The monolayers were characterized by occasional irregular clear areas. Figure 6B shows the monolayer after 48 h of incubation. Many of the clear areas have been overgrown with cells, although some clear areas remained throughout the life of the monolayer. Cells from the same inoculum grew into normal confluent monolayers on plates that had been treated by glow discharge with their covers open at the same time as the closed plates.

The location of materials within the vacuum evaporator during glow discharge operations did not seem to be an important factor. There were no noticeable differences in Vero cell monolayers grown on plates subjected to glow discharge at distances from 4 to 30 cm from the electrode.

The length of time a plate was subjected to glow discharge also did not seem to be critical. Plates subjected to glow discharge for more than 1 min consistently supported confluent Vero cell monolayers, comparable to those grown from the same inoculum on Falcon 3002 tissue culture plates. Plates subjected to glow discharge for less than 1 min generally supported confluent monolayers; some of the monolayers, however, had occasional small clear areas similar to those shown in Fig. 6.

DISCUSSION

Cells inoculated onto different types of plastic products that were treated by glow discharge produced consistent growth, equivalent to that in similar products commercially prepared for tissue culture. The shape of the materials seemed to have little effect on the efficiency of the glow discharge treatment. For instance, cell growth at the bottom of a microtitration plate well was equivalent to that on a more open, 100-mm diameter petri plate. The only situation that produced unacceptable cell growth was that in which the plates were treated by glow discharge with their covers closed.

Growth of several types of cells was slightly less dense on plates subjected to glow discharge than on plates commercially treated for tissue

culture, but the differences were small enough to have been caused by differences in inoculum distribution or overcharging of the plates. Otherwise, the location of materials within the bell jar or length of glow discharge treatment was not critical. These factors suggest that the preparation of plastic materials for tissue culture by glow discharge need not be an exacting process; this would be advantageous in the use of glow discharge on a large scale.

The need to have petri plates (and other containers) open during the glow discharge operation, along with the return of air into the bell jar after the operation, would necessitate sterilization of the materials after the glow discharge operation. Sterilization by ultraviolet radiation or ethylene oxide after treatment produced no adverse effects on cell growth. The only requirement was that the plates sterilized by ethylene oxide had to be held several days to permit adequate dissipation of the gas.

One of the more important factors that determines the usefulness of glow discharge is how long the materials will remain charged. Cells grown on plates subjected to glow discharge 7 months before inoculation were no different from those grown on recently charged plates or on commercially prepared plates. This suggests that treatment by glow discharge creates a long-lasting, if not permanent, change in the treated plates.

At this time, the mechanism of change in glow discharge-treated materials can only be speculated on. Glow discharge is a phenomenon long known to physicists and has been extensively studied (2). It consists of the passage of electrons between two electrodes through a gas at low pressure, creating an ionized atmosphere. Common applications of glow discharges include fluorescent and neon lights. The ionized atmosphere present during glow discharge could affect plastic surfaces as follows. (i) The stream of electrons in the chamber could impart a negative charge on the polystyrene surface which would facilitate cell attachment. The chemical structure of polystyrene is fairly simple; impurities usually present during the manufacture of polystyrene, however, can be incorporated into the overall structure after polymerization (1). These impurities could then hold the negative charge necessary for cell attachment. This theory is supported by Rubin (4), who reported that the negative charge on ordinary plastic surfaces is too low to permit cell spreading and that his sulfuric acid-sodium carbonate treatment sufficiently increased this charge. (ii) Like charges could be imparted on both the styrene molecules and the previously mentioned impurities, causing removal of the

FIG. 6. Vero cells 24 h after inoculation on a Falcon 1007, 60-mm petri plate treated by glow discharge with the cover left on (A). The same monolayer 48 h after inoculation (B).

impurities. The glow discharge attachment on the vacuum evaporator was designed to degas impurities from the bell jar in this manner. A recent study using scanning electron microscopy (3) suggests that the removal of cells from a surface by trypsin is caused by the physical deformation of the cells by the trypsin rather than the dissolving of an adhesive substance. Impurities present in untreated polystyrene could also cause cell deformation, preventing attachment; therefore, removal of the impurities by glow discharge treatment would allow the cells to attach normally.

The usefulness of a glow discharge technique for treatment of plastic surfaces has been established. Although more investigation into the possible limitations of glow discharge is warranted, this technique, as it now stands, has advantages over chemical techniques and is useful in providing an emergency source of tissue culture-compatible plasticware. The main limitation is the necessity of having available a glow discharge unit-equipped vacuum evaporator or similar device; however, evaporators are fairly common items, especially in laboratories involved in electron microscopy. Also, since an extremely high vacuum is not required, a glow discharge chamber could easily be fabricated

out of readily available materials.

The glow discharge treatment of plastic materials, if not already being used, may be applicable commercially. For commercial application of glow discharge, some modification of equipment and technique would be needed, such as establishing a large scale capacity. Nevertheless, glow discharge offers sufficient advantages over chemical techniques that its commercial application should be investigated.

ACKNOWLEDGMENT

This work was supported in part by Public Health Service grant FD-00618 from the Food and Drug Administration.

LITERATURE CITED

1. Albright, L. F. 1974. Processes for major addition-type plastics and their monomers. McGraw-Hill, New York.
2. Llewellyn-Jones, F. 1966. The glow discharge. John Wiley and Sons, New York.
3. Revel, J. P., P. Hoch, and D. Ho. 1974. Adhesion of culture cells to their substratum. *Exp. Cell Res.* 84:207-218.
4. Rubin, H. 1966. Altering bacteriological plastic petri dishes for tissue culture use. U.S. Public Health Serv. Rep. 81:843-844.
5. Sullivan, E. J., and M. J. Rosenbaum. 1967. Methods for preparing tissue culture in disposable microplates and their use in virology. *Am. J. Epidemiol.* 85:424-430.