Multisurface Glass Roller Bottle for Growth of Animal Cells in Culture

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A multisurface glass roller bottle has been constructed for the growth of animal cells in culture. This bottle contains five concentrically placed glass cylinders that provide additional surfaces for the growth of animal cells. The bottle occupies the same space as a standard roller bottle, but it contains nine times the surface area of a standard bottle. L and HeLa cells can be grown in the bottle with cell yields 5- to 10-fold greater than in a standard bottle. L cells can be induced to produce interferon in the multisurface bottle.

As the biochemistry and physiology of animal cells becomes better understood, the demand for large production of such cells in culture increases. However, the growth of animal cells in culture on a large scale is a problem that is not always easily solved. Many cell strains and lines, unlike bacteria and other microorganisms, grow only in monolayers when attached to a surface. The scale-up of monolayer cultures is not easily accomplished, since a doubling of surface area is required to double the number of cells. This doubling in surface area can be achieved by either of two methods: (i) simply doubling the number of flasks or bottles in which the cells are grown, or (ii) increasing the surface area available within a container while keeping the total volume constant. The former has disadvantages because of the space required, and because the time required for maintenance of the culture is directly proportional to the increase in the number of culture containers. The latter has disadvantages because the maintenance and transfer of the cells necessarily becomes more complex. Numerous methods and apparatus have been designed for the in culture production of large quantities of animal cells, and many of these methods have been recently reviewed (4).

In our laboratory, we have used monolayer culture of mouse L cells for the production of interferon. In order to grow more cells and thus produce more interferon, we have sought containers for the growth of cells on a larger scale that would require only a small increase in labor and space. This report describes a simple glass roller bottle, in which the surface area for cell growth is increased approximately ninefold above a standard roller bottle. In this multisurface bottle, mouse L cells can be grown, and interferon subsequently produced.

MATERIALS AND METHODS

Cells and viruses. Mouse L cells and HeLa "O" cells were grown in suspension culture medium (Joklik modified) supplemented with 7% fetal calf serum and 7% horse serum, respectively. Cells were maintained between $1 \times 10^6$ and $8 \times 10^6$ cells/ml. Monolayer cultures of L cells were grown in Eagle minimum essential medium supplemented with 7% fetal calf serum. Monolayer cultures of HeLa O cells were grown in McCoy 5A medium, by Hsu's modification, supplemented with 7% horse serum. All cultures contained the antibiotics penicillin and streptomycin.

Interferon production and assay. Interferon was assayed by a microassay technique (1, 4). In standard roller bottles, interferon was produced by mouse L cells by induction with MM virus, a strain of encephalomyocarditis virus, as previously described (1, 3). For interferon production in the multisurface roller bottle, the following procedure was used: (i) when a bottle contained approximately $1 \times 10^9$ cells, crude L interferon was added to a final concentration of 10 U/ml to prime the cells; (ii) after 6 h at 37°C, the medium was poured off and discarded; (iii) MM virus in 700 ml of serum-free medium was added, with an MM virus multiplicity of infection of one; (iv) with no further change of medium, the cells produced interferon at 37°C for 20 h; (v) after 20 h, the medium (700 ml) was collected and stored at 4°C as crude interferon. At all times when the bottle was at 37°C, it was rotated at the rate of one revolution per 4 min.

Construction of multisurface roller bottle. An increase in surface area was achieved by concentrically placing five equally spaced glass cylinders inside a bottle made of Pyrex glass with a screw cap. The glass of the bottle and the cylinders is 0.2 cm thick. The bottle is 28 cm long (including the neck).
and 11 cm wide. These dimensions are approximately the same as those of a standard roller bottle, 26 by 11 cm, purchased from New Brunswick Scientific Co., New Brunswick, N. J., and made of disposable mold-blown lime glass.

The five concentric glass cylinders are 21 cm long and are, respectively, from the center, 3.5, 5.0, 6.5, 8.0, and 9.5 cm in diameter. The cylinders are separated from each other and the outside wall by glass rods fused to the wall and to the cylinders. The glass rods are approximately 0.5 cm long. This spacing between the cylinders allows good movement of liquid. The cylinders are not attached to the top or the bottom of the bottle. The multisurface bottle weighs 930 g. Figure 1A shows a full view of the bottle; figure 1B shows a top view; and figure 1C is a bottom view. This multisurface bottle was constructed to our specifications by Labglass, Inc., Vineland, N. J.

Cleaning procedure for multisurface bottle. The bottle must be cleaned manually, but this can be done easily and simply. Our cleaning procedure consists of the following consecutive steps: (i) add a 10 to

Fig. 1. Multisurface roller bottle. (A) full view; (B) top view; (C) bottom view.
20% solution of Clorox, and allow it to stand at room temperature for 30 min to detach all cells; next (ii) add a 1% (wt/vol) solution of Impact detergent (Economics Laboratory, Inc.), and allow this to stand at room temperature for 30 min to thoroughly clean all surfaces; then (iii) thoroughly rinse with distilled water, and (iv) sterilize by autoclaving. We have had four bottles in almost constant use for 2.5 years without the loss of a bottle through breakage.

RESULTS

Comparison of multisurface bottle to standard bottle. The reason for constructing a glass multisurface roller bottle was to increase the surface area for cell growth, and thus increase the ultimate number of cells, without increasing the roller space requirements or the time necessary for manipulating the bottle during the inoculation and growth of cells. This has been accomplished with the multisurface bottle. The surface area available for cell growth in a standard bottle is approximately 530 cm², whereas the multisurface bottle contains approximately 4,900 cm². Since cells can grow on both sides of the concentric cylinders, the surface area has been increased greater than nine-fold over the standard roller bottle. The minimum volume of liquid necessary to cover all surfaces is 600 ml.

Growth of L and HeLa cells in multisurface bottle. L cells were grown by inoculating $1 \times 10^6$ of the suspension culture cells contained in 700 ml of monolayer growth medium into a bottle. This was routinely done by mixing 100 to 200 ml of suspension culture cells with 500 to 600 ml of monolayer growth medium. The bottle was then placed on a roller apparatus and turned one rotation per 4 min for cell attachment and subsequent growth. The L cells reached a maximum density of $1 \times 10^9$ cells per bottle in 72 h (Table 1). At this point, the cells can be removed by trypsinization or induced for interferon production.

HeLa cells grown in suspension were inoculated and grown by the same procedure described for L cells, i.e., $1 \times 10^4$ cells in 700 ml of medium. HeLa cells grew to a maximum density of $5 \times 10^6$ to $6 \times 10^6$ cells per bottle (Table 1). At densities above $5 \times 10^8$ to $6 \times 10^8$, the cells began to detach from the surface. For both HeLa and L cells, two monolayer surfaces can be observed with a standard inverted microscope. A comparison, between the standard and the multisurface bottle, of the maximum number of cells that can be obtained is shown in Table 1.

Production of interferon in the multisurface bottle. L-cell interferon was produced in the multisurface bottle as described in Materials and Methods. The data in Table 2 compare interferon yields from one standard bottle and one multisurface bottle. We have consistently obtained, on the average, about twice as much interferon per cell in the multisurface bottle as in the standard bottle (Table 2). Stated another way, one multisurface bottle yielded approximately the same amount of interferon as 20 standard bottles.

DISCUSSION

The need to grow animal cells in culture on a large scale is likely to accelerate. The large scale culture of cells in monolayer is a much more difficult task than the large scale culture of cells in suspension. Simple, relatively inexpensive containers for the scale-up of monolayer cultures of animal cells are particularly useful. This report describes the design and use of such a multisurface glass roller bottle. The surface area for cell growth of this bottle is ninefold greater than that of a standard roller bottle. The multisurface bottle occupies one roller space and requires the same handling time during cell inoculation and growth as one standard roller bottle. The multisurface glass bottle is not intended to compete with more sophisticated methods, such as the multisurface stacked plate propagator (5). It is similar in design to a commercially available plastic bottle (Cooke Engineering Co., Alexandria, Va.), which contains a spiral of plastic film inside the bottle to increase the surface area (2). We were unable, however, to grow our mouse L cells in this bottle.

### Table 1. Growth of mouse L cells and HeLa cells in multisurface glass roller bottles

<table>
<thead>
<tr>
<th>Cell type</th>
<th>No. of cells inoculated</th>
<th>No. of cells counted</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Standard bottle</td>
<td>Multisurface bottle</td>
</tr>
<tr>
<td>L</td>
<td>$1 \times 10^7$</td>
<td>$1 \times 10^8$</td>
</tr>
<tr>
<td>HeLa</td>
<td>$1 \times 10^7$</td>
<td>$1 \times 10^8$</td>
</tr>
</tbody>
</table>

### Table 2. Production of interferon by mouse L cells in a multisurface glass roller bottle

<table>
<thead>
<tr>
<th>Bottle type</th>
<th>No. of cells</th>
<th>Vol</th>
<th>Interferon (U/ml)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard bottle</td>
<td>$1 \times 10^6$</td>
<td>50</td>
<td>4,000*</td>
<td>200,000</td>
</tr>
<tr>
<td>Multisurface bottle</td>
<td>$1 \times 10^6$</td>
<td>700</td>
<td>6,400*</td>
<td>4,480,000</td>
</tr>
</tbody>
</table>

* Average interferon units per milliliter from 200 bottles, 10,000 ml of crude interferon.
* Average interferon units per milliliter from 100 bottles, 70,000 ml of crude interferon.
Mouse L cells and HeLa cells were grown in the multisurface bottle with cell yields 5- to 10-fold greater than that in a standard roller bottle. L cells were grown routinely in the bottle, two cultures per week for 2 years, and consistently produced good yields of interferon. It is not suggested that this bottle can be used to grow all lines and strains of cells. Each cell type must be tried on an individual basis.

In conclusion, the multisurface bottle provides a simple, relatively inexpensive way to scale-up L and HeLa monolayer cultures by using existing roller equipment and significantly reducing handling time on a per bottle basis.

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LITERATURE CITED