SILICONE-TUBE CELL CULTURE DEVICE

In Ho KIM* and Tai Hyun PARK**

Lucky Central Research Institute, P.O. Box 10, Dae Duk, Korea
(Received 24 September 1986 • accepted 15 December 1986)

Abstract—Silicone tube was used to facilitate gas transport in mammalian cell culture. A bundle of silicone tubes provides anchorage dependent cells (BHK 21 cells) with surface. It was found that the surface characteristic of silicone tube for the cell attachment was the same as that of commercial tissue culture dish. Also high O_2 permeability of silicone membrane gives rise to a faster metabolism of cells.

INTRODUCTION

In recent ten years, culture devices for anchorage-dependent mammalian cells have been extensively developed to attain high cell densities [1]. Knazek et al. [2] described a perfusion system that is comprised of artificial capillaries encased in a plastic shell. To maximize ratio between surface area and the total culture volume, Van Wezel grew cells on microcarrier beads suspended in liquid culture media [3].

The idea of using a gas-permeable plastic film to form a culture vessel was first suggested by Munder et al. [4]. Cells attach to the plastic film, and their oxygen and CO_2 supply comes from diffusion through the plastic. Liquid nutrients come from medium that directly contacts with cells. Jensen's IL 410 cell culture system [5] was designed with FEP-teflon film (O_2 permeability: 4.9×10^{-10} cm^3(STD)cm/cm^2.s.cmHg, CO_2 permeability: 12.7×10^{-10} cm^3(STD)cm/cm^2.s.cmHg at 25°C) to facilitate gas transport [6].

The present study describes an alternation of the IL 410 svstem by using silicone tubes (O_2 permeability: 6.05×10^{-8} cm^3(STD)cm/cm^2.s.cmHg, CO_2 permeability: 3.24×10^{-7} cm^3(STD)cm/cm^2.s.cmHg at 25°C) as well as modifying the geometry of culture device.

IL 410 system employed a tubular spiral geometry that has a disadvantage of nutrients gradient along axis due to a long length of tube [7].

We introduced the geometry of Knazek's perfusion device by making a bundle of silicone tubes which would have higher S/V_t than the tubular spiral geometry.

* To whom all correspondence should be addressed

** Present address: Department of Chemical Engineering, Purdue University

MATERIALS AND METHOD

Baby syrian hamster kidney(BHK-21) cells were grown in Dulbecco's modified Eagle's(DME) medium (Gibco, Grand Island, NY, USA) supplemented with 10% fetal calf serum(Hyclone, Sterile Systems, Inc., Logan, UT, USA), 10^5 units/liter penicilline, and 10^5 μg/liter streptomycin(Gibco). All culture were incubated at 37°C in 10% CO_2 atmosphere.

Two kinds of silicone rubber(Shinetsu KE 441 T and KE 45W, Tokyo, Japan) were used to coat glass petri dishes. The silicone was solidified overnight, and the coated glass dishes were washed with distilled water after boiling in detergent solution. A small piece of silicone tubing(Silastic® tubing, Dow Corning Corp., Midland, MI, USA) was also attached on the surface of petri dish to study cell attachment on the silicone surface. Petri dishes were sterilized overnight under ultraviolet light.

Silicone-tube cell culture device(Fig. 1) was comprised of 4 silicone tubes (Silastic® tubing, length = 19cm, inner diameter = 0.355 cm, outer diameter = 0.465cm) embeded by epoxy at both ends. Orifices at the ends of the device were attached to a medium reservoir by polyethylene tubing. Cells cultured in tissue culture dishes(Falcon® , Becton, Dickinson & Co., Oxnard, CA, USA) were trypsinized to inoculate the device. The trypsinized cells(2×10^5cells/ml) were filled in the silicone tubes by a peristaltic pump. The pump was located at the outlet of medium to prevent cell breakage. After the inoculated cells were attached inside the tube walls, new medium was recirculated at a rate of 4ml/min. Medium volume was 120ml.

Comparative cell counts were made either by enumerating cells under a microscope in a fixed area (silicone coated dishes and silicone tube) or by using a hemocytometer after trypsinizing cells(culture dishes).

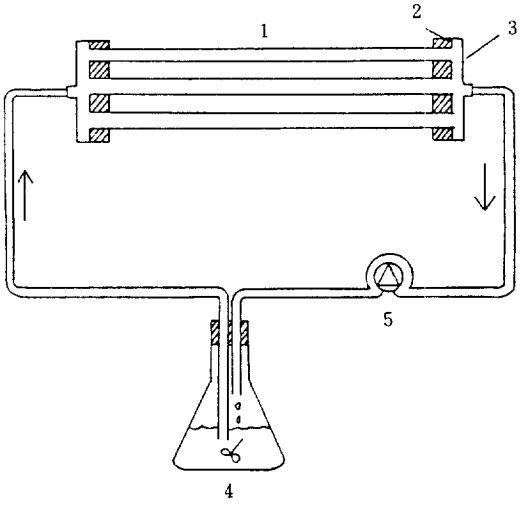

Fig. 1. Schematic diagram of experimental apparatus.

1. Silicone tube 2. Polyurethane
3. Glass cap 4. Reservoir
5. Peristaltic pump

Gluose concentration was measured by the glucostat method, and lactic acid was assayed using L-lactic dehydrogenase.

RESULTS AND DISCUSSION

First of all, the growth of cells in commercially available tissue culture dish was studied. Fig. 2 shows the growth of BHK cells on tissue culture dish. The dish area was 19.6cm^2 and 5ml of medium was added to each dish. Specific growth rate was 0.068h^{-1} and maximmum cell density was 1.3×10^5 cells/cm^2 (2.5×10^6

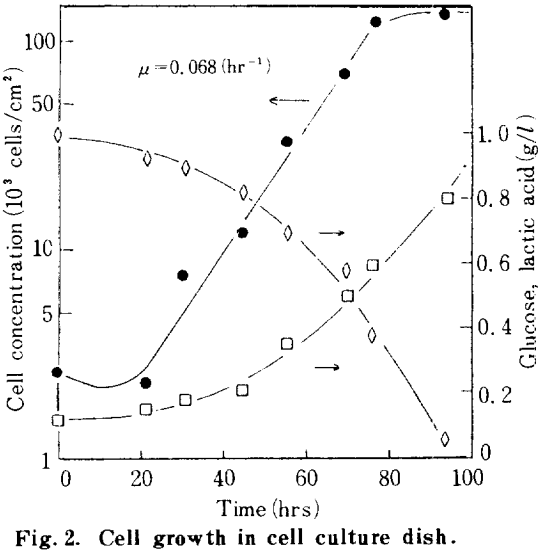

Fig. 2. Cell growth in cell culture dish.

◇ : glucose, □ : lactic acid, ● : cell concentration, Cell : BHK-21, Medium : DME with 10% FCS, Condition : 37℃ in 10% CO_2.

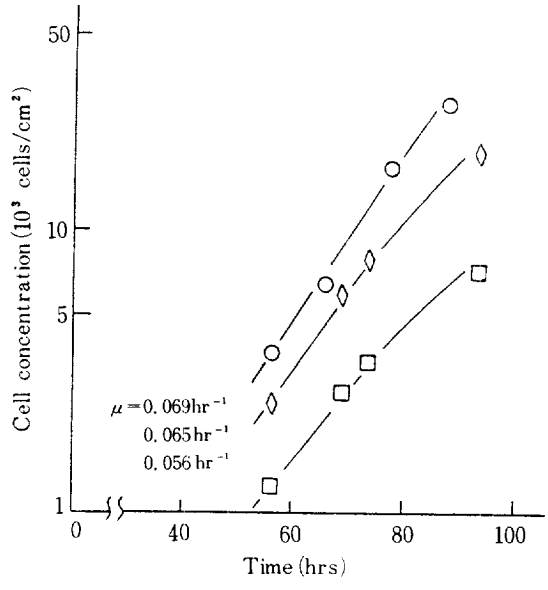

Fig. 3. Cell growth on silicone surfaces.

○ : Silastic tube, ◇ : KE 45-W Silicone, □ : KE 441T Silicone, Experimentals as the same in Fig. 2.

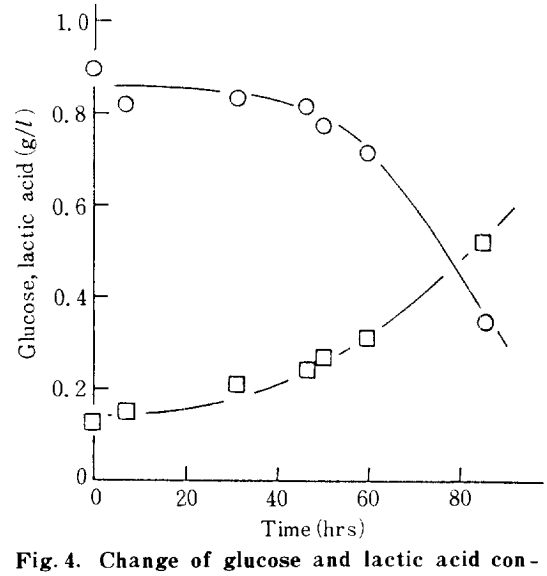

Fig. 4. Change of glucose and lactic acid concentrations in the silicone-tube cell culture device.

○ : glucose, □ : lactic acid

cells/50mm ID dish). The production rate of lactic acid was nearly proportional to the consumption rate of glucose, that is, two moles of lactic acid was produced as one mole of glucose was consumed.

Fig. 3 illustrates the growth of cells on three kinds of silicone material. Counting of cells before 50 hours was not possible because cells detached from the counting area with the movement of medium when we draw medium to count cells with microscope. It is ascribed to the fact that silicone has hydrophobic surface, and consequently medium rolls on the surface with cells. Once cells occupied the whole surface of petri dish, there did not occur detachment of cells. Since the initial cell numbers in counting area (0.2cm × 0.2cm) were different for the three cases, specific growth rates were compared. Shinetsu KE 45W, Silastic tube, and tissue culture dish had almost the same specific growth rate.

The performance of silicone-tube cell culture device is shown in Fig. 4. Referring to the surface area and medium volume in Table 1, we can see that tissue culture dish has a larger S/V_m ratio than the silicone-tube cell culture device. And the comparison of concentration profiles between Fig. 2 and Fig. 4 shows that glucose decay rates have a similar pattern. This means that the cells in the silicone-tube cell culture device metabolize glucose faster than the cells of tissue culture dish due to the high permeability of silicone membrane. If we increase the S/V_m ratio by using more silicone tubes having small diameter, the glucose consumption rate may be increased with the increase of the S/V_m ratio.

Table 1. Comparison of geometry between a tissue culture dish and the silicone-tube cell culture device.

Culture system	Surface Area (cm^2)	Medium Volume V_m (cm^3)	S/V_m	S/V_t
Tissue culture dish	19.6	5	3.92	-
Silicone-tube culture system	84.8	120	0.706	11.3

NOMENCLATURE

S : Surface area (cm^2)
V_m : Medium Volume (cm^3)
V_t : Tube Volume (cm^3)

REFERENCES

1. Glacken, M.W., Fleischaker, R.J. and Sinskey, A.J.: *Trends in Biotechnol.*, **1,** 102 (1983).
2. Knazek, R.A., Gullino, P.M., Hohler, P.O. and Dedrick, R.L.: *Science*, **178,** 65 (1972).
3. Wezel, A.L. van: *Nature*, **216,** 64 (1967).
4. Munder, P.G., Modolell, M. and Wallach, D.F.H.: FEBS Lett., **15,** 191 (1971).
5. Jensen, M.D.: *Biotechnol. Bioeng.*, **23,** 2703 (1981).
6. Brandrup, J., Immergut, E.H. and McDowell, W. (eds.): "Polymer Handbook", III-229, 2nd ed., New York, John Wiley & Sons (1975).
7. Feder, J. and Tolbert, W.R.: *Sci. Amer.*, **248,** 24 (1983).