# CELL CULTURE ON MODIFIED POROUS CELLULOSE BEADS

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A method of cell culture on microcarriers, which has many advantages over methods used previously for this purpose, has been suggested in recent publications. In particular, it enables more cells to be produced with less consumption of medium and time [3-5, 7].

Adhesion of cells to particles of a microcarrier and their growth on these particles depend on the chemical composition and structure of the microcarrier, and also on the type of cells to be cultured. Several types of microcarriers, based on dextran [8], glass [7], polyacrylamide gel [4], and plastic [3] have been described. Even so, research into the synthesis of new microcarriers is promising, for there are advantages in using different carriers for growth of different cells and for the solution of different problems.

The writers previously [1] suggested a method of synthesizing immunosorbents based on porous cellulose beads (PCB) [1]. This paper gives data on chemical modifications of PCB and the use of modified beads (cyto-PCB) for cell culture.

## EXPERIMENTAL METHOD

PCB measuring 70-100 or 100-250 μ, prepared from hygroscopic cotton [1], were used as the basis for cyto-PCB. After oxidization the PCB were modified by the addition of various ligands containing amino groups. PCB modified by diaminohexane were used in most experiments. Experiments were carried out on three cell lines which were cultured in the following media: human melanoma (MeVo) cells in medium PRMI (Research Institute of Poliomyelitis and Viral Encephalitis, Academy of Medical Sciences of the USSR) containing 10% embryonic calf serum (N. F. Gamaleya Research Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR), 0.03% glutamine (Research Institute of Poliomyelitis and Viral Encephalitis, Academy of Medical Sciences of the USSR), and 40 μg/ml of gentamicin (Mosmedpreparaty Combine); spontaneously transformed embryonic hamster cells (STHE), in a mixture containing 50% of Eagle's medium (Moscow Research Institute of Virus Preparations), 40% of 0.5% solution of lactalbumin hydrolysate in Hanks' solution (Moscow Research Institute of Virus Preparations), 10% bovine serum (Kiev Meat Combine), and 40 μg/ml of gentamicin; mouse tumor L cells in medium 199 containing 10% bovine serum, 0.03% glutamine, and 40 μg/ml of gentamicin. HEPES was added to all media up to a final concentration of 20 mM. The cells were cultured in 20-ml potassium-free glass flasks (diameter 2.5 cm). To each flask 0.6 ml of a 50% suspension of cyto-PCB in the corresponding medium, in a dose of (0.5-1.0) × $10^6$ cells, the total volume of the mixture was adjusted to 1.6 ml, after which the sample was thoroughly mixed, centrifuged for 5 min at 1000 rpm, and incubated for 30 min at 37°C. This procedure was repeated two more times, after which the samples were allowed to stand at 37°C. Next day 3.2 ml of fresh medium was added to each sample. A 50% change of medium was carried out at intervals of 1-3 days depending on its acidity. Cells growing on cyto-PCB were stained with a solution of azure-eosin by the Giemsa method [2]. To determine the number of cells not adherent to the cyto-PCB, the beads were separated from the cells on a No. 76 kapron sieve. To determine the number of cells growing on cyto-PCB, the beads were treated with 0.25% trypsin solution (Spofa, Czechoslovakia) before fractionation. The ratio between the numbers of living and dead cells was determined by staining with a mixture of trypan blue and eosin. Cells were counted in a Goryaev's chamber.

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TABLE 1. Cell Growth on PCB Modified by Addition of Various Ligands

| Name of ligand | Quantity per gram of PCB | Cell line | Cell growth |
|---|---|---|---|
| — | — | STHE | — |
| Glycine | 5 mmoles | » | — |
| Leucine | 5 mmoles | » | — |
| Glutamic acid | 5 mmoles | » | — |
| Glutamine | 5 mmoles | » | — |
| Lysine | 5 mmoles | » | — |
| Arginine | 5 mmoles | » | — |
| Tyrosine | 5 mmoles | » | — |
| Cysteine | 5 mmoles | » | — |
| Uridine | 5 mmoles | » | — |
| Polylysine | 100 mg | » | + |
| Diaminopentane | 1 mmoles | » | ± |
| | | MeVo | — |
| | 5 mmoles | STHE | + |
| | | MeVo | + |
| | 10 mmoles | STHE | + |
| | | MeVo | + |
| | 25 mmoles | STHE | + |
| | | MeVo | + |
| Diaminohexane | 2,9 mmoles | STHE | + |
| | 5,8 mmoles | STHE | + |
| | | MeVo | + |
| | 8,6 mmoles | STHE | + |
| | | MeVo | + |
| | | L cells | + |
| | 11,5 mmoles | STHE | + |

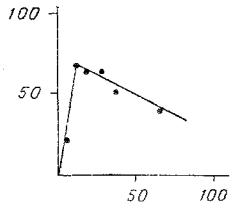


Fig. 1. Dependence of number of adherent cells on total surface area of cyto-PCB. Abscissa, total surface area of cyto-PCB (in cm $^2$); ordinate, number of adherent cells (in %).

## EXPERIMENTAL RESULTS

Cells of the overwhelming majority of lines do not adhere to cellulose. We therefore attempted to find cellulose derivatives to which the cells we studied would adhere. Cells were found to adhere well to cellulose modified by diaminopentane, diaminohexane, or polylysine (Table 1). Since growth of cells on surfaces coated with polylysine has been described by many workers, the subsequent research was aimed at studying this process on PCB modified by diamines. Cell growth has been described [6] on polyacrylamide microcarriers modified with these same ligands.

In the present experiments good cell growth was observed after addition of the diamines in the proportion of 5-10 mmoles to 1 gram of dialdehyde-cellulose.

Adherence of the cells to cyto-PCB depended on several conditions: the volume of mixture, the area of the phase boundary between cyto-PCB and the cell suspension, the ratio between the number of cells and the number of particles of microcarrier. Dependence of the number of adherent cells on the total surface area of cyto-PCB is illustrated in Fig. 1. In this experiment 70% of the added cells adhered to the microcarrier, i.e., $10^5$ cells to 1 cm$^2$ of surface of the beads.

Both cyto-PCB on which cells could be seen to be spread out, and also beads (about 25%) with no cells on their surface were discovered microscopically in the samples 24 h after the beginning of incubation. As incubation continued the number of beads not contain-

TABLE 2. Comparison of Conditions and Results of Cell Growth in a Glass Flask and in a Flask Containing Cyto-PCB

| Vessel | Capacity of vessel, ml | Area of bottom of vessel, $cm^2$ | Area of surface to which cells adhere, $cm^2$ | Volume of incubation mixture, ml | Volume of medium used up, ml | Number of cells seeded | Yield of cells on 7th day | Increase in number of cells, % |
|---|---|---|---|---|---|---|---|---|
| Flask | 100 | 30,5 | 30,5 | 10 | 10 | $2 \cdot 10^6$ | $10 \cdot 10^6$ | 500 |
| Flask containing cyto-PCB | 20 | 4,9 | 28,0 | 4,8 | 7,2 | $10^6$ | $8 \cdot 10^6$ | 800 |

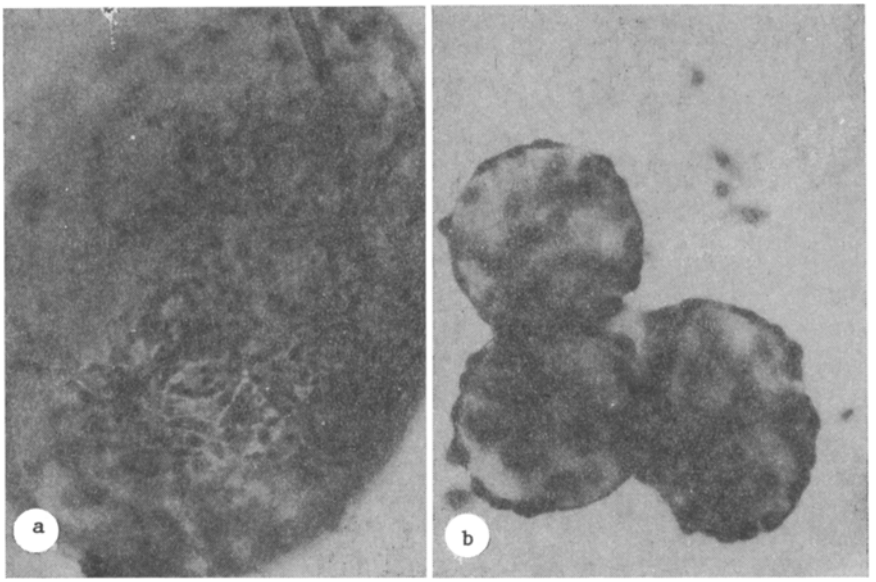


Fig. 2. Cell growth on cyto-PCB: a) MeVo cells, b) L cells. Stained by Giemsa's method. 200×.

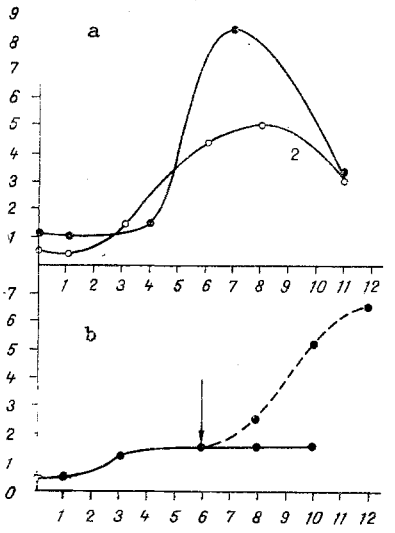


Fig. 3. Curves of cell growth on cyto-PCB (a) and effect of addition of fresh portion (b). Abscissa, days after beginning of culture; ordinate, yield of cells. a) Cells seeded on 0.6 ml of 50% suspension of cyto-PCB: 1) L cells ($10^6$ cells were seeded), 2) STHE ($0.5 \times 10^6$ cells were seeded); b) $0.5 \times 10^6$ L cells were seeded on 0.2 ml of a 50% suspension of cyto-PCB: continuous line — cell growth without addition of cyto-PCB; broken line — cell growth after addition of fresh portion of cyto-PCB. Arrow indicates addition of fresh portion of cyto-PCB (0.4 ml of 50% suspension).

ing cells decreased, down to 0.2% on the 7th day. Growing cells, covering the surface of the bead, could be seen on the majority of cyto-PCB (Fig. 2).

In a special experiment the results of cell culture under ordinary conditions (in a glass flask) and in flasks on cyto-PCB were compared. It will be clear from Table 2 that the use of cyto-PCB resulted after 7 days in a considerable increase in the number of cells (800%) in a vessel with small volume.

Curves showing cell growth on cyto-PCB are given in Fig. 3a. On the day after seeding the number of cells adherent to the carrier was less than the number of cells seeded. Cells not adherent to the beads were dead. By the 7th day of culture the number of cells on cyto-PCB was 5-10 times greater than on the 1st day. Later the number of cells ceased to rise. Moreover, some of the cells spread out on the microcarrier became round, separated from the substrate, and died. Cessation of cell growth could not be prevented by the addition of fresh portions of medium. However, the addition of fresh portions of cyto-PCB led to resumpton of cell growth. In the experiment illustrated in Fig. 3b, addition of a second dose of microcarrier resulted in a 12-fold increase in the number of cells (compared with the 1st day of culture).

Improvement of the conditions of culture (for example, mixing, culture in a continuous flow of medium) evidently enables a further increase in the number of cells to be obtained. The preparation described above has several advantages over other microcarriers. In particular, cyto-PCB differ from blast or plastic microcarriers in being permeable for nutrient medium, as a result of which this medium is accessible for the regions of the cell adherent to the substrate. Meanwhile cellulose, unlike dextran, is not degraded by enzymes present in animal cells. Another important advantage of the cellulose base is that many chemical modifications of it are possible, so that microcarriers suitable for the solution of different analytical and preparative problems can be obtained.

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