CHARACTER OF CELL GROWTH ON A SURFACE OF LIMITED AREA

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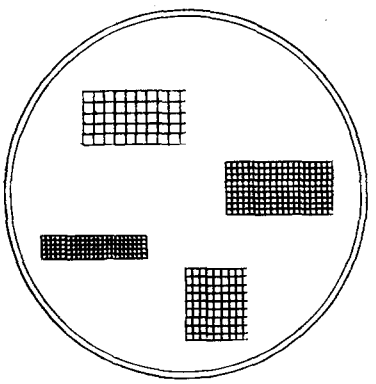
The phenomenon of the effect of substrate relief on cell growth was established in 1952 [9]. Quantitative characteristics of responses of different cells to substrates with regular relief, in the form of parallel grooves [1, 6] were studied later. It was subsequently shown that cell growth can be influenced not only by the configuration of the substrate, but also by its size: Single cells did not divide if the area of substrate was under 300 μm^2 [7], and growth of BHK-21 cells on glass granules 25 μm in diameter was inhibited [8].

This paper describes a study of the effect of size of substrate on growth of cells of different origin.

EXPERIMENTAL METHOD

Cells were seeded in plastic **petri** dishes of 35•10 type (from Flow Laboratories, England), with grooves marked on their floor, forming squares from 0.01 to 1.0 mm^2 in area (Fig. 1). Grooves 50-80 μm wide and up to 100 μm deep were cut with a steel cutter, mounted in a micromanipulator. Part of the surface of the dishes not marked with grooves served as the control.



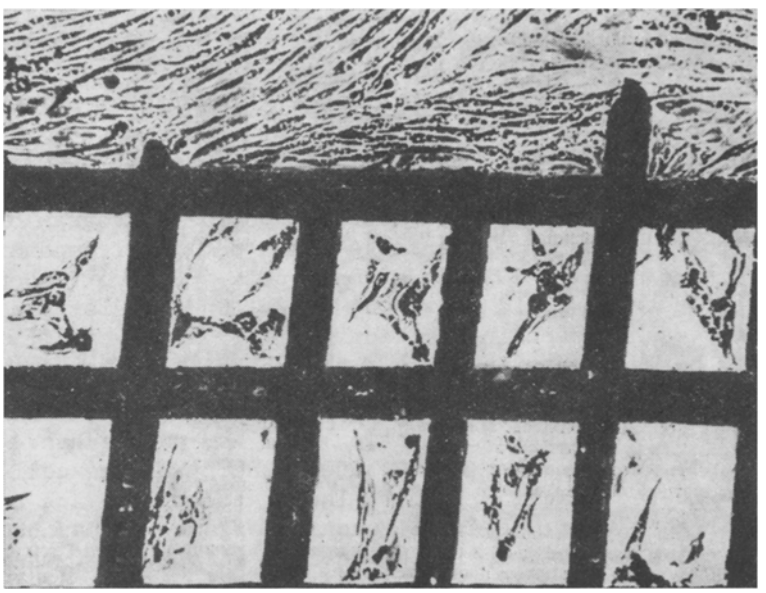


Fig. 1. Arrangement of grooves forming squares of different area on surface of plastic **petri** dishes.

Fig. 2. Growth of diploid human embryonic fibroblast-like cells (M-7) on squares 0.02 mm^2 in area. Growth of cells inhibited on substrates bounded by grooves. Individual cells have uncharacteristic polygonal shape, cytoplasm is **vacuolated.** Monolayer of cells with characteristic fibroblast-like shape was formed on control surface (outside squares). 70 ×.

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TABLE 1. Growth of Diploid Human Embryonic Skin and Muscle Fibroblasts on Surface of Limited Area

Parameter	Area of squares, mm²												
	1,0	0,81	0,64	0,49	0,36	0,25	0,16	0,09	0,06	0,04	0,03	0,02	0,01
Seeding dose of cells per square	200	162	128	96	72	50	32	18	12	8	6	4	2
Number of cells growing in squares (M ± m)	998,9± 77,3	812± 62,5	636± 58,9	494,4± 47,2	368,2± 35,8	248,2± 25,4	128,7± 26,2	68,1± 14,7	37,2± 8,8	22,9± 4,9	12± 3,2	4,1± 1,1	2± 0,7

Primary chick embryonic fibroblasts (CEF), primary cultures of green monkey kidney cells (GMKC) (both epithelium-like and fibroblast-like cells were represented), diploid fibroblast-like human embryonic skin and muscle cells (lines M-1 and M-7) at the 18th and 32nd passages, and fibroblast-like cells of human embryonic lung diploid line (line HEL) at the 29th and 34th passages, and green monkey kidney heteroploid epithelial-like cells (line 4647) [2] at the 96th passage were used. All cell cultures used in the work were obtained in the Institute of Poliomyelitis and Virus Encephalitis, Academy of Medical Sciences of the USSR. Cells of lines M-1, M-7, 374, and 4647 were seeded with a density of $2 \cdot 10^4$ cells/cm² in 3 ml of Eagle's minimal medium with 10% calf serum, and GMKC and CEF were seeded with a density of 10^5 cells/cm² in 3 ml of mixture of equal volumes of Eagle's minimal medium and 0.5% lactalbumin hydrolysate in Hanks' solution with the addition of 5% calf serum. The cultures were incubated at 37°C in a humid atmosphere with 5% CO_2. After 4-5 days the cells were fixed with 70° ethanol and stained with methylene blue. The cells were counted by means of an ocular grid and on photographs.

EXPERIMENTAL RESULTS

Epithelium-like cells of both the diploid line 374 and the heteroploid line 4647 spread out to form a monolayer in the course of 4-5 days on squares ranging in area from 1.0 to 0.01 mm². Fibroblast-like cells of lines M-1 and M-7 formed a monolayer in the course of 4-5 days on the control surface of the dishes, and also on the surface of squares with an area of 1.0-0.16 mm². On squares 0.09-0.04 mm² in area these cells arranged themselves along the diagonals of the squares. Cells growing on squares 0.09-0.04 mm² in area preserved their mutual orientation and their fibroblast-like shape. On squares 0.03-0.01 mm² in area cells of lines M-1 and M-7 assumed an uncharacteristic shape and were arranged haphazardly; they did not form monolayers (Fig. 2). The results of counting cells of the M-7 line, growing on squares 1.0-0.01 mm² in area, are given in Table 1 and diagrammatically in Fig. 3. The character of growth of HEL cells on squares with different area varied by lesser degree than that of M-1 and M-7 cells, possibly on account of the ability of such cells to form monolayer cultures [4].

Primary CEF formed a monolayer on squares of all areas at the same times as in the control, but on squares 0.01-0.02 mm² in area many of the cells were polygonal in shape, and on squares 0.03-1.0 mm² in area practically all cells were fibroblast-like in shape.

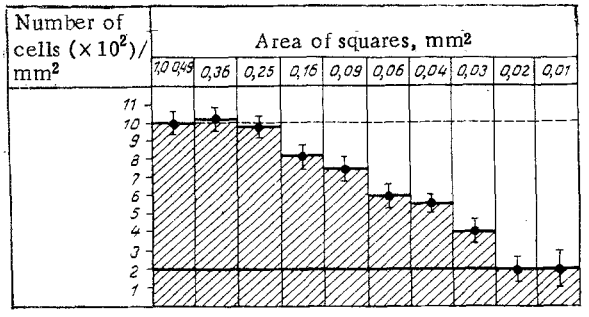

Fig. 3. Standardized values of number of human embryonic diploid cells (M-7) growing on squares of different area. Horizontal broken line denotes density of cell population in control, continuous line denotes seeding density of cell culture.

TABLE 2. Ability of Cells of Different Origin to Grow on Substrates of Limited Area

Cell culture	Cell morphology	Area of squares, mm²							
		1.0—0.25	0,16	0,09	0,06	0,04	0,03	0,02	0,01
M-7	Fibroblast-like	0	1	1	2	2	2	3	3
HEL		0	0	0	0	1	1	1	2
CEF		0	0	0	0	0	0	0	1
GMKC	Mixed culture	0	0	0	0	0	0	1	1*
								0	0**
4647	Epithelium-	0	0	0	0	0	0	0	0
374	like	0	0	0	0	0	0	0	0

Legend. 0) No inhibition of cell growth; 1) weak inhibition (monolayer forms but density of cell population is lower than in control); 2) marked inhibition (cell manolayer does not form, but number of cells increased); 3) complete inhibition of cell growth. *) Cultures of primary GMKC in these squares consisted of fibroblast-like cells, **) cultures of primary GMKC in these squares consisted of epithelium-like cells.

Primary GMKC formed mixed cultures during growth on substrates with an area of 1.0-0.03 mm² and on the control surface, whereas epithelium-like cells predominated on substrates with an area of 0.02-0.01 mm² (Table 2).

Inhibition of growth during culture of M-7 and M-1, HEL, and primary fibroblast-like GMKC cells on squares with an area of 0.02-0.01 mm² and changes in shape of the cells and vacuolation of their cytoplasm resembled the phenomena observed during the action of toxic samples of nutrient media on the cells. However, this hypothesis can hardly be accepted, for cells growing on squares of different area were otherwise under identical conditions. Granularity of the cytoplasm of cells cultured on a surface with an area of 0.02-0.01 mm² could be attributable to the accumulation of certain metabolites. For example, accumulation of fat has been described in the cytoplasm of contact-inhibited 3T3 cells [5].

The suggested method and the results obtained by means of it can be used to study substrate—cell and cell—cell interactions, and may prove useful for practical purposes. In particular, such a system of culture could be used for cloning cells and for selecting epithelium-like cells. Data on inhibition of growth of particular types of cells on a surface of limited area can be used to establish optimal dimensions of microcarriers. This problem is becoming particularly important at the present time with the expanding use of the method of cell culture on microcarriers for both production and research purposes.

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