Effect of Extracellular Matrix on Cultured Epidermocyte Adhesion and Proliferation

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The necessity of rapidly obtaining a large cellular mass in a limited amount of time requires careful selection of the substrate for culturing. Various types of collagen [2-4,9-11] or fibronectin [1,6,7] are now most frequently used to improve cell adhesion to the culture flask surface. It is still unclear which substrate is best for epidermocyte growth, to what degree this or that substrate promotes adhesion, and, still more important, does a particular substrate influence subsequent proliferation of these cells?

Here we compare the effects of various substrates: types I and III collagen and fibronectin on cultured epidermocyte adhesion and proliferation.

MATERIALS AND METHODS

Type I collagen (Sigma, C 9879, USA), group 1, type III collagen (Sigma, C 3511, USA), group 2, and fibronectin (Sigma, F 2008, USA), group 3, were used as substrates for epidermocyte culturing. The surface of plastic culture flasks (Costar, USA) were treated before culturing in accordance with published recommendations [5]. A primary human epidermocyte culture was prepared from burn patients' skin fragments obtained during autodermoplasty operations [8]. In all cases the number of cells for culturing was 10^5 per cm^2 surface. Epidermocytes isolated from the same sample were cultured simultaneously on all examined substrates and without them on plastic (control group). More than 250 primary epidermocyte cultures were examined. The cells were cultured in DMEM medium (Flow, England) with 2% ultraser (IBF, LKB), 2% glutamine, and antibiotics in a CO incubator (Flow, England). The effect of the substrate on adhesion was assessed by counting the relative numbers of adhering cells after 2 and 24 h of culturing. The effects of the substrates on cell proliferation were assessed by scintillation of cells incorporating ^{3}H-thymidine after 2 and 4 hours and 3 and 6 days of culturing zthe index of ^{3}H-thymidine-labeled cells was estimated. The isotope was added to the culture medium in a dose of 5 μCi/ml and incubated for one hour. The data were statistically processed and compared between the experimental groups and with the control group in which epidermocytes were cultured on plastic.

RESULTS

Reliable differences in adhesion parameters in the experimental and control groups were evident as soon as after 2 h of culturing (Table 1). The use of substrates regularly resulted in much higher counts of cells adhering to and spread on the culture flask surface as against the control. The num-

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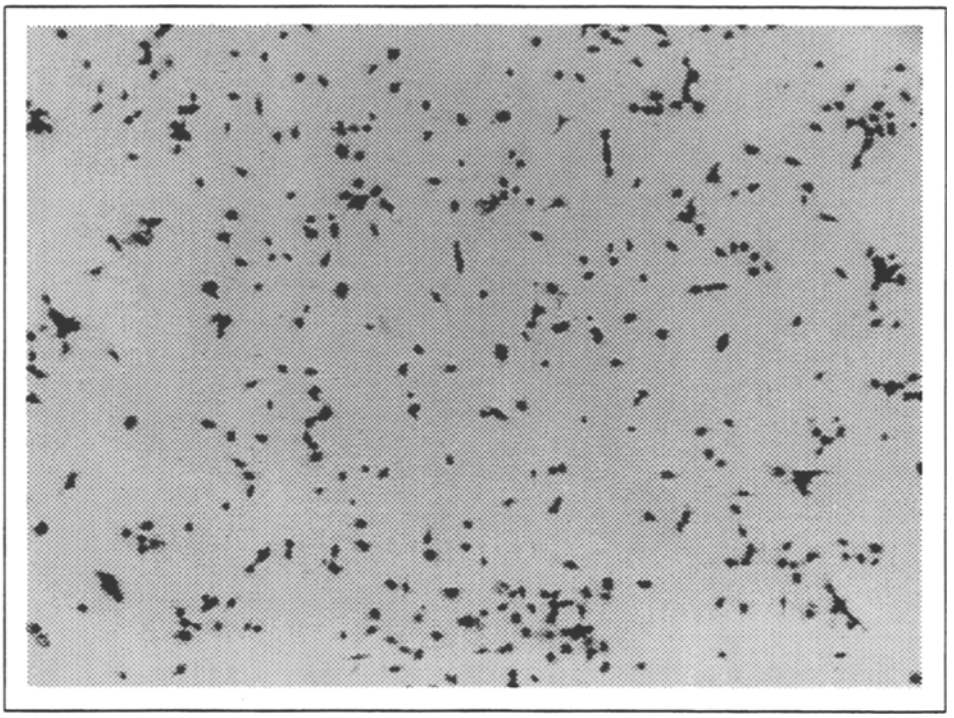

Fig. 1. Low density of adhering and spread epidermocytes after 24 h of culturing, controls (on plastic). Phase contrast, ×240.

ber of such cells was highest with fibronectin used as the substrate, surpassing not only the control value, but the values in the other two experimental groups as well. With types I and III collagen cell adhesion was also higher than in the control, but the values did not differ much.

After 24 h of culturing cell adhesion and spreading in the primary epidermocyte culture was virtually over. The quantitative parameters of cell adhesion were reliably increased in comparison with those after 2 h of culturing in all the groups, being still higher than the control in the groups with substrates and highest of all in the group with cell culturing on fibronectin. Differences of cell density per unit of culture flask surface were the direct result of differences of cell adhesion in the tested groups at this point during the observations, being even upon visual inspection lowest in the control, far inferior to the experimental samples (Figs. 1 and 2).

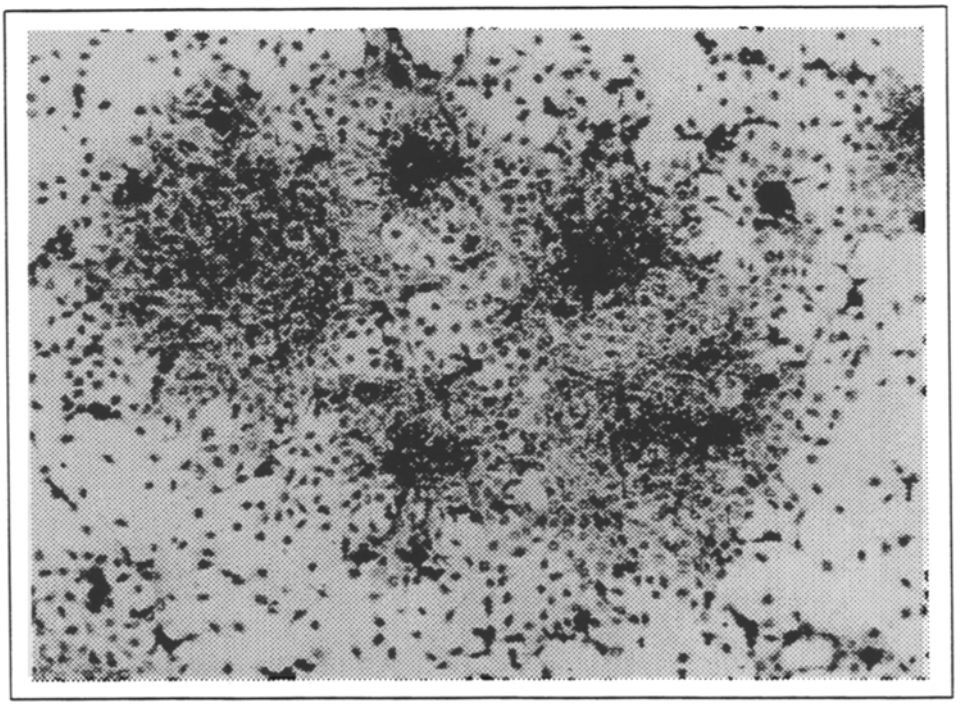

Fig. 2. Numerous epidermocytes adhering to and spread on fibronectin matrix (group 3) After 24 h of culturing. Phase contrast, ×240.

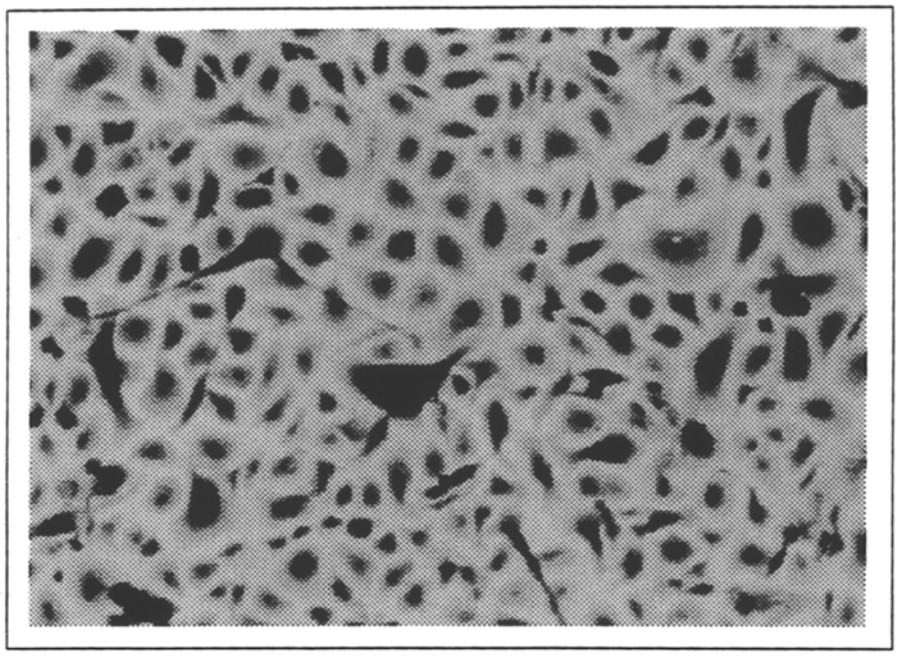

Fig. 3. Epidermocyte monolayer on fibronectin matrix after 3 days of culturing (group 3). Phase contrast, ×240.

Analysis of the counts of epidermocytes incorporating ^{3}H-thymidine at various stages of the experiment showed a changed level of epidermocyte proliferative activity in tissue cultures with substrates (Table 2).

After 2 h of culturing we failed to identify ^{3}H-thymidine-labeled cells in either the experimental or control groups. Solitary cells with incorporated label appeared after 24 h of culturing on collagen and somewhat higher counts of such cells in cultures grown on fibronectin. Such cells were not found in control cultures.

TABLE 1. Epidermocyte Adhesion on Test Substrates, % of Adhering Cells

Group	Period of investigation, h	
	2	24
1	53.2±3.45	69.25±8.4
2	51.6±4.3	70.65±1.7
3	79±5.1	91.3±1.67
Control	30.8±7.2	44.25±7.7

The differences in the index of ^{3}H-thymidine-labeled cells became significant by day 3 of culturing. The level of epidermocyte proliferation in

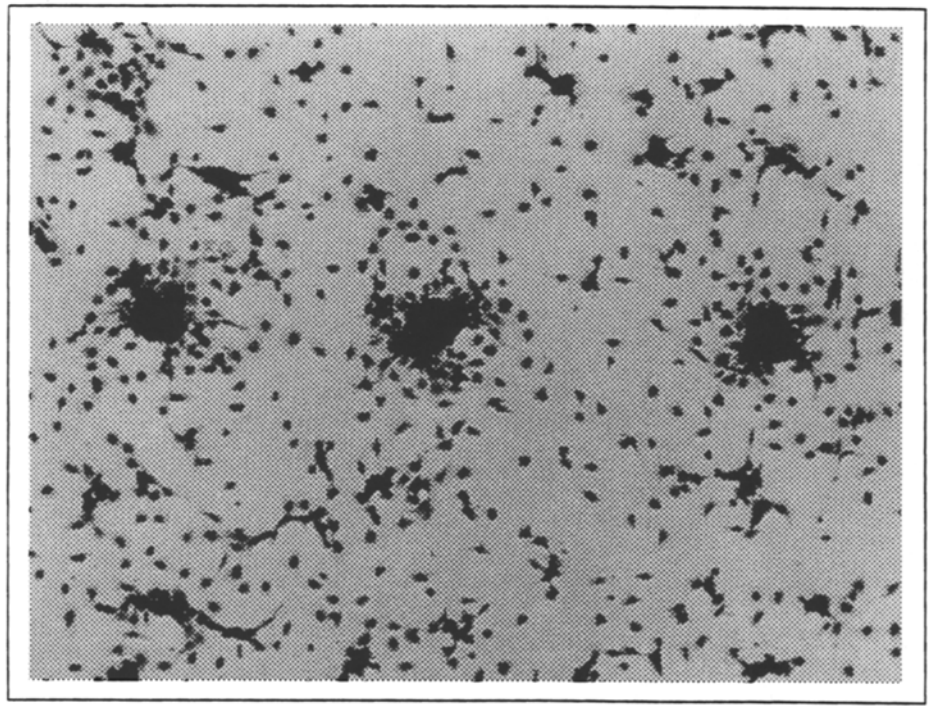

Fig. 4. Islet growth of epidermocytes after 3 days of culturing in control group (on plastic). Phase contrast, ×240.

TABLE 2. Index of ^3H – Thymidine – Labeled Cells with Types I and III Collagen and Fibronectin Used as Substrates

Group	Period of investigation			
	2 h	1 day	3 days	6 days
1	–	0.9	12.23±2	7.25±1.1
2	–	0.3	9.8±1.6	8.2±1.7
3	–	3.3±1.2	16±3.15	15.85±2.7
Control	–	–	5.75±2.24	9.2±2.7

cultures grown on substrates was definitely higher than in the control cultures, being similar in the cultures with types I and III collagen and highest in the fibronectin group. As a result of the differences of epidermocyte proliferation in the experimental and control groups, a cell monolayer formed by the end of the third day of culturing in the experimental groups, whereas only islet culture growth was verified in the control (Figs. 3 and 4).

The differences in the index of ^3H-thymidine-labeled cells in the collagen groups and control had leveled off by the end of the sixth day of cultivation, presumably due to contact proliferation inhibition in the experiment and continued epidermocyte mitosis in the less mature control cultures. The higher index of ^3H-thymidine-labeled cells in cultures grown on fibronectin was unexpected. This index reliably surpassed the proliferation values in the rest of the groups, although it was somewhat lower than at the previous stage of the observations.

Our data indicate that epidermocyte adhesion and proliferation are two related processes. Improvement of cell adhesion conditions may later result in a higher proliferation level; however, with such substrates as types I and III collagens the parameters of epidermocyte proliferative activity, though rapidly (on day 3) attaining the maximal level vs. the control, do not surpass it in terms of their absolute values in later periods.

On the other hand, fibronectin was associated with a significantly higher level of epidermocyte proliferation at all stages of the experiment in comparison with the control and other experimental groups.

Hence, the extracellular matrix is highly significant in the development of epidermocytes cultured *in vitro*, not only promoting adhesion, but accelerating proliferation as well. The effect off fibronectin in this respect was the most expressed.

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