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ROLE OF EXTRACELLULAR MATRIX IN WOUND REPAIR BY CULTURED GASTRIC MUCOSAL CELLS

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Effects of extracellular matrix on wound repair of cultured gastric epithelial cells were assessed. Artificial wounds were made by mechanical cell denudation in confluent rabbit gastric epithelial cell sheets which were formed on different types of extracellular matrix (e.g., collagen type I and type IV, laminin, fibronectin and Matrigel). Changes in wound size were analyzed quantitatively. Cell migration and proliferation were observed in stages of the wound repair process. The speed of wound repair was different with each extracellular matrix studied and was fastest on Matrigel. The type of extracellular matrix used in this study modulated both cell migration and proliferation. Therefore, it is concluded that extracellular matrix plays an important role in rates of gastric mucosal wound healing.

The restoration of gastric mucosa after damage involves both cell proliferation and migration(1). However, these two processes are difficult to analyze *in vivo*. Therefore, we established a new, convenient wound repair model using primary cultured gastric epithelial cells which allows quantitative analysis of both migration and proliferation(2,3,4,5). In this model, wound repair occurred with initial migration and later proliferation stages and was stimulated by hepatocyte growth factor(5). The cellular cytoskeleton plays an important role in this process(4). Although extracellular matrix was once believed only to support cells and fill the free space of tissue, it is now widely accepted to be involved in cell adhesion morphology, motility and maturity(6). Further, different types of extracellular matrix may have different effects on cells. For example, it was reported previously that fibronectin increased

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the length of the path of the rabbit corneal epithelial layer, but laminin did not(7). Therefore, this study was undertaken to clarify the role of several different types of extracellular matrix in gastric epithelial restoration, particularly in cell migration and proliferation.

MATERIALS AND METHODS

Preparation and Culture of Primary Cultures of Gastric Cells

Gastric mucosal cells were isolated from male Japanese white rabbits (2kg) according to methods described previously(2,3,4,5). Briefly, the oxyntic mucosa was minced into small pieces and incubated in a medium containing 0.07% type I collagenase (Sigma Chemicals Co., St. Louis, MO) for 15 min at 37°C. Tissues were washed with Ca^{2+}- and Mg^{2+}- free Hanks Balanced Salt Solution (HBSS) containing 1mM EDTA. These procedures were repeated twice and cells were washed in HBSS containing 1mM EDTA and 0.1% bovine serum albumin. Cell viability was assessed by monitoring cytoplasmic exclusion of 0.1% trypan blue. Cell viability was 96 ± 2.6% at the time of cell inoculation. Isolated cells were inoculated at a concentration of $5x10^6$ cells / dish onto plastic dishes (Corning Glass Works, Corning, NY) coated with collagen type I(8) (control), collagen type IV(8), laminin(9), fibronectin(10) or 10% Matrigel(11) and cultured in Ham's F-12 medium as described previously(2,3,4,5). In this study, collagen type I coated dishes were used as a control as we reported previously(2,3), because inoculated cells did not form perfect complete monolayer cell sheets without extracellular matrix coating on the dish. After 48 h of inoculation, cells formed complete monolayer sheets. As previously reported(2,3,4,5), 90.2% of the cells were mucous cells as indicated by PAS staining.

Effect of Extracellular Matrix on Gastric Mucosal Restoration

Restoration experiments were performed after the formation of a confluent monolayer cell sheet. A wound was made as described previously(2,3,4,5) in the gastric cell sheet using a rotating silicon tip resulting in a cell-free area of constant size. The effects of extracellular matrix were assessed in Ham's F-12 medium with 10% fetal calf serum. The cell-free area was measured using an IBAS image analyzer (Carl Zeiss, Germany). The measurement was performed in triplicate from four separate cell preparations (n=4). The process of restoration was monitored using a time-lapse videodisc recorder (SONY LVR-3000N, Tokyo). All results were expressed as mean ± S.D. and statistical analysis was performed using analysis of variance and non-paired Student's t-test.

Detection of Cell Proliferation

Proliferating cells were detected by indirect immunohistochemical methods using monoclonal anti-5-bromodeoxyuridine (BrdU; Sigma Chemical Co.)(12) antibody. In this study, five groups were compared. In the first group, BrdU was added to the culture medium at a concentration of 10 μg/ml immediately after the wound was made followed by incubation for 12 h (0-12 h group). In the second group, BrdU was added 12 h after wound formation and incubation was continued for 12 additional hours (12-24 h group). In the third group,

BrdU was added 24 h after wound formation and incubation was continued for 12 additional hours (24-36 h group). In the fourth group, BrdU was added 36 h after wounding and incubation was continued for 12 h (36-48 h group). In the fifth group, BrdU was added 48 h after wounding and incubation was continued for 12 h (48-60 h group). Samples were stained for BrdU by standard techniques. The BrdU labeling index in controls and experimental extracellular matrix groups was calculated in the unit area ($0.109mm^2$) of the wound edge (n=4).

RESULTS

Wound Repair of Gastric Epithelial Cells

In collagen type I coated controls, the cells at the edge of the wound began to form pseudopodia-like structures (lamellipodia) several minutes after wounding and to repopulate toward the center of the wound with a continuous ruffling movement. The cell-free area ($2.6mm^2$) was repopulated gradually via migration of epithelial cells and was repaired completely within 36-48 h after wounding (Figure 1). In each experimental extracellular matrix group, the healing rate was different. The healing rate was significantly faster in cells inoculated on Matrigel than in controls and was significantly slower in collagen type IV, fibronectin and laminin groups (Figure 2).

In controls, BrdU-positive cells were rarely detectable in the 0-12 h group and 12-24 h group. However, in the 24-36 h group, the number of BrdU-positive cells increased. Subsequently, proliferating cells decreased as wound recovery occurred and they were not detected after complete repair (36-48 h group) (Figure 3). Disappearance of BrdU positive cells was retarded in collagen type IV, fibronectin and laminin groups. In all groups, 60 h after wounding no cell-free areas and BrdU-positive cells were observed. These morphological observations were quantitated by calculation of the BrdU-labeling index (Figure 4).

DISCUSSION

Normal gastric mucosa restores a mucosal defect by sheet migration of surrounding surviving cells *in vivo* and *in vitro*(1,13,14). Recently, we established a new quantitative wound repair model to investigate the process of wound healing of primary cultured gastric

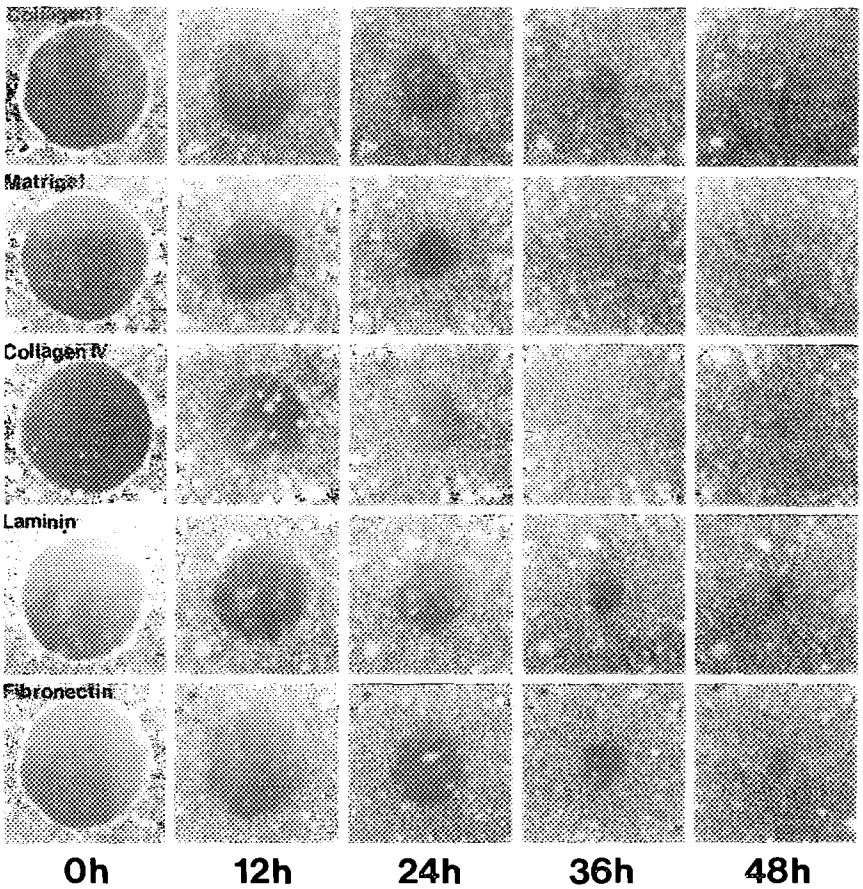

Figure 1

Phase contrast photomicrographs showing the process of wound repair
Inoculated cells formed complete monolayer cell sheet on each different extracellular matrix In controls, wound repair completed within 48 h In Matrigel group, wound repair was significantly accelerated In collagen type IV, laminin, and fibronectin groups, the speed of the wound repair was slower in comparison with controls
original magnification 40x.

mucosal cell sheets as a model for gastric mucosal injury(2,3,4). We found that gastric epithelial cells repaired the wound with an initial cell migration followed by a later cell proliferation.

Recent reports indicate that the extracellular matrix plays an important role in cell migration and cell proliferation. The extracellular matrix is known to regulate neoplastic(15) and nonneoplastic(16) cell migration in other cell systems and to alter adhesion(17) and

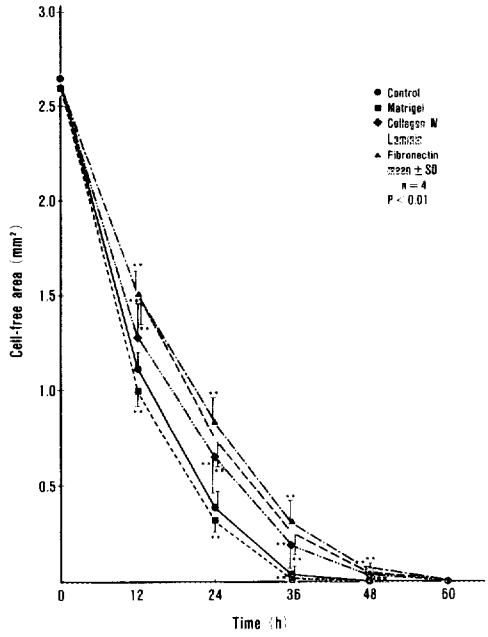

Figure 2

Quantitative analysis of the effects of different extracellular matrix on the mucosal restoration. Matrigel significantly caused the acceleration of mucosal restoration and collagen type IV, laminin, and fibronectin showed slower restoration speed.

** $p < 0.01$.

differentiation(18) in some poorly or moderately well-differentiated enterocyte cell lines. In the immune system, the extracellular matrix and its adhesion receptors regulate the migration of lymphocytes and interactions of activated cells during immune responses(19).

When gastric mucosal injury occurs, a variety of factors contribute to the mucosal restoration. Among them, the extracellular matrix might be very important. In this study, extracellular matrix modulated both cell migration and proliferation. Mucosal restoration on collagen type IV, fibronectin and laminin was slower than on Matrigel, which is a mixture of these components. These results indicate that the contribution of each extracellular matrix component to wound repair of gastric epithelial cells is different. From the aspect of cell proliferation, labeling index after wounding was not significantly different for cells grown on the various extracellular matrix components at 12 h to 36 h. However, a difference was observed

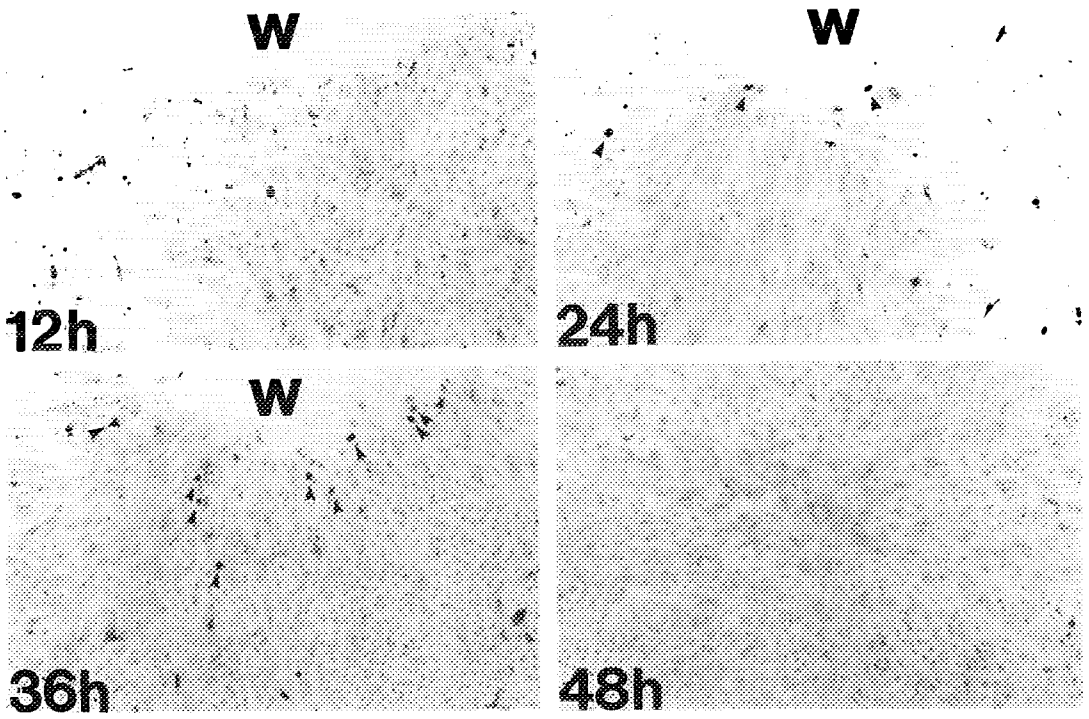

Figure 3.
Photomicrographs of BrdU staining of cultured gastric mucosal cells
This series of photomicrographs shows the immunohistochemical detection of BrdU-positive cells during the process of wound repair.
BrdU was added in the medium and incubated for 12 hours with different cultures as described in detail in Materials and Methods.
BrdU-positive proliferative cells are mainly detected in 24-36 h groups in control.
W: wound.
arrows: BrdU-positive cells.

in 48 h groups of collagen type IV, fibronectin and laminin. BrdU-positive cells were still observed in these groups. Cells inoculated on Matrigel showed a faster rate of wound repair than control, perhaps because, the major component of Matrigel is laminin, followed by collagen type IV, and heparan sulfate proteoglycans, and it also contains TGF-beta, fibroblast growth factor and other growth factors(11). Thus, Matrigel is a solubilized basement membrane, not a single extracellular matrix. Matrigel might accelerate cell migration more than the others. The reason the wounds on collagen type IV, laminin and fibronectin were restored more slowly than control will be evaluated in future studies. Laminin has previously

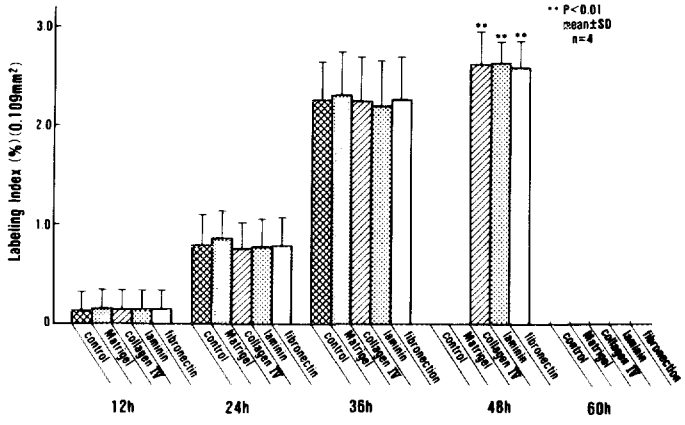

Figure 4

BrdU labeling index around the wound

There are no significant differences among each extracellular matrix tested in 12 h, 24 h, 36 h groups. In collagen type IV, laminin and fibronectin groups, labeling index showed the maximal value in 48 h group because of incomplete restoration of the wound.

** $p < 0.01$.

been reported to act as a promoter of differentiation and as a relative inhibitor of migration in other cell types(20). Thus the finding of a lower cell migration rate on laminin may reflect a generalized tendency for some cells on laminin to assume a less motile and more differentiated phenotype. In this cell culture model, cell migration rather than proliferation is the major factor responsible for wound healing, as we previously reported(5). The modulation of migration speed is an important reason for the different restoration rates in experiments with each extracellular matrix component. In the previous report (21), the cytoskeltal system plays a key role in cell migration. Therefore, it is reasonable that the extracellular matrix modulates the cytoskeletal system because the cell surface proteins which bind these ligands (integrins) are known to form connections with the cytoskeleton on the opposite side of the cell membrane(6,22,23).

In conclusion, the extracellular matrix might play an important role in gastric mucosal restoration in gastric ulcer disease.

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