A PATTERN OF EPIDERMAL CELL MIGRATION DURING WOUND HEALING

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ABSTRACT

Epidermal repair during wound healing is under investigation at both the light and electron microscopic levels. Suction-induced subepidermal blisters have been employed to produce two complementary model wound healing systems. These two model systems are: (a) intact subepidermal blisters, and (b) opened subepidermal blisters (the blister roof was removed immediately after induction, leaving an open wound). From these studies a pattern of movement for epidermal cells in wound healing is proposed. This pattern of movement is the same for both model systems. Epidermal cells appear to move by rolling or sliding over one another. Fine fibers oriented in the cortical cytoplasm may play an important role in the movement of these epidermal cells. Also instrumental in mediating this movement are intercellular junctions (desmosomes) and a firm attachment to a substrate through hemidesmosomes. In the intact subepidermal blisters hemidesmosomal attachment is made to a continuous and homogeneous substrate, the retained basal lamina. In the opened subepidermal blisters contact of epidermal cells is made to a discontinuous substrate composed of sporadic areas of fibrin and underlying mesenchymal cells.

INTRODUCTION

During the healing of cutaneous wounds, epidermal repair is manifested by the progressive extension of a "tongue" or stratified sheet of epidermal cells from the peripheral epidermis across the wound (2, 5, 6, 10, 25). Two important phenomena instrumental in this extension are migration and mitosis of epidermal cells. Mitotic activity, however, is not observed at or close to the leading margin of the epidermal sheet but in cells distal to this area (2, 5, 10, 22). It would appear, then, that cellular migration plays a very important role in restoring epidermal continuity.

Winter (38) has suggested that epidermal repair in wound healing is effected by the movement of epidermal cells over each other upon the surface of the wound. From in vitro studies, however, Vaughan and Trinkaus (33) have reported that the migration of epithelial cells occurs as a monolayered sheet. Observations by Lash (20), during the in vitro repair of wounded amphibian skin, indicate a cessation of movement for migrating epidermal cells upon mutual contact. Also, Abercrombie and Heaysman (1) have observed that fibroblasts moving toward each other from opposite directions on a cover slip ceased their movement once they contacted each other. Movement of one fibroblast over a contiguous fibroblast was not observed.

In an attempt to better understand the mechanism of epidermal cell migration in wound healing and to clarify the aforementioned divergent findings on cell movement, two complementary model wound healing systems have been developed. These model systems permit sequential observa-
tions of epidermal repair during the healing of intact and opened subepidermal blisters. The light and electron microscopic studies reported here show that normal epidermal cells do pass over one another during epidermal repair of wound healing. Furthermore, while the environment of the epidermal cells differs in both model systems, the pattern of movement appears the same. From the representative observations presented, a suggested pattern of movement for epidermal cells during wound healing is advanced.

METH O D S

Wounding

By the use of a variation of a technique first reported by Kiistala and Mustakallio (18) the epidermis was detached from the dermis with suction. The following device has been developed for these studies and provides a quick and uncomplicated way of inducing subepidermal blisters. A 4 cm portion from the tip of a Pasteur pipette is connected via a length of polyethylene tubing to an evacuated test tube (Vacutainer, Becton, Dickinson and Co., Rutherford, N.J.). This suction device and the techniques employed to obtain the two model wound healing systems are diagrammatically depicted in Fig. 1.

2-day old mice of the Webster strain (Department of Infectious Diseases Animal Farm, Harvard Medical School) were subjected to the aforementioned suction device until a clear elevation at the point of suction was observed. The device was applied for about 3–5 min and removed immediately after the blister was formed. Blisters were induced in the dorsal thoracic skin and averaged 1.0–1.5 mm in diameter. Immediately after subepidermal blister induction, animals were handled in one of two ways: (a) the blisters were allowed to remain intact, or (b) the blister roof was removed, leaving an open wound. All animals were biopsied at sequential periods of time after wounding.

The terms “intact” blister (blister roof left on) and “opened” blister (blister roof removed) will be

FIGURE 1  Diagrammatic illustration of techniques employed to obtain the two model wound healing systems described in the text.
used throughout the text of this paper to distinguish the two wound healing systems used in these studies.

**Tissue Preparation**

Biopsies of intact blisters were obtained immediately after wounding or at 12, 18, and 24 hr thereafter. Biopsies of opened blisters were obtained immediately after blister roof removal or at 1, 4, 12, 18, and 24 hr thereafter. Biopsies of intact or opened blisters were placed in one of the following fixative solutions: (a) 2% redistilled glutaraldehyde (7, 8) containing 50 mM monosodium phosphate buffer, pH 7.3 at 4°C for 1 hr. After a buffer wash, the tissue was secondarily fixed in 1% OsO₄ containing the same buffer for 1 hr at 4°C; (b) 4% formaldehyde (generated from the cyclic trimer trioxymethylene, Fisher Scientific Company, Pittsburgh, Pa.) containing 0.1 M monosodium phosphate buffer for 2 hr at room temperature. This tissue was postfixed for 2 hr at room temperature with 1.5% OsO₄ containing the same buffer; (c) a diluted version of Karnovsky's fixative (15) (i.e., 2% newly generated formaldehyde plus 2% redistilled glutaraldehyde) in 0.1 M monosodium phosphate buffer for 1 hr at room temperature. These tissues were postfixed at 4°C for 1 hr in 0.1 M monosodium phosphate-buffered 1% OsO₄. After secondary fixation all tissue specimens were treated en bloc with 0.5% uranyl acetate (9), rapidly dehydrated, and embedded in Epon (21).

Thick sections (1.0–1.5 µ) were cut on a Porter-Blum MT-1 ultramicrotome with glass knives and stained with borate-buffered toluidine blue for light microscopic study. Thin sections were cut on a Porter-Blum MT-2 ultramicrotome with a diamond knife and mounted on formvar-coated grids. These sections were doubly stained with aqueous uranyl acetate and lead citrate (34). Observations were made with a Zeiss EM9A electron microscope.

**RESULTS**

**Normal Mouse Skin**

Normal mouse skin (Fig. 2) at 2 days after birth has a cornified epidermis two to three cells thick. Cells of the basal layer are cuboidal in shape, while those of the upper layers have a flattened appearance. The dermo-epidermal junction appears relatively smooth and flat, except in areas where epidermal cells are contiguous with cells of the hair follicle root sheath. Blood vessels, fibroblasts, hair follicles, nerves, and occasionally leukocytes can be seen in the dermis.

**Intact Subepidermal Blisters**

A biopsy specimen removed immediately after blister formation (Fig. 3) shows detachment of the epidermis from the dermis at the dermo-epidermal junction, resulting in the formation of a subepidermal blister. Ultrastructurally (Fig. 4), the dermis immediately beneath the newly formed blister shows no gross disruption of architecture. Additionally, the basal lamina has remained with the dermis and forms the uppermost dermal...
FIGURE 4 An ultrastructural view of the blister base from an intact subepidermal blister biopsied immediately after induction. The dermal architecture (D) does not appear adversely affected by the application of the suction device. Blister cavity (BC). Formaldehyde-glutaraldehyde/OsO₄ fixation. Scale line, 1 µ. × 4760.

FIGURE 5 A biopsy obtained immediately after subepidermal blister induction shows that the basal lamina (BL) forms the uppermost boundary of the dermis. Projecting from the dermal side are fibers (arrows) usually associated with a cutaneous basal lamina. Mesenchymal cell (MC). Formaldehyde-glutaraldehyde/OsO₄ fixation. Scale line, 0.50 µ. × 55,800.
Figure 6 Biopsy of an intact subepidermal blister removed 12 hr after induction. Epidermal cell movement proceeds from the epithelial root sheath (ES) of the hair follicle over the denuded dermis. Fibrin and portions of degenerating epidermal cells from the blister roof have settled out from the blister cavity (BC), forming a layer of debris (D) upon the epidermal cells and the denuded dermal surface. Formaldehyde-glutaraldehyde/OsO₄ fixation. Scale line, 1 μ. × 5075. Inset: Light micrograph of an Epon-embedded section from an intact subepidermal blister removed 12 hr after induction. The blister roof (BR) remains intact at this time. Epidermal (E) cell movement appears as a wedge-shaped projection from the epithelial roof sheath of the hair follicle (HF) out over the denuded dermal (D) surface. Borate-buffered toluidine blue stain. Formaldehyde OsO₄ fixation. × 400.
boundary (Fig. 5). The basal lamina is easily recognized by its characteristic appearance and the fibers which project from its dermal side (26).

Primary movement of epidermal cells proceeds from the epithelial root sheath of the hair follicles (Fig. 6 and inset). At 12 hr after wounding, this movement manifests itself as a wedge-like projection from the follicle sheath over the denuded dermal surface. This wedge-shaped group of epidermal cells is two to three cells deep nearest the follicle and tapers to one elongated cell at the free epidermal margin. Fibrin from the blister fluid and portions of degenerating epidermal cells from the blister roof form a layer of debris on the epidermal cells and the denuded dermal surface. The blister roof is intact, but epidermal cells of the blister roof appear to be degenerating.

The most advanced epidermal cell extends a long narrow process out over the denuded dermal surface (Figs. 7 and 7 a). This cellular extension is in close proximity to the basal lamina, the uppermost boundary of the dermis. At selected points along the basal plasma membrane of this foremost epidermal cell, hemidesmosome formation is observed (Fig. 8). This same cell exhibits a cortically oriented band of fine fibers extending from the main part of the cell out into the narrow cellular process. Occasionally, at the free epidermal edge, the foremost extension of one epidermal cell can be observed overlapping the extended process of another epidermal cell (Figs. 7 a and 9).

18 hr after wounding, basally positioned epidermal cells back from the free edge (asterisk) have changed shape from low and elongated to an oval or round form (Fig. 10). These basal epidermal cells also display a greater interdigitation with the basal lamina as compared with cells at the free epidermal margin. Epidermal cells positioned superior to these basal cells have a flattened and elongated appearance. The cortical band of fine fibers can still be seen in superiorly oriented cells but this cortical band is not observed in the oval or round basal cells.

At 24 hr after wounding (Fig. 11), the original blister roof is still intact but the dermis is now completely covered by a noncornified epidermis three to four cells thick.

**Opened Subepidermal Blisters**

When the blister roof is removed the denuded dermal surface is exposed. A biopsy at this time (0 hr) reveals that no epidermal cells have remained (Fig. 12), and that the superior border of the dermis is formed by the basal lamina (Fig. 13). 1 hr after blister roof removal the exposed dermis appears to be degenerating as a result of dessication (Fig. 14). The basal lamina is not evident 1 hr after the removal of the blister roof, and the superior border of the dermis has an amorphous appearance (Fig. 15). A 24 hr biopsy (Fig. 16) shows that a coagulum composed of degenerating leukocytes and mesenchymal cells covers the wound area. Extending from the peripheral epidermis beneath this coagulum is a stratified sheet of epidermal cells (arrows). This sheet of cells is three to four cells deep at the peripheral epidermis and tapers to one or two epidermal cells at the free epidermal margin. The foremost cell of this stratified sheet is touching strands of fibrin (arrows, Fig. 17) and other cells of the dermis. Since a basal lamina is not present, areas of the basal plasma membrane are exposed to the dermal milieu. Located within the cytoplasm of this cell are...

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**Figure 7** The leading edge of an epidermal cell at the free epidermal margin, 12 hr after blister induction, is depicted (arrow). This cell process extends out upon the “old” basal lamina (BL) which still forms the upper boundary of the denuded dermis. Dermis (D). Formaldehyde glutaraldehyde/NaO4 fixation. Scale line, 1 µ. X 16,800.

**Figure 7 a** A more complete view of the epidermal cell in Fig. 7 is shown. The foremost portion of the cell (EC3) closely approximates the basal lamina (BL) and overlaps another epidermal cell (ECi). The direction of epidermal cell movement is indicated by the arrow. Dermis (D). Formaldehyde glutaraldehyde/NaO4 fixation. Scale line, 1 µ. X 16,800.

**Figure 8** An area on a foremost epidermal cell farther back from the free epidermal margin shows hemidesmosomal (HD) attachment of the epidermal cell to the old basal lamina (BL). In the cytoplasm, adjacent to the upper plasma membrane of the cell, a cortical band (CB) of fine fibers extend from the body of the cell out into the cellular extension. Formaldehyde-glutaraldehyde/NaO4 fixation. Scale line, 0.50 µ. X 41,400.
FIGURE 9  The narrow elongated processes of two advancing epidermal cells at the free epidermal margin are depicted. Epidermal cell (EC2) is farther advanced and its foremost cytoplasmic extension overlaps the extended process of another epidermal cell (EC1). Movement is in the direction of the arrow. Dermis (D); basal lamina (BL). Formaldehyde-glutaraldehyde/OsO₄ fixation. Scale line, 1 µ. × 16,800.

FIGURE 10  Biopsy of an intact subepidermal blister removed 18 hr after induction. A basally positioned cell (*) appears rounder or more oval in shape than superiorly positioned epidermal cells. The basal epidermal cells also have a greater interdigitation with the dermis (D) as compared with the epidermal pseudopod at the free margin. The cells above the basal layer still have a low and elongated profile. Movement is in the direction of the arrow. Formaldehyde-glutaraldehyde/OsO₄ fixation. Scale line, 1 µ. × 4025.

FIGURE 11  Light micrograph of an Epon-embedded intact subepidermal blister biopsied 24 hr after induction. The dermis (D) is now completely covered by a noncornified epidermis (E). Borate-buffered toluidine blue stain. Formaldehyde/OsO₄ fixation. × 400.

Vacuoles (V₁ and V₂) containing an amorphous electron-opaque material similar in appearance to the extracellular fibrin. Such deposits probably represent fibrin that has been phagocytized by the epidermal cells. Similar observations have been made by other investigators (11, 25). Along the dermal aspect of the epidermal sheet, close proximity of more than one epidermal cell to strands of fibrin is not uncommon (Fig. 18). The inset for Fig. 18 shows fibers having a banding periodicity of approximately 240 Å, equivalent to that of fibrin.

Although not depicted, a cortical band of fine fibers is also seen in epidermal cells at the free margin of the stratified sheet.

Hemidesmosome formation suggests attachment of the basally positioned epidermal cells to areas of

FIGURE 13 Corresponding electron micrograph of an area shown in Fig. 12. The basal lamina (BL) forms the upper boundary of the dermis (D). A portion of a mesenchymal cell and collagen fibers are depicted. Formaldehyde-glutaraldehyde/OsO₄ fixation. Scale line, 1 µ. X 15,000.

FIGURE 14 Light micrograph of an Epon-embedded opened blister. Biopsy obtained 1 hr after subepidermal blister induction and removal of the blister roof. The upper dermal boundary is a coagulum (C) composed of decomposed dermal elements. Borate-buffered toluidine blue stain. X 190.

FIGURE 15 Corresponding electron micrograph of area shown in Fig. 14. The upper dermal boundary (UDB) has an amorphous appearance and fine cellular detail is missing (arrows). No basal lamina is evident. Formaldehyde-glutaraldehyde/OsO₄ fixation. Scale line, 0.5 µ. X 47,000.
fibrin or newly formed basal lamina (arrows, Fig. 19). Many areas opposite the basal plasma membrane of these cells are devoid of either fibrin or basal lamina (single arrows, Fig. 21). Selected portions of the cell membrane along this aspect of the cell show a close approximation of the epidermal cell to subjacent macrophages or other mesenchymal cells (double arrows, Fig. 20).

**FIGURE 16** 24 hr biopsy of an opened blister. The coagulum (C) composed of degenerating leukocytes and mesenchymal cells covers the wound area. A stratified sheet of epidermal cells (arrows) extends from the peripheral intact epidermis beneath the coagulum. Borate-buffered toluidine blue stain. Formaldehyde/glutaraldehyde/OsO4 fixation. X 300.

**FIGURE 17** Biopsy specimen of an opened subepidermal blister removed 24 hr after wounding. Along its basal plasma membrane the foremost epidermal cell (EC) shows areas of close contact with strands of fibrin (arrows). A basal lamina is not present. Within the cytoplasm, vacuoles (V1 and V2) can be observed partially or completely filled with electron-opaque material, similar in consistency and appearance to extracellular fibrin (F). Formaldehyde-glutaraldehyde/OsO4 fixation. Scale line, 1 µ. X 13,500.
FIGURE 18 Three epidermal cells (EC1, EC2, and EC3) are depicted in close proximity to a sheet of fibrin (F). Scale line, 1 µm x 20,700. Inset: Higher magnification of extracellular fibrin containing fibers that show a banding of approximately 240 Å. Fixation of both areas, glutaraldehyde/OsO₄, X 40,800.

FIGURE 19 Along the basal plasma membranes of two epidermal cells (EC1 and EC2) hemidesmosomes (arrows) can be observed opposite areas of fibrin (f), or newly formed basal lamina. Glutaraldehyde/OsO₄ fixation. Scale line, 1 µm x 21,300.

FIGURE 20 The close proximity of an epidermal cell (EC) with a dermally oriented macrophage (M) is depicted. Some areas along the basal plasma membrane of the epidermal cell (single arrows) are completely devoid of either fibrin, basal lamina, or other cells. At other points close approximation of the epidermal cell's basal plasma membrane to the subjacent macrophage is evident (double arrows). Glutaraldehyde/OsO₄ fixation. Scale line, 1 µm x 20,400.

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Basally positioned epidermal cells (EC$_1$ and EC$_2$, Fig. 21) farther back from the advancing edge have a more oval or cuboidal appearance. Cells superior to these still have a low and extended profile.

**Speed of Epidermal Repair**

Epidermal repair takes place sooner in the intact subepidermal blisters than in the opened subepidermal blisters. At 24 hr after wounding, for example, the dermis of the intact subepidermal blisters is completely covered by a noncornified epidermis three to four cells thick, whereas at 24 hr after wounding in the opened subepidermal blisters, initiation of epidermal repair is observed at the lateral border of the wound. This appears as the extension of a stratified sheet of epidermal cells from the peripheral epidermis beneath the coagulum.

**DISCUSSION**

**Cellular Migration and Mitosis**

Restoration of normal epidermal architecture during wound healing results from both mitosis and migration of epidermal cells. Cellular migration, though, appears to be a primary event and the major factor in closing the epidermal hiatus (2, 5, 10, 22). Increased mitotic activity of epidermal cells is not usually observed until 1–2 days after restoration of epidermal continuity (22, 23, 35).

Interrupting mitotic activity by the administration of colchicine or vinblastine sulfate, immediately after subepidermal blister induction, produces no difference in epidermal cell migration in intact subepidermal blisters at 12, 18, or 24 hr after wounding. However, increasing numbers of epithelial root sheath cells arrested in the metaphase stage of mitosis are observed in animals receiving these stathmokinetic agents.

**Pattern of Epidermal Cell Movement**

From the representative observations presented in this paper the following suggested pattern of epidermal cell migration during epidermal repair of intact and opened subepidermal blisters is diagrammatically depicted in Figs. 22 and 23.

In the intact subepidermal blisters, the foremost epidermal cell extends a narrow pseudopod and through de novo formation of hemidesmosomes attaches to the basal lamina. The trailing portion of this cell then moves forward with epidermal cells positioned above and behind it. Subsequently, an elongated extension of an overlying epidermal cell rolls or slides over the basal cell below, contacts

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1. Krawczyk, W. S. Unpublished observations.
two flattened epidermal cells form the advancing margin while the epidermis behind is two to four cells deep. As advancing cells contact fibrin or subjacent mesenchymal cells, they assume an oval or cuboidal shape while superiorly positioned cells remain low and elongated. Superiory positioned cells move forward by sliding or rolling over basally positioned epidermal cells.

The pattern of movement for epidermal cells effecting restoration of epidermal continuity in both model systems seems to be the same. Epidermal cells appear to move by rolling or sliding over one another. Instrumental in mediating epidermal cell movement are the intercellular junctions (desmosomes) and an attachment to a substratum through the hemidesmosomes.

**Rate of Epidermal Wound Healing**

The difference in epidermal repair between the two model systems is striking. Restoration of epidermal continuity occurs faster in the intact system as compared to the opened system. A number of factors could influence this rate differential.

First, epidermal cells in the intact blister system contact a continuous substratum, the basal lamina. The phenomenon of "contact guidance" as proposed by Weiss (36, 37) may be contributing to the movement of these cells on the basal lamina. In the opened blister system there is no similar predetermined path along which the migrating epidermal cells can travel.

Secondly, the rapid repair in the intact system may partially result from the absence of any gross impediment in movement. Cellular debris and fibrinous deposits that have settled on the basal lamina appear to be deflected away. This may be due to a ruffling of the advancing cell membrane. Ruffling activity of this sort has been observed during the in vitro movement of epithelial cell sheets (33). The area around migrating epidermal cells in the opened system appeared quite congested with fibrin and cellular fragments and, as a result of this, forward progress is probably slower.

Thirdly, phagocytosis may slow cellular movement in the opened system. Inclusions containing deposits similar in appearance to extracellular fibrin are more commonly observed in epidermal cells of the open system than those of the intact system. Fibrin, cellular debris (13, 25, 28), and tracers like thorotrast (11) are phagocytized by epidermal cells in wounds like the opened subepi-
Figure 23 Diagrammatic representation of epidermal cell movement in opened subepidermal blisters. Relative progression of cells indicated by letters A, B, C, D, E, F. Explanation in text.
Cell Organelles and Movement

It is generally believed that hemidesmosomes have a major role in mediating the attachment of the epidermis to the connective tissue (4, 16, 17, 24). From our observations, we have no doubt that de novo formation of hemidesmosomes occurs along the basal plasma membrane of the foremost epidermal cell in the intact system, and it would appear that formation of hemidesmosomes in this model wound healing system is an important factor in mediating the movement of epidermal cells. What we cannot tell is whether these complexes are stationary or transient junctions. However, the correlated observations of an increasing interdigitation of the cell with the substrate (e.g., the basal lamina, Figs. 9 and 10) and a change in cell shape may indicate that these junctions are stationary. In healing wounds similar to the opened subepidermal blisters, Tarin and Croft (30) have observed close contact between epidermal cells and mesenchymal cells at the time when it was thought that epidermal invasion had ceased. It is their belief that this close contact of mesenchymal cells with epidermal cells may be important in reestablishing epidermal differentiation.

The nature of the intracytoplasmic forces that are instrumental in cell migration still remains obscure. Suggestions have been advanced that a fibrillar contracting mechanism may be effective in cytoplasmic movement (14, 40). The association of fine filaments with morphogenesis and cell form has been demonstrated in epithelial (29, 41) and other tissues (3, 12). Other fine filaments in the form of microtubules have been shown to be involved in axopodial extension (32) and maintenance of cell form (31). Cytoplasmic flow in migrating cells appears to have the greatest velocity near the cortical cytoplasm (14, 27, 40). The cortical band of fine fibers that we have consistently observed in both the intact and opened subepidermal blisters may have a significant role in the mechanisms of epidermal cell movement. These fibers do not appear to be tonofilaments, which are usually sparse in these advancing epidermal cells. A more detailed report on these fine fibers and their involvement during epidermal repair is in preparation.

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