Parakeratotic corneocytes play a unique role in human skin wound healing

Marcia L. Usui, Robert A. Underwood, Philip Fleckman, and John E. Olerud
Department of Medicine/Division of Dermatology, University of Washington, Seattle, WA, USA

Upon revisiting of our large library of archived human skin wound specimens, we observed that stratum lucidum interfollicular parakeratotic corneocytes appear to take an active role in epidermal wound healing by expanding, migrating and bifurcating to interact directly with and secure the wound scab in place. Our observations are based on the morphologic analysis of 240 acute, normal, full-thickness, incisional, 1-to 21-day human upper arm and lower leg skin wounds obtained from 30 healthy volunteers, average age 64±8 years (mean±SD). Wound tissue was either ½ Karnovsky's-fixed, PolyBed®-embedded and Richardson's-stained or Carnoy-fixed, paraffin-embedded and hematoxylin and eosin-stained. All wounds were obtained in accordance with the Declaration of Helsinki and the University of Washington Human Subjects Institutional Review Board.

In interfollicular epidermis, terminal differentiation begins in the granular layer of the epidermis, resulting in the formation of a cornified stratum corneum (SC), often described as the “dead” outer layer of the skin (Candi E et al., 2005). The SC, however, has been shown to be a dynamic and metabolically interactive tissue acting as a biosensor to regulate metabolic responses in the underlying nucleated cells layers (Elias PM, 1996). The SC appears to be composed of three structurally (Brody I, 1962) and functionally (Richter T et al., 2004) identifiable sublayers: 1) the outermost layer composed of corneocytes that undergo desquamation (stratum disjunctum); 2) an intermediary zone of uniform-sized, tightly-compacted anucleate corneocytes (stratum compactum); and 3) the zone immediately adjacent to the stratum granulosum populated with parakeratotic corneocytes (stratum lucidum) (Kligman A, 1964). Stratum lucidum parakeratotic corneocytes retain rod-shaped nuclei, ribosomes, lysosomes, mitochondria, Golgi apparatus, numerous granules, fibrillar structures (Ebling F and Rook A, 1972), and have “fragile” rather than “rigid” cornified envelopes identified in the upper layers of the SC (Haftek M et al., 2011). The stratum lucidum is approximately 1–2 cell-layers thick in normal interfollicular skin and is more prominently visible (~8–10 layers) in palmoplantar skin and lip (Ebling F and Rook A, 1972).
Stratum lucidum corneocytes in interfollicular skin have been distinguished from the upper SC corneocytes by use of unique tissue fixations and histochemical stains (Montagna W et al., 1992) but are poorly discernable in routine hematoxylin and eosin-stained tissue. Organelles are only sporadically identifiable in corneocytes in this transition zone even using transmission electron microscopy (Montagna W et al., 1992). Teleologically, a transient zone must exist where major cellular changes (loss of nuclei, organelles, and keratohyalin granules) take place, however, the difficulty of identifying the zone lies in the fact that this metamorphosis occurs with great speed (Kligman A, 1964).

Upon injury to the skin, the clotting cascade is initiated in order to stop the hemorrhaging of damaged vessels (Singer AJ and Clark RA, 1999). A clot filled with fibrin, blood components, wound debris (collagen and elastin fragments), and glycoproteins (Singer AJ and Clark RA, 1999) provides a “sticky” plug that covers and fills the wound bed. As wound healing progresses, the clot appears to compartmentalize into two identifiable regions, with the upper region of the clot separated from the lower region by a “polyband” layer of polymorphonuclear leukocytes (Jonkman MF et al., 1988). This upper clot region desiccates, forming a scab, crust or eschar that sloughs eventually during tissue repair while the lower fibrin-rich region of the clot serves as the wound bed in which granulation tissue forms (Singer AJ and Clark RA, 1999).

In this study we show that in acute human skin wounds, a unique population of parakeratotic corneocytes interacts directly with scabs, appearing to secure a temporary barrier to cover a wound until the underlying epidermis fully epithelializes, terminally differentiates, and restores a permanent skin barrier. Along the wound margin of a 6-μm tissue section of a 1-day wound immunolabeled with a pancytokeratin antibody (Dako, Carpenteria, CA), parakeratotic corneocytes above granular layer corneocytes expand in number and begin to migrate laterally towards the wound exudate (Figure 1a). In the 2-day wound (1b), the parakeratotic corneocyte population then bifurcates with a second population of parakeratotic corneocytes appearing to stream ventrally down along the underlying epidermis. In the 3-day wound (1c), the ventral branch parakeratotic corneocytes travel beyond the migrating tip (arrowhead) of the underlying nucleated migrating epidermis. By 7 days (1d), a mature scab has formed above an epithelialized new wound epidermis, showing parakeratotic corneocytes interacting with the upper region of the scab and undermining the base of the scab. Figure 1e shows a schematic illustration of the relationship between parakeratotic corneocytes and scabs as we observed.

Forty four percent (106/240) of the wounds appeared to have identifiable scabs (many immature wounds not yet forming scabs) of which 93% (98/106) of the wounds with scabs showed a pattern of scab/parakeratotic corneocyte interaction (Figure 1f). On occasion, scabs mechanically (either from tissue processing or from handling during tissue harvest) separated from the underlying new epithelium. These scabs appeared to be attached to the SC of the wound tissue sample (Figures 1g –i). This finding suggests that scabs adhere to the wound bed not merely by their “stickiness”, but by direct interaction with the SC.

The earliest observation of wound keratinocyte interaction with the scab was made by Leo Loeb in 1898, who described and illustrated in great detail that the granular and “horny”
(SC) layers resolved into a homogeneous multinucleated protoplasmic layer, completely independent of the underlying epithelial tongue. This protoplasmic layer, for which he saw no cellular borders, bifurcated, with the upper “arm” quickly integrating with or covering the scab while the lower “arm” more slowly migrated to undermine the scab ahead of the underlying Malphigian (nucleated) keratinocytes. These protoplasmic cells adhered tightly to the scab until the scab was sloughed (Loeb L, 1898).

Zahir suggested that Loeb’s “upper protoplasmic layer was either coagulated exudate forming the most superficial part of the scab or layers of collagen fibers at the surface of the scab”. Zahir also described the branch extending over the upper surface of the scab as less well-developed cells with pyknotic nuclei (Zahir M, 1965). Rather than necrotic, pyknotic cells, our studies show that parakeratotic corneocytes appear to be viable, as indicated by their ability to migrate towards the wound.

Viziam et al. stated that the expanded parakeratotic corneocyte population seen in wounds emanated from rapid differentiation of new wound suprabasal and stratum granulosum keratinocytes. They did not observe parakeratotic cells in the proximal portion of the migratory wound epithelial tongue, and concluded that the actively migrating epithelium had not yet undergone differentiation (Viziam CB et al., 1964).

In contrast to studies by Viziam et al. we observed presence of parakeratotic corneocytes beyond the tip of the underlying, migrating, nucleated tongue of early, 1–3-day wounds. The presence of parakeratotic corneocytes not in direct association with underlying granular layer corneocytes suggests that these parakeratotic corneocytes are not derived from an accelerated keratinocyte differentiation pathway of the new wound epidermis but appear to be derived as a much earlier response to injury.

It appears that keratinocytes in the granular layer in response to injury continue to produce parakeratotic corneocytes that expand in number possibly by ceasing to differentiate into anucleate corneocytes. It is this expanded population of parakeratotic corneocytes that we believe independently interacts with the scab. The expansion of the parakeratotic population appears not to be by mitosis since there is no evidence of Ki67 immunostaining within this cell population (Usui ML et al., 2005). These unique parakeratotic corneocytes may play a role in epidermal repair separate from the role of underlying differentiated suprabasal keratinocytes (keratinocytes not yet cornified) that have been postulated to actively participate in re-epithelialization by rolling onto the wound bed (Usui ML et al., 2005).

Securely attaching a scab as a temporary barrier not only protects the wound bed, but formation of an attached transitory scab may have additional benefits in protecting the host from infection. Studies have shown that 99% of the bacterial population found in wounds is sequestered in scabs and not on or within wounds beds (Barnett A et al., 1986; Zhao G et al., 2010).

These morphologic observations necessitate additional characterization and mechanistic studies. The origin of the parakeratotic corneocytes emanating from the stratum lucidum is based strictly from our many static morphological images and is therefore hypothetical. We defined the parakeratotic keratinocytes we observed as being “corneocytes” however, lipid
membrane immaturity, fragility of cornified envelopes, presence of corneodesmosomes and ultrastructural components (cornified envelopes and organelles) characteristic of normal parakeratotic corneocytes must be further evaluated to clearly determine if they are, in fact, corneocytes. In addition, studies need to be conducted to determine the mechanism by which corneocytes migrate (cytoskeletal machinery) and adhere (surface receptors) to the desiccating scab matrix.

Though this was strictly a morphological and not a mechanistic study in which our observations show that wound parakeratotic keratinocytes interact directly with scabs, we concur with Kligman’s philosophy about morphological observations: “The usual sequence of biological knowledge is from the anatomical to the physiological” (Kligman A, 1964).

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Abbreviation

| SC   | stratum corneum |

REFERENCES

Barnett A, Dave B, Ksander GA, et al. A concentration gradient of bacteria within wound tissues and scab. J Surg Res. 1986; 41:326–32. [PubMed: 3762140]

Brody I. The ultrastructure of the horny layer in normal and psoriatic epidermis as revealed by electron microscopy. J Invest Dermatol. 1962; 39:519–28. [PubMed: 14015700]

Candi E, Schmidt R, Melino G. The cornified envelope: a model of cell death in the skin. Nat Rev Mol Cell Biol. 2005; 6:328–40. [PubMed: 15803139]

Ebling, F.; Rook, A. Disorders of keratinization. In: Rook, A.; Wilkinson, D.; Ebling, F., editors. Rook's Textbook of Dermatology. 3rd ed.. Vol. Vol. 2. Blackwell Scientific Publications; Oxford, London, Edinburgh, Melbourne: 1972.

Elias PM. Stratum corneum architecture, metabolic activity and interactivity with subjacent cell layers. Exp Dermatol. 1996; 5:191–201. [PubMed: 8889466]

Haftek M, Callejon S, Sandjeu Y, et al. Compartimentalization of the human stratum corneum by persistent tight junction-like structures. Exp Dermatol. 2011; 20:617–21. [PubMed: 21672033]

Jonkman MF, Bruin P, Hoekema EA, et al. A clot-inducing wound covering with high vapor permeability: enhancing effects on epidermal wound healing in partial-thickness wounds in guinea pigs. Surgery. 1988; 104:537–45. [PubMed: 3413682]

Kligman, A. The biology of the stratum corneum. In: Montagna, W.; Lobitz, W., editors. The epidermis. Academic Press; New York and London: 1964.

Loeh L. Über regeneration des epithels. Archiv für entwicklunsmechanik der organismen. 1898; 6:297–364.

Montagna, W.; Kligman, A.; Carlisle, K. Atlas of Normal Human Skin. Springer-Verlag; New York, Berlin, Heidelberg, London, Paris, Tokyo, Hong Kong, Barcelona, Budapest: 1992. p. 384

Richter T, Peuckert C, Sattler M, et al. Dead but highly dynamic--the stratum corneum is divided into three hydration zones. Skin Pharmacol Physiol. 2004; 17:246–57. [PubMed: 15452411]

Singer AJ, Clark RA. Cutaneous wound healing. N Engl J Med. 1999; 341:738–46. [PubMed: 10471461]
Usui ML, Underwood RA, Mansbridge JN, et al. Morphological evidence for the role of suprabasal keratinocytes in wound reepithelialization. Wound Repair Regen. 2005; 13:468–79. [PubMed: 16176455]

Viziam CB, Matoltsy AG, Mescon H. Epithelialization of small wounds. J Invest Dermatol. 1964; 43:499–507. [PubMed: 14234856]

Zahir M. Formation of Scabs on Skin Wounds. Br J Surg. 1965; 52:376–80. [PubMed: 14286988]

Zhao G, Hochwalt PC, Usui ML, et al. Delayed wound healing in diabetic (db/db) mice with Pseudomonas aeruginosa biofilm challenge: a model for the study of chronic wounds. Wound Repair Regen. 2010; 18:467–77. [PubMed: 20731798]
Figure 1.
(a–d) Pan-cytokeratin antibody labeled tissue sections of 1-, 2-, 3-, and 7-day wounds respectively show parakeratotic corneocytes (PC) (blue dashed-lines) interacting with scabs (*). Black dashed lines = dermal-epidermal-junctions, arrowhead = tip of migrating epidermal tongue. Inserts a°–d°, red dashed lines outline wound beds, blue boxes indicate regions in (a–d). (e) Illustration of a wound shows PC (magenta) bifurcation with neutrophil polyband outlined in blue. (f) Table showing scab analysis. (g) H&E-stained 7-day wound shows scab (*) attached to the stratum corneum (SC). Arrows indicate split in SC. (h–i) Higher magnification of boxed regions in (g) shows PC attachment to scab (*), (blue outline) (h). Mag bars (a°, b°, c°) = 200 μm, (d°) = 500 μm, a–d = 50 μm, g = 200μm, h–i = 50μm.