

Supplement to the September 2005

WOUNDS

A Compendium of Clinical Research and Practice



Targeting the Science Within Wounds

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Continuing Education

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1. Determine the biochemical signals and pathways associated with wound healing
2. Review the behavior of epidermal-dermal communication
3. Discuss the structure and function of cell therapy in wound healing
4. Discuss the rationale for utilizing advanced technology for the chronic wound.

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Keratinocyte Cross-Talks in Wounds

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This article will provide an overview of the epidermis and discuss the abnormalities that are found in a nonhealing wound with a focus on the biology of keratinocytes. Keratinocytes and fibroblasts are the 2 major cellular compounds that comprise the human skin equivalent known as bilayered cell therapy (Apligraf, Organogenesis Inc., Canton, Mass). Because keratinocytes play a significant role in wound healing, it is also important to have an understanding of what is in their biology that participates in the normal process and how it is affected in the pathogenesis of the chronic wound.

KERATINOCYTE SPECIFICS

As epidermal cells, keratinocytes are derived from the epidermal stem cells that reside in the bulge area of the hair follicle. From there, they migrate into the basal layers of epidermis, where they give rise to their progenitors of transit-amplifying cells. Then, keratinocytes become proliferative and differentiating, giving rise to a tissue called epidermis (**Figure 1**). The basal layer of keratinocytes is the only layer that can mitotically divide. As keratinocytes leave the basal membrane, they start to differentiate and lose their nuclei, synthesize insoluble proteins that cross-link, and form a protective, cornified layer to fulfill their major function—barrier formation—which keeps pathogens out and water in.¹⁻⁴

The process of differentiation is perpetual; the epidermis is replenished by the epidermal stem cells and their progenitors. During wound healing, this normal process of differentiation is interrupted, and keratinocytes change their phenotype, becoming activated.

Activated keratinocyte phenotype indicates that these cells are migratory and hyperproliferative, producing, secreting, and responding to extracellular matrix components and signaling polypeptides. It is inherent in their biology to not only maintain the barrier but also to inform neighboring cells when the barrier has been broken and when there is possible pathogen penetration.

KERATINOCYTE BEHAVIOR

Keratinocytes have pre-stored interleukin-1 (IL-1). As soon as a wound occurs and the barrier is disrupted, the pro-inflammatory cytokine of IL-1 is released.

endothelial cells and, even though they are not in direct contact with all these cell types, they communicate with each other by responding to specific signaling molecules such as growth factors and cytokines including keratinocyte growth factor (KGF), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), granulocyte macrophage colony-stimulating factor (GM-CSF), nerve growth factor (NGF), insulin-like growth factor (IGF), IL-1, tumor necrosis factor (TNF), and transforming growth factor (TGF) (**Figure 2**). In return, keratinocytes are equipped to respond to growth factors that are secreted by other

As a result of keratinocyte biology, epidermis as a tissue has the ability for complete regeneration.³⁻⁵

As a result, the keratinocytes respond to IL-1 stimulation by entering the activation cycle that stimulates their migration and proliferation to restore the broken barrier. Therefore, as a result of keratinocyte biology, epidermis as a tissue has the ability for complete regeneration.³⁻⁵

As a response to wounding and keratinocyte activation, a variety of cells (primarily fibroblasts) in the vicinity of the wound site receive signals from keratinocytes.⁵ As a consequence, these cells start releasing multiple growth factors, beginning the wound-healing process. Keratinocytes communicate with dermal fibroblasts, lymphocytes, granulocytes, platelets, neurons, macrophages, and

cells. For example, fibroblast-producing KGF specifically targets keratinocytes, its primary responding cells. In response to all of these stimuli, keratinocytes start migrating and proliferating.

Adhesion molecules direct the biology of the cell within the structure of the epidermis in a way that, at any given moment, keratinocytes “know” their position throughout the epidermis. Understanding how keratinocytes respond to extracellular matrix molecules (ECMs) is particularly important because, by virtue of tissue engineering, one can change the matrix molecules. It is necessary to predict cellular response as a consequence of that change. A

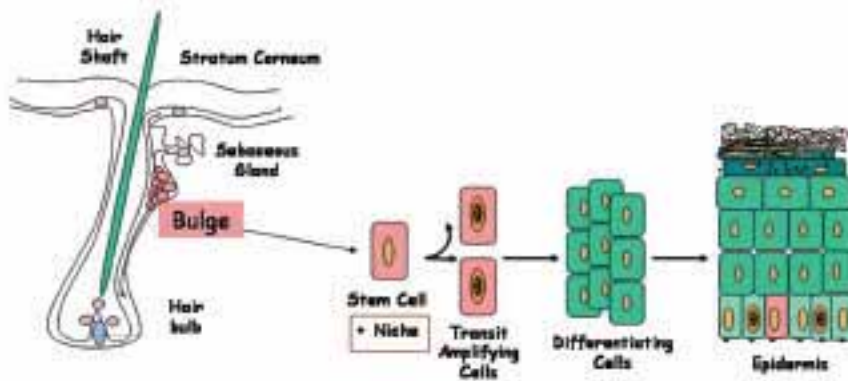


FIGURE 1: From epidermal stem cells to differentiated epidermis. The graph illustrates the cellular pathway that leads to fully differentiating epidermis.

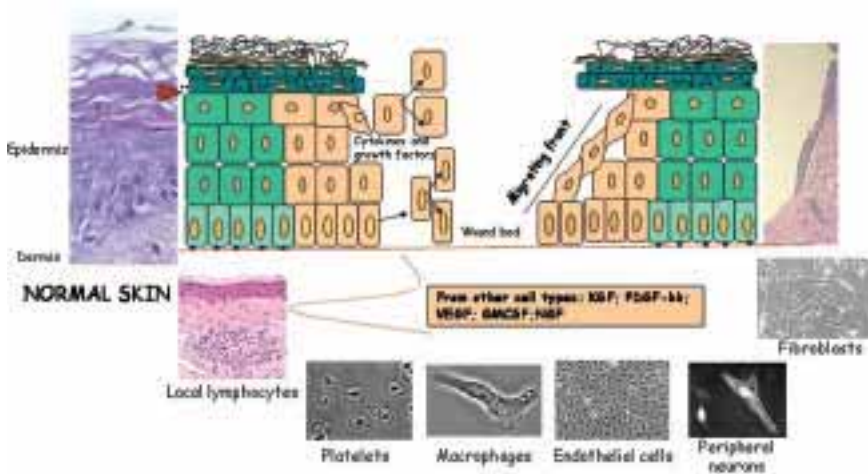


FIGURE 2: Cellular cross-talk between keratinocytes and a number of other cell types in the site of the wound triggers keratinocyte migration and proliferation, thus maintaining the wound-healing process.

recent article by Ortiz-Urda et al.⁶ provides another interesting example of different keratinocyte response—in this case, to collagen VII. If exposed to a truncated form of collagen VII, keratinocytes will respond incorrectly, leading to a higher incidence of squamous cell carcinomas.

KERATINOCYTES AND WOUND HEALING

Multiple growth factors and cytokines produced and secreted by keratinocytes and other surrounding cell types regulate transcription of specific genes that guide and govern the process of wound healing. This is achieved by multiple transcription factors that translocate to the nuclei, targeting particular epidermal genes (eg,

keratin-6 [K-6]) that participate in wound healing. This gene is an intermediate filament molecule that, together with its partner K-16, participates in the formation of the cytoskeleton. It is believed that filaments formed by K-6/K-16 soften the cytoskeleton, providing the flexibility of the cellular scaffold to allow them to migrate. Keratin-6 is an early wound-healing gene that is activated upon normal acute wound healing.

Lee et al.⁷ have used K-6 as a paradigm of keratinocytes specific transcriptional regulation during wound healing (ie, how particular genes in the epidermis are targeted by these multiple growth factors that orchestrate the wound-healing process). The authors studied the K-6

promoter and identified a variety of molecules that bind as a cluster of transcription factors in response to early signals of wound healing, such as tumor necrosis factor-alpha (TNF- α), EGF, and IL-1. This cluster further interacts with groups of proteins designated as co-activators that are responsible for “opening” the chromatin leading to the strong activation of transcription. If, at the same time, cells are exposed to corticosteroids, which are wound-healing-inhibitor compounds, epithelization is either inhibited or markedly delayed. On a molecular level, tools are in place to both induce and repress this gene and its function within the process. Therefore, the outcome (cellular behavior) depends on the balance of these positive and negative factors, resulting in either migration or its inhibition.

A LOOK AT AN ANIMAL MODEL

Samuels et al.⁸ generated a transgenic mouse, which, on a molecular level, shifts the balance of wound-healing molecules toward repression by eliminating a copy of 1 of the co-activators. This means that EGF response is altered, and as a result, the mouse develops a chronic wound phenotype.⁸ Keratinocytes from these mice do not migrate and do not respond to EGF stimuli, leading to inhibition of epithelization and development of chronic wounds. Results from the mouse model have also been seen in the clinic. On a molecular level, this translates into failure of keratinocyte activation that leads to lack of epithelization and impaired wound healing. The next question is: What is the keratinocyte phenotype in a chronic wound environment?

Histological analyses of biopsies from patients with chronic ulcers reveal that the epidermis clearly looks different. It is hyper-proliferative (in addition to the basal cells, suprabasal cells are also proliferating and are mitotically active, which is consistent with keratinocyte activation). However, these cells are for some reason unable to migrate. Therefore, keratinocytes at the chronic wound edge reveal only partial (incomplete) activation.⁹ In addition, the cornified layer is thick and shows the presence of nuclei, unlike in a normal epi-

dermis, indicating that, in addition to incomplete activation, the differentiation process is also not fully completed and is incapable of proceeding to full determination.⁹ Therefore, keratinocytes at the chronic wound edge are partially activated and partially differentiating, not able to complete either of the 2 processes.

CHRONIC WOUND PHENOTYPE

Several studies have shown that, in transgenic mouse models, overexpression of c-myc in the basal layer of epidermis causes chronic wound phenotype and depletion of local epidermal stem cell population.¹⁰⁻¹² Over-expression of c-myc in the super basal layer of epidermis causes hyper-proliferation and hyper-keratosis, which is evident in patient biopsies. Taken together, this would suggest possible activation of c-myc in the chronic wound environment, resulting in similar cellular behavior. When patients' biopsies were tested, activation of the c-myc in keratinocytes at the nonhealing edge of chronic wounds was apparent.⁹

C-myc is a known downstream target of a factor called beta-catenin, which normally participates in adherens junction formation. When Stojadinovic et al.⁹ examined biopsies from patients with chronic ulcers for the localization of the beta-catenin, the authors found it to be nuclear in the keratinocytes of the non-healing edge of the chronic wound in the same location where as c-myc activation.

Specific phenotype of keratinocytes on the nonhealing edge of a chronic wound is characterized by incomplete activation and differentiation in part resulting from the activation (nuclear presence) of beta-catenin and expression of c-myc, leading to the lack of appropriate response to growth factors and cytokines. Cells grown from the nonhealing edge fail to respond to growth factor stimuli, whereas the cells from the adjacent epidermis that histologically looks normalized respond properly to such stimuli. Those cells do not have c-myc expression/beta-catenin nuclearization characteristics and are the target cells, which will be able to respond to the therapy.

Molecular markers, such as beta-cat-

nein and c-myc, can be utilized to detect the location of the chronicity and the cells that are permissive and responsive to the therapy. The area of the wound with marked reduction of the cells positive for c-myc and nuclear beta-catenin presence can be identified with these markers.

POTENTIAL OF TISSUE ENGINEERING

Today, cells can be grown *in vitro*, their genome engineered, and skin created in the culture dish.¹²⁻¹⁶ Plus, the epidermal stem cells can be isolated and engineered to secrete growth factors and cytokines.¹²⁻¹⁶ An epidermal stem cell can be engineered to sustain expression of a "foreign" gene (transgene) *in vitro*. Such a stem cell can give rise to the epidermis in which all their daughter cells maintain the expression of that transgene, thus creating an epidermis that continuously expresses a molecule originating from a transgene.¹⁵⁻¹⁶ This engineering technology creates huge potential, given that tissue-engineered products in the form of human skin equivalents are a current FDA-approved treatment modality for chronic wounds.¹⁷

CONCLUSION

Understanding the molecular mechanism and pathogenesis of keratinocytes will provide insight into why wounds fail to heal. Also, utilizing current new technologies focused on such an approach will allow for identification of the potential molecular targets and therapeutic approaches.¹⁸ Bringing such knowledge to tissue engineering further provides clinicians with the ability to utilize live cells as a delivery system to the wound site. Also, patient's keratinocytes can already be grown and combined with human skin equivalents to make skin reconstructed from the patient's cells. This creates a number of possibilities, such as customized therapy for specific types of ulcers and eventually customized products for individual patients. ■

References

1. Fuchs E, Tumber T, Guasch G. Socializing with the neighbors: stem cells and their niche. *Cell*. 2004;116(6):769-778.
2. Koster MI, Roop DR. Genetic pathways required for epidermal morphogenesis. *Eur J*

3. Tomic-Canic M, Agren M, Alvarez O. *Cell Biol*. 2004;83(11-12):625-629.
4. Epidermal repair and the chronic wound. In: Rovee D, Maibach H, eds. *Epidermal Wound Healing*. Boca Raton, Fla: CRC Press; 2004:25-59.
5. Morasso M, Tomic-Canic M. Epidermal stem cells: the cradle of epidermal determination, differentiation and wound healing. *Biol Cell*. 2005;97(3):173-183.
6. Freedberg IM, Tomic-Canic M, Komine M, Blumenberg M. Keratins and the keratinocyte activation cycle. *J Invest Dermatol*. 2001;116(5):633-640.
7. Ortiz-Urda S, Garcia J, Green CL, et al. Type VII collagen is required for Ras-driven human epidermal tumorigenesis. *Science*. 2005;307(5716):1773-1776.
8. Lee B, Im MJ, Vouthounis G, Stojadinovic O, Brem H, Tomic-Canic M. From enhanceosome to repressosome: molecular antagonism between corticosteroids and EGF that leads to inhibition of wound healing. *J Mol Biol*. 2005;345(5):1083-1097.
9. Mahajan MA, Das S, Zhu H, Tomic-Canic M, Samuels HH. The nuclear hormone receptor co-activator NRC is a pleiotropic modulator affecting growth, development, apoptosis, reproduction, and wound repair. *Mol Cell Biol*. 2004;24(11):4994-5004.
10. Stojadinovic O, Brem H, Vouthounis C, et al. Molecular pathogenesis of chronic wounds: the role of beta-catenin and c-myc in the inhibition of epithelialization and wound healing. *Am J Pathol*. 2005;167(1):59-69.
11. Honeycutt KA, Roop DR. c-myc and epidermal stem cell fate determination. *J Dermatol*. 2004;31(5):368-375.
12. Waikel RL, Kawachi Y, Waikel PA, Wang XJ, Roop DR. Deregulated expression of c-Myc depletes epidermal stem cells. *Nat Genet*. 2001;28(2):165-168.
13. Arnold I, Watt FM. c-myc activation in transgenic mouse epidermis results in mobilization of stem cells and differentiation of their progeny. *Curr Biol*. 2001;11(8):558-568.
14. Bickenbach JR. Isolation, characterization, and culture of epithelial stem cells. *Methods Mol Biol*. 2005;289:97-102.
15. Ghazizadeh S, Taichman LB. Multiple classes of stem cells in cutaneous epithelium: a lineage analysis of adult mouse skin. *EMBO J*. 2001;20(6):1215-1222.
16. Ghazizadeh S, Doumeng C, Taichman LB. Durable and stratum-specific gene expression in epidermis. *Gene Ther*. 2002;9(19):1278-1285.
17. Garlick JA, Fenjves ES. Keratinocyte gene transfer and gene therapy. *Crit Rev Oral Biol Med*. 1996;7(3):204-221.
18. Brem H, Young J, Tomic-Canic M, Isaacs C, Ehrlich HP. Clinical efficacy and mechanism of bilayered living human skin equivalent (HSE) in treatment of diabetic foot ulcers. *Surg Technol Int*. 2003;11:23-31.

The Science of Bilayered Cell Therapy

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Over the past several decades, prognostic indicators have been recognized that can predict which ulcers are likely to heal. Substantial data exist for a variety of wound types, and universal to predicting healing with treatment involving standard of care are the wound's baseline size and duration.^{1,2} Additionally, surrogate endpoints, such as reduction in wound size after 4 weeks of treatment, can also predict eventual healing.³⁻⁵ Implied in the concept of predicting healing is that

mon chronic wounds are refractory to healing. For example, a meta-analysis of control groups of clinical trials involving diabetic neuropathic ulcers found that only 24% of patients healed with standard of care.⁸ Among patients treated in curative wound centers, analysis of that database found that only one-quarter of patients healed after 12 weeks of care and only 31% of patients healed after 20 weeks.⁸

Whether VLUs, which heal in the range of 40–70% with standard care in

the VLU, and the pressure ulcer. A number of therapies currently in practice aim to address these underlying features associated with nonhealing and include antimicrobial and antiproteolytic dressings, growth factors, and bioengineered skin. Among the latter is the only bilayered cell therapy approved by the US Food and Drug Administration (Apligraf, Organogenesis Inc., Canton, Mass) for treating chronic wounds.

Controlled, randomized trials in VLUs²⁸ and DFUs²⁹ have shown significantly improved healing when bilayered cell therapy was added to standard of care. In VLUs, healing was even more impressive in a statistically significant way compared to control patients with refractory, hard-to-heal wounds²⁹ and for DFUs, not only was healing improved (RR >2) but other outcomes, such as occurrence of osteomyelitis and amputation numbers, improved as well with bilayered cell therapy.³⁰

*A substantial number of
common chronic wounds are
refractory to healing.*

differential features exist between healing and nonhealing wounds and that addressing these features might lead to healing for those refractory wounds.⁶ This article will review some of these differential features and discuss how bilayered cell therapy may obviate these factors.

THE NONHEALING WOUND

Using the venous leg ulcer (VLU) model as an example of the aforementioned data, ulcers present longer than 6 months and those larger than 5 cm² are factors associated with an increased likelihood of nonhealing. When seen in combination, only 13% of patients were found to heal with standard of care (multilayered compression bandages).⁷

Overall, a substantial number of com-

mon chronic wounds are refractory to healing. For example, a meta-analysis of control groups of clinical trials involving diabetic neuropathic ulcers found that only 24% of patients healed with standard of care.⁸ Among patients treated in curative wound centers, analysis of that database found that only one-quarter of patients healed after 12 weeks of care and only 31% of patients healed after 20 weeks.⁸ Whether VLUs, which heal in the range of 40–70% with standard care in clinical practice,⁹ or diabetic foot ulcers (DFUs), which heal in the range 30–45% in clinical practice,¹⁰ a significant number of common chronic wounds fail to heal in a timely fashion. Common to each chronic wound is an abnormal wound environment. There are common features to all chronic wounds that do not heal. For nonhealing wounds, even those with an adequate blood supply in a well-nourished person, features associated with a refractory nature include unresponsive or senescent cells, an inflammatory and proteolytic wound environment, deficient or unavailable growth factors, and the presence of bacteria, either in form or number, that may inhibit healing.¹¹⁻²⁷ These factors seem to be common to a variety of chronic wounds—the DFU,

THE SCIENCE OF THE TECHNOLOGY

Bilayered cell therapy provides a variety of important factors to a nonhealing wound including the various cell types themselves as well as growth factors and other cytokines produced by these cell types, natural antibiotics produced by the keratinocytes, matrix proteins, and proteoglycans. *In toto*, although the exact mechanism of action remains unknown, it is theorized that this therapy works by not only providing a temporary barrier function, both physical and biologic, but also serving as a dermal matrix for cell migration and adsorbing proteolytic enzymes.

The manufacturing process begins with neonatal cells, both keratinocytes

and fibroblasts. After extensive screening for infectious organisms, fibroblasts are initially placed in a bovine, type-I collagen matrix. Over a 6-day period, these fibroblasts proliferate, and they begin to secrete their own matrix, in essence developing a bilayered cell therapy-derived neodermis. After 6 days in culture, neonatal foreskin keratinocytes are placed over the developing dermal matrix and, for the following 4 days, migrate as a monolayer to cover the neodermis. Subsequently, the submerged cells are raised to an air-liquid interface, allowing keratinocyte stratification. After 10 days, a stratified, functional epithelium exists, ready for patient application.

CELL FUNCTION

Although implied, application of healthy, proliferating neonatal cells appears critical to the success of bilayered cell therapy. As previously noted, cellular senescence is a cardinal feature of non-healing wounds. Fibroblasts and keratinocytes have been shown to be morphologically and functionally abnormal in refractory wounds. Conceptually, replacing both of these cell types with bilayered cell therapy application appears worthwhile. The complex actions of healthy fibroblasts include cell proliferation and migration, extracellular matrix (ECM) production, and growth factor and cytokine production, which lead to angiogenesis and protease release. A similarly complex “job description” for keratinocytes also exists. Cultured keratinocytes, applied to the nonhealing wound, deliver a “prefabricated,” mature, stratified, differentiated epidermal layer attached to an intact basal membrane layer and securely held to the dermis. Animal studies³¹ have demonstrated barrier function at the time of application, but this permeability barrier continues to improve even after application. By post-application Day 7, its barrier function is the same as that of normal skin.

Evidence of the healthy and vital keratinocyte layer in bilayered cell therapy is the expression of certain keratins, such as keratin-6, -16, and -19, by bilayered cell therapy. The first 2 keratins are normally expressed in proliferative skin, and

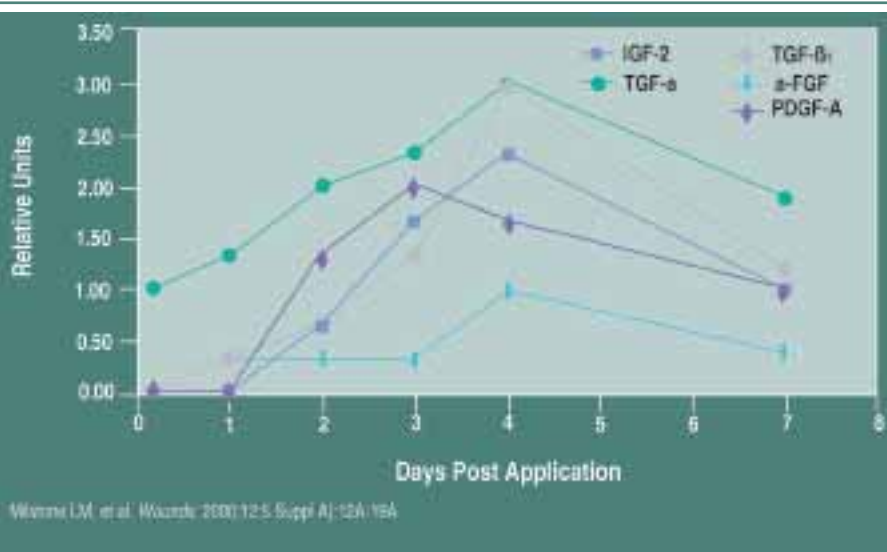
Figure 1: Cytokine production in bilayered cell therapy and human skin

	Human Keratinocytes	Human Dermal Fibroblasts	Apligraf	Human Skin
FGF-1	+	+	+	+
FGF-2	-	+	+	+
FGF-7	-	+	+	+
ECGF	-	+	+	+
IGF-1	-	-	+	+
*IGF-2	-	+	+	+
*PDGF- α	+	+	+	+
*PDGF- β	+	+	+	+
TGF- α	+	-	+	+
IL-1 α	+	-	+	+
IL-6	-	+	+	+
IL-8	-	-	+	+
IL-11	-	+	+	+
TGF- β 1	-	+	+	+
*TGF- β 3	-	+	+	+
VEGF	+	+	+	+

*Enzyme-linked immunosorbent assay. FGF = fibroblast growth factor; ECGF = endothelial cell growth factor; IGF = insulin-like growth factor; PDGF = platelet-derived growth factor; TGF = transforming growth factor; IL = interleukin; VEGF = vascular endothelial growth factor.

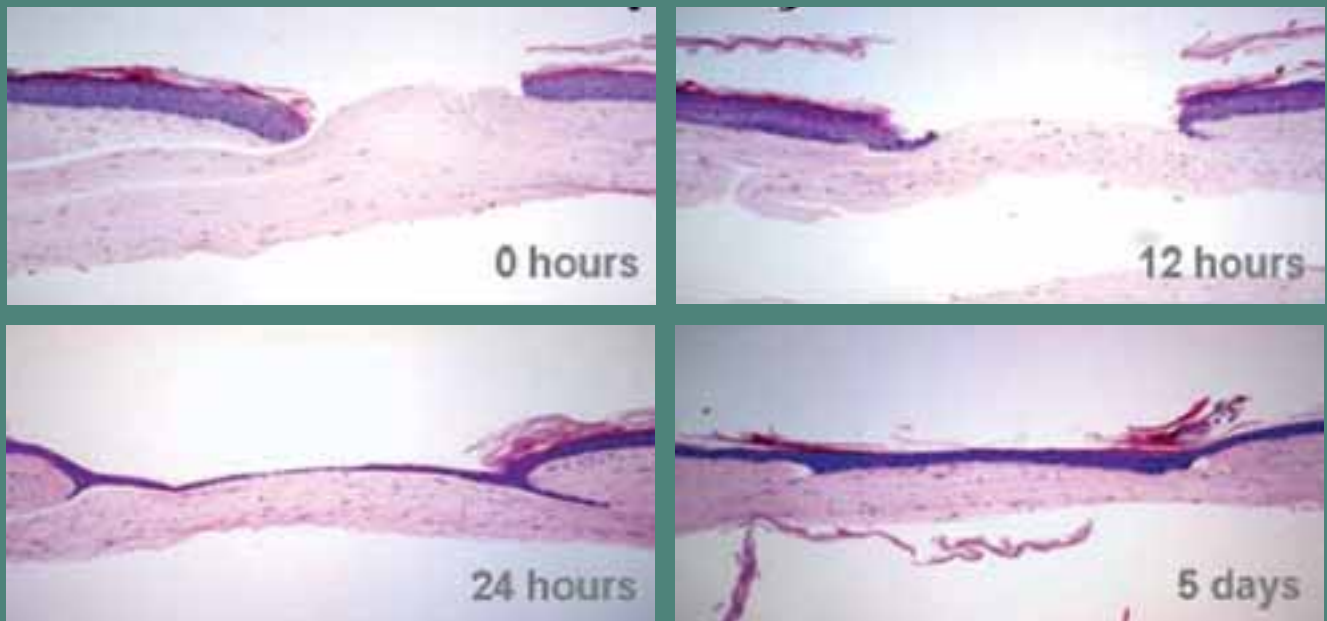
Brem H, Young J, Tomic-Canic M, et al. Clinical efficacy and mechanism of bilayered living human skin equivalent (HSE) in treatment of diabetic foot ulcers. *Surg Technol Int* 2003;11:23-31.

Figure 2: Expression of growth factor mRNA in healing bilayered cell therapy *in vivo*



Wattson LM, et al. *Wounds* 2000;12(5 Suppl A):12A-15A

Figure 3: Bilayered cell therapy wound-healing capacity



Reprinted with permission from Milstone LM et al. *WOUNDS*. 2000;12(5 Suppl A):12A-19A.

the latter keratin is normally expressed in neonatal tissue.³¹ Demonstrative of the proliferative activity is expression of a proliferation marker Ki67, a protein expressed by dividing keratinocytes and expressed in greater quantity in the keratinocytes on bilayered cell therapy than normal skin. Sustained delivery of these cells to the wound occurs as demonstrated by cellular persistence for 4 weeks and beyond in a variety of studies.³²

GROWTH FACTOR DELIVERY

Among the other features associated with a nonhealing wound is the relative deficiency or unavailability of growth factors. Bilayered cell therapy provides a number of growth factors to a wound (Figure 1). Interestingly, in comparison to either keratinocytes or fibroblasts grown in culture by themselves, bilayered cell therapy secretion profile more resembles that of normal skin, producing in some cases, cytokines that neither fibroblasts or keratinocytes in culture alone produce and thus, in essence, a synergy occurs. Exemplified, is insulin-like growth factor-I, (IGF-1), neither produced by fibroblasts or keratinocytes in culture, but when fibroblast and keratinocytes are combined,

either naturally in human skin or engineered in bilayered cell therapy, IGF-1 cytokine production occurs.

As would be expected, cytokine production is continuous after bilayered cell therapy application (Figure 2). Among growth factors produced are transforming growth factor (alpha and beta), fibroblast growth factor, and platelet growth factor. For the majority, peak cytokine expression is seen several days after application, demonstrative of the living nature of the construct. *In vivo*, tissue used for bilayered cell therapy stains for both vascular and epithelial growth factors, thus these factors are delivered to the wound bed. A concrete result of cytokine production is the fact that wounded bilayered tissue heals (Figure 3). Migration of keratinocytes is shown as early as 12 hours after wounding. By 5 days, restoration of the epidermis is seen.

BACTERIAL INFECTIONS

Bacteria may also be causal in nonhealing wounds, supported by ample evidence that controlling wound bacterial bioburden is important.³³ After wounding of normal skin, an innate defense mechanism exists through up-regulation of certain antimicro-

bial peptides, such as beta defensin, that protect against infection in acute wounds. In a similar fashion, bilayered cell therapy regularly produces many of the same peptides including beta defensin as the skin does, suggesting an antimicrobial function of bilayered cell therapy. However, different than normal skin, which produces antimicrobial peptides only in a wounded states, bilayered cell therapy constitutively expresses these peptides.

PROTEOLYTIC ENZYMES

The proteolytic environment of the nonhealing wound is created, in part, by the excess expression of pro-inflammatory cytokines, specifically interleukin-1 (IL-1) and tumor necrosis factor-alpha (TNF-alpha).^{34,35} This pro-inflammatory cytokine environment produces excessive amounts of matrix metalloproteases (MMP) and a reduction of their natural inhibitors, tissue inhibitors of metalloproteases (TIMP). As a result, an imbalance of proteases in a nonhealing wound exists. One of the myriad functions of bilayered cell therapy is to replace abnormal dermis with a normal dermis. As previously mentioned, the dermal layer of bilayered cell therapy contains human fibroblasts harvested from

healthy neonatal tissue. Over time, those cells produce their own ECM including procollagen I, which provides part of the structural integrity of the wound; fibronectin, an important component of granulation tissue; and tenascin, important for cell motility. Additionally and likely importantly, bilayered cell therapy also produces its own protease inhibitor-2 (TIMP-2). Bilayered cell therapy, in essence, delivers a protease inhibitor to the wound, potentially reversing the proteolytic excess.

After quenching excessive proteases, the production of cytokines (mentioned previously), such as TGF beta, stimulates new healthy ECM. The new normal neodermis imparted to the wound may help explain, in part, the effectiveness of bilayered cell therapy in treating disease states characterized by deficiencies in certain types of collagen, such as junctional or dystrophic epidermolysis bullosa.^{36, 37}

CONCLUSION

Differential features exist between healing and nonhealing wounds, and addressing features that differ between healing and nonhealing wounds might lead to healing for refractory wounds. A review of the characteristics of bilayered cell therapy addresses a number of these features consistently seen in a variety of nonhealing wounds. Delivery of neonatal cells that have a high proliferative healing capacity; differentiate to produce a complex dermis and dermal matrix with proteolytic activity; produce a complex epidermis with antimicrobial and functional barrier activity; and persist and can respond to a wound leads to a construct that works in concert to address features of a nonhealing wound and results in a healing wound. ■

References

- Phillips TJ, Machado F, Trout R, Porter J, Olin J, Falanga V. Prognostic indicators in venous ulcers. *J Am Acad Dermatol*. 2000;43(4):627-630.
- Margolis DJ, Allen-Taylor L, Hoffstad O, Berlin JA. Diabetic neuropathic foot ulcers: predicting which ones will not heal. *Am J Med*. 2003;115(8):627-631.
- Robson MC, Hill DP, Woodske ME, Steed DL. Wound healing trajectories as predictors of effectiveness of therapeutic agents. *Arch Surg*. 2000;135(7):773-777.
- Kantor J, Margolis DJ. A multicentre study of percentage change in venous leg ulcer area as a prognostic index of healing at 24 weeks. *Br J Dermatol*. 2000;142(5):960-964.
- Sheehan P, Jones P, Caselli A, Giurini JM, Veves A. Percent change in wound area of diabetic foot ulcers over a 4-week period is a robust predictor of complete healing in a 12-week prospective trial. *Diabetes Care*. 2003;26(6):1879-1882.
- Hess CT, Kirsner RS. Orchestrating wound healing: assessing and preparing the wound bed. *Adv Skin Wound Care*. 2003;16(5):246-259.
- Margolis DJ, Berlin JA, Strom BL. Which venous leg ulcers will heal with limb compression bandages? *Am J Med*. 2000;109(1):15-19.
- Margolis DJ, Kantor J, Berlin JA. Healing of diabetic neuropathic foot ulcers receiving standard treatment. A meta-analysis. *Diabetes Care*. 1999;22(5):692-695.
- Meyer FJ, Burnand KG, Lagattolla NR, Eastham D. Randomized clinical trial comparing the efficacy of two bandaging regimens in the treatment of venous leg ulcers. *Br J Surg*. 2002;89(1):40-44.
- Margolis DJ, Kantor J, Santanna J, Strom BL, Berlin JA. Effectiveness of platelet releasate for the treatment of diabetic neuropathic foot ulcers. *Diabetes Care*. 2001;24(3):483-488.
- Hochberg I, Hoffman A, Levy AP. Regulation of VEGF in diabetic patients with critical limb ischemia. *Ann Vasc Surg*. 2001;15(3):388-392.
- Mayrovitz HN, Sims N. Effects of support surface relief pressures on heel skin blood flow in persons with and without diabetes mellitus. *Adv Skin Wound Care*. 2004;17(4 Pt 1):197-201.
- Teixeira AS, Andrade SP. Glucose-induced inhibition of angiogenesis in the rat sponge granulo-ma is prevented by aminoguanidine. *Life Sci*. 1999;64(8):655-662.
- Doxey DL, Ng MC, Dill RE, Iacopino AM. Platelet-derived growth factor levels in wounds of diabetic rats. *Life Sci*. 1995;57(11):1111-1123.
- Duckworth WC, Fawcett J, Reddy S, Page JC. Insulin-degrading activity in wound fluid. *J Clin Endocrinol Metab*. 2004;89(2):847-851.
- Edwards R, Harding KG. Bacteria and wound healing. *Curr Opin Infect Dis*. 2004;17(2):91-96.
- Armstrong DG, Lavery LA, Wu S, Boulton AJ. Evaluation of removable and irremovable cast walkers in the healing of diabetic foot wounds: a randomized controlled trial. *Diabetes Care*. 2005;28(3):551-554.
- Stanley A, Osler T. Senescence and the healing rates of venous ulcers. *J Vasc Surg*. 2001;33(6):1206-1211.
- Phillips TJ, al-Amoudi HO, Leverkus M, Park HY. Effect of chronic wound fluid on fibroblasts. *J Wound Care*. 1998;7(10):527-532.
- Smith PD. Update on chronic-venous-insufficiency-induced inflammatory processes. *Angiology*. 2001;52(Suppl 1):S35-S42.
- Lauer G, Sollberg S, Cole M, et al. Expression and proteolysis of vascular endothelial growth factor is increased in chronic wounds. *J Invest Dermatol*. 2000;115(1):12-18.
- Taylor RJ, Taylor AD, Smyth JV. Using an artificial neural network to predict healing times and risk factors for venous leg ulcers. *J Wound Care*. 2002;11(3):101-105.
- Cullum N, Nelson EA, Fletcher AW, Sheldon TA. Compression for venous leg ulcers. *Cochrane Database Syst Rev*. 2001;(2):CD00265. Review.
- Edsberg LE, Natiella JR, Baier RE, Earle J. Microstructural characteristics of human skin subjected to static versus cyclic pressures. *J Rehabil Res Dev*. 2001;38(5):477-486.
- Schubert V, Heraud J. The effects of pressure and shear on skin microcirculation in elderly stroke patients lying in supine or semi-recumbent positions. *Age Ageing*. 1994;23(5):405-410.
- Diegelmann RF. Excessive neutrophils characterize chronic pressure ulcers. *Wound Repair Regen*. 2003;11(6):490-495.
- Van Marum RJ, Meijer JH, Ooms ME, Kostense PJ, van Eijk JT, Ribbe MW. Relationship between internal risk factors for development of decubitus ulcers and the blood flow response following pressure load. *Angiology*. 2001;52(6):409-416.
- Falanga V, Margolis D, Alvarez O, et al. Rapid healing of venous ulcers and lack of clinical rejection with an allogeneic cultured human skin equivalent. Human Skin Equivalent Investigators Group. *Arch Dermatol*. 1998;134:293-300.
- Veves A, Falanga V, Armstrong DG, Sabolinski ML. Apligraf Diabetic Foot Ulcer Study. *Diabetes Care*. 2001;24(2):290-295.
- Falanga V, Sabolinski M. A bilayered living skin construct (APLIGRAF) accelerates complete closure of hard-to-heal venous ulcers. *Wound Repair Regen*. 1999;7(4):201-207.
- Data on file. Organogenesis, Inc., Canton, Mass.
- Griffiths M, Ojeh N, Livingstone R, Price R, Navsaria H. Survival of Apligraf in acute human wounds. *Tissue Eng*. 2004;10(7-8):1180-1195.
- Browne AC, Vearncombe M, Sibbald RG. High bacterial load in asymptomatic diabetic patients with neurotrophic ulcers retards wound healing after application of Dermagraft. *Ostomy Wound Manage*. 2001;47(10):44-49.
- Baker EA, Leaper DJ. Proteinases, their inhibitors, and cytokine profiles in acute wound fluid. *Wound Repair Regen* 2000;8(5):392-398.
- Murphy G, Willenbrock F, Crabbe T, O'Shea M, Ward R, Atkinson S, O'Connell J, Docherty A. Regulation of matrix metalloproteinase activity. *Ann NY Acad Sci*. 1994;732:31-41.
- Schmid P. Immunohistologic characterization of Graftskin (Apligraf®). *WOUNDS*. 2000;12(5 Suppl A):4A-11A.
- Falabella AF, Valencia IC, Eaglstein WH, Schachner LA. Tissue engineered skin (Apligraf) in the healing of patients with epidermolysis bullosa wounds. *Arch Dermatol*. 2000;136(10):1225-1230.

Putting Bilayered Cell Therapy to Use

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Given a patient who has lost his leg to diabetes versus a patient whose limb was salvaged at a partial foot amputation level, which patient is more likely to sustain recurrent ulceration, recurrent amputation, and early death? The answer is obvious in clinical practice, and the preliminary scientific data concur that the more distal the amputation, the less likely the patient is to sustain further complications.

Limb-salvage procedures are often-

medical management. Additionally, for wound healing to be expected, there must be an adequate blood supply to the location (on the micro- and macro-vascular levels), and there must be an absence of infection at the wound base. In general, 80% of newly presenting diabetic neuropathic foot ulcerations will heal with 6 weeks of conservative care (eg, regular debridement, offloading of pressure, and moist dressings). In the early phases, watch the wound for signs

to the high-risk patient subgroup in whom there is a history of prior amputation or nonhealing wounds. These patients require aggressive management and generally require a much quicker progression from “good” wound care to “advanced” wound care technologies.

Wound bed preparation is important when considering the use of advanced wound technologies simply because most advanced wound products stimulate active healing and therefore require a viable, vascular, and clean wound base in which to interact with the cellular wound function. For example, if a viable wound bed or an optimized surface is not available for grafting, bilayered cell therapy (Apligraf, Organogenesis Inc., Canton, Mass) probably should not be considered until the wound has been optimized and prepared. This preparation can include minimizing exudate, managing periwound edema, and controlling biochemical factors in the wound base (such as matrix metalloprotease, which, in addition to harming growth factor viability, can prove disruptive to bilayered cell therapy).¹

Limb-salvage procedures are oftentimes also life-saving procedures.

times life-saving procedures when secondary morbidity (eg, infection, ischemia, and worsening of problematic chronic disease states) can be prevented. Complicated wound healing and foot infection cases that have not achieved the desired outcome are common. What are the reasons for this failure? What are some additional technologies that can be used to intervene? This article will address these questions.

DEALING WITH NONHEALING WOUNDS

In order to begin the healing cascade in a diabetic foot wound, a treatment plan for the patient’s overall disease state must first be established. The plan must include the control of diabetes through adequate diet and aggressive

of healing on a weekly basis. If there is no significant advancement trend over the first several weeks, the wound healing trajectory is failing and the wound is becoming stagnant. When this cellular senescence is observed in the wound, it is important to change the treatment plan appropriately. Oftentimes, this change will include the addition of advanced healing technologies for active wound management. It is important to not wait 4–6 weeks for the wound to “fail standard care” but rather to observe this trend early in the wound history so that the treatment course can be adjusted appropriately and with minimal lost time. The sidebar “Identify Problem Wounds Early and Transition to Advanced Therapy” particularly applies

BILAYERED CELL THERAPY

The classifications and product types in advanced wound therapy modalities are rapidly becoming confused by practitioners. Part of this confusion is resultant from the rapid evolution of new products in this arena. Bioengineered tissue can be broadly defined to include much of the new wound care technologies that are being advocated today. It is important, however, to make the key differentiation between living tissue products and nonliving/acellular tissue products. Both the living and

nonliving wound technologies can play a major role in problem wound healing, but they need to be properly understood to realize the proper timing and placement of these distinct technologies.

Some examples of nonliving advanced wound technologies include Oasis Wound Matrix (Healthpoint, Ltd., Fort Worth, Tex), Integra Bilayer Matrix Wound Dressing (Integra Lifesciences Corp., Plainsboro, NJ), and Graft Jacket (LifeCell Corporation, Branchburg, NJ). Currently, there are only 2 living tissue- engineered wound products available: Apligraf (Organogenesis Inc.) and Dermagraft (Smith & Nephew, Largo, Fla). It is important to keep in mind that although these are living human cell products, they are more than “skin graft substitutes.” This wording creates the wrong impression of what is to be expected from a living tissue product. Labeling these as “sheets of growth factors” helps define the proper patient selection and timing for their placement. (See “Bioengineered Products for Wounds” at right for more information.)

There are many reasons why practitioners will hesitate to utilize advanced therapy bioengineered tissues for problem wounds. Some feel that doing so will slow down their practice, is too cumbersome or requires special instrumentation, and must be done in an operating room, or takes too much time. In reality, there is much flexibility that can be found when considering the use of bioengineered tissues. The application of these technologies is generally something that can be done in the office setting and with minimal time and special instrumentation.

FOCUSING ON THE GRAFT

Bilayered cell therapy (Apligraf) arrives in a clear, plastic-sealed pouch. The color of the agar medium should be compared to the pH/color chart to ensure that the graft has remained viable during shipping. Once the site has been debrided, prepped, and rinsed, the pouch is opened, and the 7.5-cm diameter graft is placed onto either a saline-moistened sponge or a skin-meshing cartridge. If foregoing the skin graft mesher, a #15 blade can easily be utilized to fenestrate the graft randomly on a saline-moistened, 4x4 sponge approximately 60 to 70

Identify Problem Wounds Early and Transition to Advanced Therapy

“Good” Wound Care

- History
- Assessment
- Debridement
- Warm, moist environment
- Offloading
- Topical care

“Advanced” Wound Care

- Hyperbaric medicine
- Growth factors
- Bioengineered tissues
- Negative pressure wound therapy
- Biologic dressings
- Active topicals
- Plastic surgery
- Curative surgery

Bioengineered Products for Wounds

Growth Factor/Cytokine Topicals

- Becaplermin/platelet-derived growth factor (PDGF; Regranex Gel 0.01%, Johnson & Johnson Wound Management, a division of Ethicon, Inc., Somerville, NJ)

Biologic Wound Adjuncts

- Porcine intestinal submucosa (Oasis Wound Matrix, Healthpoint, Ltd., Fort Worth, Tex)
- Bovine collagen and chondroitin-6-sulfate (Integra Bilayer Matrix Wound Dressing, Integra Lifesciences Corp., Plainsboro, NJ)
- Gamma-irradiated human allograft skin (GammaGraft, Promethean LifeSciences, Inc., Pittsburgh, Pa)
- Human allograft product (Graft Jacket, LifeCell Corporation, Branchburg, NJ)

Living Tissue Products

- Living human dermal fibroblasts and epidermal keratinocytes in bovine collagen matrix (Apligraf, Organogenesis Inc., Canton, Mass)
- Living human fibroblast dermal substitute (Dermagraft, Smith & Nephew, Largo, Fla)

times. The meshing or fenestrating is important because the punctures will allow drainage through the graft and help prevent hematoma or seroma, and the process of injuring this “tissue” will stimulate keratinocyte and fibroblast activity as well as growth factor production.

The graft is then applied directly to the wound, at which point it is imperative to make sure that there is intimate adherence of the graft to the wound bed. Any portion of the graft that becomes “tented” over the wound bed will be devoid of blood supply and will likely fail. A simple compressive dressing, sutures, or staples can be used to anchor the graft in place.

There is a significant learning curve to

what the 1-, 2-, and 3-week post-op courses look like with bioengineered tissue grafting, and there is also a significant learning curve in the decision making post-graft. In general, do not debride the base of a bilayered cell-grafted wound site for 3–4 weeks post procedure. Rather than debride a wound that has a yellow, somewhat gelatinous matrix, leave it intact—especially if it has hydrated in the wound site and appears viable with no clinical signs of infection. At 4 weeks, a curette may be used to debride the wound base and then the determination should be made as to whether a repeat grafting procedure is indicated or if alternative therapy should

Figure 1: Efficacy of bilayered cell therapy in patients with venous leg ulcers > 1 year duration



Figure 2: Efficacy of bilayered cell therapy in diabetic foot ulcers



times more effective than the conservative therapy was in healing these wounds (Figure 1). A significant improvement in the bilayered cell therapy versus the control group is evident.² When bilayered cell therapy is given to the worst VLU's (those greater than 1 year in duration), the conservative measures for those are fairly abysmal in their success rates, whereas combining compression with bilayered cell therapy can be significant in its 8- and 24-week results for VLU's.

Diabetic wounds. In a large, multicenter, randomized control trial involving 208 patients, 67 patients had Type 1 diabetes and 139 had Type 2 diabetes.³ (Only 5% of all diabetic patients are Type 1, so this study had a disproportionately large share of challenging patients with Type 1 diabetes.) Mean ulcer size was about 3 cm. Looking at the 8- and 12-week data, a significant difference is observed in the healing of diabetic foot wounds: 56% healed in the bilayered cell therapy group versus 39% healed in the control group (Figure 2). Even more important is the rapidity toward closure. The decreased number of days needed to heal wounds in the bilayered cell therapy group resulted in decreased rates of infection, osteomyelitis, and amputation.

CASE STUDIES

Case 1. A man presented with an advanced diabetic foot infection that, in many institutions, would have been treated with a primary amputation (Figure 3). An aggressive, radical debridement was attempted with the goal of saving the leg. Eventually, with a stable wound site, the patient was prepped and transitioned into bilayered cell therapy. He progressed to closure.

Case 2. A 52-year-old man with a 15-year history of Type 2 diabetes had been hospitalized for gangrene of his left forefoot. Consequently, he had a guillotine mid-foot amputation (trans-metatarsal amputation), resulting in no dorsal and plantar flaps (Figure 4). This patient had a lower-extremity arterial bypass performed for revascularization of the extremity. Eventually, the granulation tissue covered the exposed metatarsal stumps. There were no signs

be considered. Also, if separation is noted between the graft and the bed post procedure, there was likely too much drainage coming from the wound site, and it may have been premature to apply the graft. An alternative in this situation would be to consider concomitant graft and negative pressure wound therapy

application to manage the exudate level post-procedure and to decrease the likelihood of graft failure.

WOUND-SPECIFIC BILAYERED THERAPY Venous leg ulcers (VLUs). Looking at the pivotal, 8-week trial data on VLU's, one can see that bilayered cell therapy is 3

Figure 3: Case 1



of soft tissue or bone infection to the open wound site. Two side-by-side pieces of bilayered cell therapy were used. At 1 week, 2 weeks, and 4 weeks, some significant contracture and partial wound closure was evident. Bilayered cell therapy was reapplied, and at approximately 12 weeks, complete closure of this wound site occurred. The key factor is that during those 12 weeks, full-thickness layer closure was being built to avoid a thin, friable layer of closure. Instead, there was a full thickness closure, and this patient can now walk while wearing a custom-molded shoe.

PREVENTING RECURRENCE

The etiology of a wound should be addressed at some point during or after the wound healing course. It is also important to attempt full-thickness tissue closure of wounds rather than simply closing them. While this progression of tissue building may take additional time, the end result will be a functional tissue base that will likely be sufficient to permit the ultimate goal of weight bearing.

Advanced technologies, including bioengineered tissues, can play a major role in wound healing and can serve to stimulate healing and the creation of a functional tissue base for eventual return to activity. It is this return to activity that extends the quality and often the length

Figure 4: Case 2



of life in many complex patients with multiple medical comorbidities. ■

References

1. Falanga V. Wound bed preparation: future approaches. *Ostomy Wound Manage.* 2003;49(5A Suppl):30-33.
2. Falanga V, Sabolinski M. A bilayered living

3. skin construct (Apligraf) accelerates complete closure of hard-to-heal venous ulcers. *Wound Repair Regen.* 1999;7(4):201-207.
3. Veves A, Falanga V, Armstrong DG, Sabolinski ML. Apligraf Diabetic Foot Ulcer Study. Graftskin, a human equivalent, is effective in the management of noninfected neuropathic diabetic foot ulcers; a prospective randomized multicenter clinical trial. *Diabetes Care.* 2001;24(2):290-295.

Post-Test Questions

Targeting the Science Within Wounds

1. Which of the following cell types does bilayered cell therapy contain?

- a) Langerhans cells
- b) Endothelial cells
- c) Keratinocytes
- d) Fibroblasts
- e) Keratinocytes and fibroblasts

2. Bilayered cell therapy cells persist for:

- a) Less than 1 week
- b) 1–2 weeks
- c) 3–6 weeks
- d) 8 weeks
- e) 12 weeks

3. Which of the following are potential mechanisms by which bilayered cell therapy heals nonhealing wounds?

- a) Antimicrobial peptides
- b) Protease inhibition
- c) Cell delivery
- d) Growth factor delivery
- e) All of the above

4. Bilayered cell therapy should be used:

- a) As a replacement for standard of care
- b) As an adjunct to standard of care

5. The epidermis of bilayered cell therapy is:

- a) Stratified
- b) Proliferative
- c) Bovine in nature
- d) A and B
- e) A, B, and C

6. What does the term “activated keratinocyte” mean?

- a) Proliferative, when they are actively dividing due to the injury
- b) Migratory, when they migrate close to wound
- c) Migratory and hyperproliferative,

when they produce, secret, and respond to extracellular matrix components and signal polypeptides

- d) Differentiated, when they leave the basal layer, stop dividing, and in turn, start differentiation

7. How do keratinocytes become activated?

- a) As a consequence of stress response, such as UV light, when they become apoptotic
- b) As a consequence of any type of injury by releasing pro-inflammatory cytokines and growth factors, they cross-talk to neighboring cells
- c) As a consequence of terminal differentiation, a process during which keratinocytes stop dividing, start cross-linking their proteins and dissolve their nuclei

8. What are the cells that participate in the cross-talk with keratinocytes?

- a) Dermal fibroblasts, because they are the only cell type in direct contact with keratinocytes
- b) Dermal fibroblasts (in direct contact) and local lymphocytes (migrate into the wound site)
- c) Dermal fibroblasts, lymphocytes, granulocytes, platelets, neurons, macrophages, and endothelial cells because, even though they are not all in direct contact, they communicate with each other by responding to specific signaling molecules, such as growth factors and cytokines
- d) Dermal fibroblasts and endothelial cells, because fibroblasts are in direct contact whereas local angiogenesis is essential for normal wound healing to occur

9. Where are the epidermal stem cells located?

- a) Their specific location is not known
- b) In the bulge area of the hair follicle
- c) Throughout the basal epidermal layer (the only epidermal layer that has the dividing cells)
- d) The major source is the bulge of the hair follicle, but they also migrate into the basal layer, where they are sporadically spread

10. What are the specific features of keratinocytes at the nonhealing edge of a chronic wound?

- a) They are senescent (quiescent), not responding to the extracellular stimuli
- b) They are normal, but the underlying granulation tissue is not appropriate for them to migrate
- c) They are hyperproliferative but not migratory, leading to a lack of epithelization
- d) They have features of incomplete activation (proliferating, but cannot migrate) and incomplete differentiation (thick cornification with nuclei present in the stratum corneum)

11. Following the application of bilayered cell therapy graft, at what point should you typically consider debridement and re-application?

- a) 2 weeks
- b) 5 days
- c) 4 weeks
- d) 10 weeks

Method of Participation: Participants must read the articles and take, submit, and pass the post-test by August 31, 2006. Participants must completely fill out the answer/evaluation form, answer at least 70% of the questions correctly, and mail or fax the answer/evaluation form to: NACCME • 83 General Warrant Blvd., Ste. 100 • Malvern, PA 19355 • Fax: (610) 560-0502

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