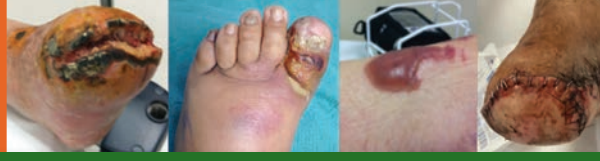

Living Cell Therapy for the Treatment of Diabetic Foot Ulcers

New and developing biologics hold the key to wound healing.

BY ADAM LANDSMAN, DPM, PHD

In the year 2000, Apligraf (Organogenesis; Canton, MA) was cleared by the FDA for use on diabetic foot ulcers. One year later, Dermagraft (Organogenesis; Canton, MA) was also approved, signaling the start of a new approach for the treatment of diabetic foot ulcers. Both of these products contain living cells, which have been prepared for application to the wound of a recipient. Apligraf contains both human keratinocytes and fibroblasts attached to a bovine collagen matrix, while Dermagraft contains human fibroblasts attached to an absorbable polyglactin absorbable mesh substrate. Aside from a few more uncommon biologic products, living cell therapy was previously only available through the use of a split thickness or full thickness autograft.

Aligraf and Dermagraft living cell therapy was something new and different. These cells were harvested and processed into a new form of biologic material that had some properties of skin and could be delivered on a large scale, direct to the clinic or office, either in a cell culture dish, or on dry ice. After preparation, the biologic material could be applied directly to the wound surface of the recipient.

Today, there are even more sources of living cell therapy to treat difficult wounds. Cell sprays, and even 3-D printed living cell materials have been proposed, but thus far, have had limited penetration in the market. In each case, these biologic products deliver some combination of living human cells, as well as other components found in skin, such as collagen and growth factors.

Each successive attempt to produce products more like skin has ultimately led to products like Theraskin (Soluble Systems, Newport News, VA), a cryopreserved split-thickness skin allograft. This is actual skin, taken from a donor, and cleansed and prepared for donation. Just like the recipient's own skin, this type of donated tissue contains living fibroblasts and keratinocytes and the full complement of collagen and growth factors normally found in skin.

It is the interaction of keratinocytes and fibroblasts that moves the wound away from the initial inflammatory response (such as after debridement), towards regenerative action.

The Role of Living Cells in the Wound Healing Cascade

Because they are most readily available, we usually think of keratinocytes and fibroblasts as the primary living cells that lead to the closure of a wound. Although they clearly play a major role, it is a more complex chain of events involving other cell lines as well. Nonetheless, the role of these two cells is particularly critical. In fact, it is the interaction of keratinocytes and fibroblasts that moves the wound away from the initial inflammatory response (such as after debridement), towards regenerative action.

The classic description of wound healing involves four phases of healing: 1) hemostasis, where essentially the bleeding is stopped, 2) inflammation, 3) proliferation, and 4) remodeling. Following hemostasis, the body moves to cleanse the wound by phagocytosing and removes any invading bacteria. Plate-Derived Growth Factor (PDGF) is released in the wound to launch the cascade of migration and cellular division that occurs in the proliferative phase.

In the proliferative phase, vascular endothelial cells form new blood vessels through a process called angiogenesis, while fibroblasts begin to form the provisional extracellular matrix (ECM) by producing collagen and fibronectin. The ECM serves as a scaffold for epithelial cells to migrate across the wound surface to provide cover for the new tissue

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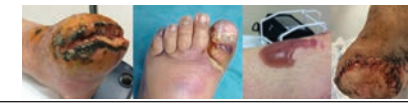

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(i.e., epithelialization).

Keratinocytes play a critical role in epithelialization. Initially, keratinocytes begin to migrate across the ECM without proliferating. The complex series of actions that determine when and where keratinocytes will migrate is highly dependent on the local environment of the wound. They move in a small envelope superficial to the granulation tissue and deep to the protective scab overlying the wound. Moist wounds are less likely to impede the migration of keratinocytes, when compared to hard, dry scabs. It is important to remember that keratinocytes can only move across living tissue.

As they migrate, keratinocytes break down parts of the clot and ECM, by excreting collagenase and proteases. The keratinocytes are followed by epithelial cells that move from the perimeter of the wound towards the middle. Because epithelialization can only occur from the perimeter of the wound, it is important to establish a granulation tissue at the perimeter (Figure 1).

Once keratinocytes cross the entire wound bed, they meet other keratinocytes and stop migrating as a result of contact inhibition. At this point, they begin to secrete proteins that form the new basement membrane.

In addition to the fibroblasts developing the ECM, myofibroblasts also begin to appear, and these act to contract the wound by gripping the edges and pulling them inward.

In the quest to achieve wound closure, the body forms tissue in a variety of directions, without regard for tension. However, once the wound has been covered, the wound enters the maturation and remodeling phase. Collagen becomes broken down and realigned, while cells that are no longer needed die through a process of cellular death and disassembly of their components that is known as apoptosis.

During the maturation and remodeling phase of healing, type III collagen, which is prevalent in the proliferative phase of healing, will gradually become replaced with type I collagen as the wound continually remodels by re-aligning collagen fibers along relaxed skin tension lines. This adds strength and flexibility to the skin as a result.

From this description, it is apparent that fibroblasts play a pivotal role in building the ECM, and keratinocytes are important for preparing the ECM for population with epithelial cells. We also see that type III collagen is critical in the maturation phase, when the skin is reforming into a strong, protective, but supple barrier.

Current living cell therapies such as Apligraf, Dermagraft, and Theraskin can provide abundant quantities of keratinocytes and/or fibroblasts. Some of the new cellular therapies in development involve concentrated mesenchymal stem cells. These cells are precursor cells that can differentiate into epidermal and dermal cells, and contain progenitor subpopulations like endothelial progenitor cells that are important for blood vessel formation. Collectively, all of these cell lines move us closer to satisfying the complete need for exogenous living cells.

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Application of "Non-self" Cells—The Role of Cellular Rejection

Before long, all clinicians who use living cell therapy will probably ask themselves, how the application of non-native cells can help

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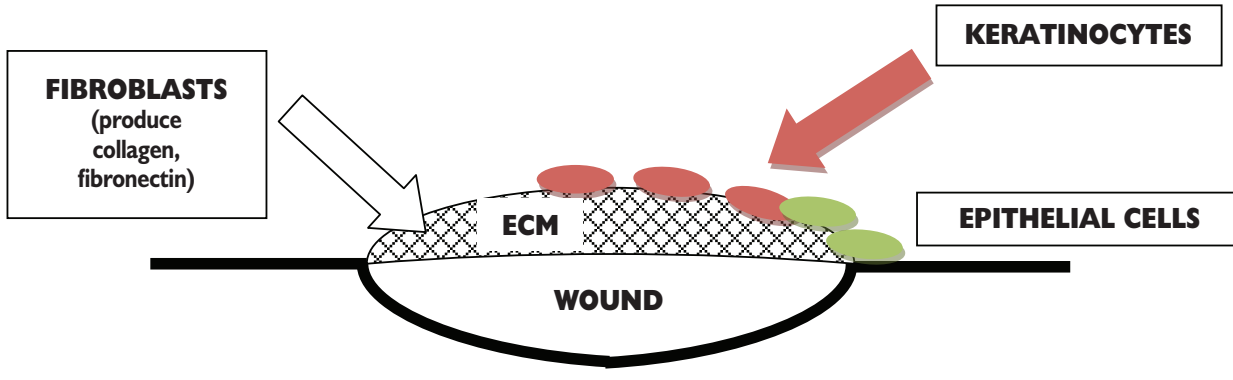
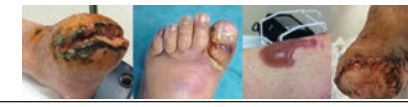
FIGURE 1

The Role of Fibroblasts and Keratinocytes in Wound Healing





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with wound healing. No doubt, many have wondered about whether or not those donated cells are rejected. The answer is that in fact they are—always. Non-self cells (i.e., cells with somebody else's genetic code) are recognized as foreign invaders and are ultimately attacked by the body. The question is where this leaves us in the wound-healing cascade if the living cells are ultimately attacked.

Research on transplantation of organs has demonstrated that the antigen presenting cells from the biologic graft side, such as macrophages, dendritic cells, and endothelial cells are the ones most likely to trigger rejection. These cells are largely absent in the biologic products described here. A second mechanism of rejection exists when the host presents antigenic cells to the graft. This process is much slower, and may allow the cellular components found in biologic grafts to survive as long as six months.[1]

Before rejection can occur, the cells used in living cell therapy are churning out proteins, growth factors, and collagen. The fibroblasts are helping to form the ECM, while the keratinocytes are setting the stage for migration of epithelial cells. The other critical attribute here is that most of the building blocks supplied by the living biologics are not antigenic at all. Collagen, growth factors, and other elements incorporated into the wound bed are indiscernible from the native materials.

Cellular Viability

Living cell therapy primarily involves viable cells which may be actively dividing and producing important materials such as proteins and growth factors, or may be undergoing apoptosis. It is important to understand that viable and apoptotic cells are present in Apligraf, Dermagraft and Theraskin.

Apligraf is a bi-laminate material in which fibroblasts and keratinocytes are seeded in a specialized cell culture dish with a bovine collagen substrate. The cells cover the collagen and remain in the dish until delivered to a clinic for application to a wound.

Dermagraft and Theraskin are both cryopreserved. Since these tissues are vastly different, the cryopreservation process is also very different, but historically 70-80% of the cells remain viable after the cryopreservation and warming process. Cryopreservation is a widely used technique for preservation of living cells. Fertility clinics have been able to store fertilized human embryos and implant them years later, with a very high degree of success.

In the case of Dermagraft, the fibroblasts are grown on an absorbable substrate in a specialized bioreactor bag, and subsequently frozen.

Conversely, Theraskin is a donated tissue, which is harvested in the early stages, post-mortem. This harvested split thickness skin is very much alive, just like the other major organs (heart, lungs, kidney, liver), which are also harvested shortly after the death of the donor.

A recent study demonstrated that natural living skin contains approximately 40,000 living cell per cubic millimeter, of which 97% are viable. In comparison, Theraskin contains about the same number of cells, after the harvesting, cleaning, cryopreservation and warming process, with over 25,000 living cells per cubic millimeter remaining viable, and the remainder still living but in various stages of apoptosis available at the time of implantation.[2]

When It Comes to Wound Healing, More Is Better

One of the greatest difficulties with getting a chronic wound to heal is to determine what is needed, and what is missing. There are certain fundamentals which are always important. For example, circulation must be adequate, mechanical forces must be dissipated (i.e., off-loading), maceration must be reduced, and bacterial count must be miniscule. Assuming these factors have been accounted for, the experienced clinician can gauge the condition of the wound by determining if there is adequate granulation tissue, indicative of good blood flow, but perhaps in need of collagen and more developed ECM.

Following the debridement of a wound, the chronic wound is "converted" to an acute wound, and the cascade begins. Once the angiogenesis begins, the fibroblasts found in all three biologics discussed here begin to produce collagen to start to form the ECM (Figure 2).[3]

Apligraf and Theraskin have the advantage of substantial amounts of

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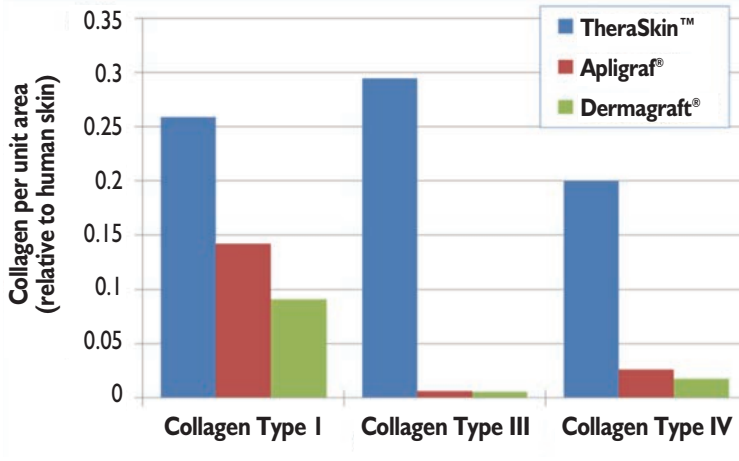
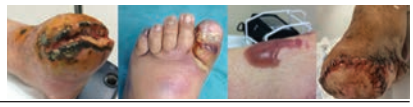
FIGURE 2

Living Cell Treatments Also Provide Significant Quantities of Collagen to the Wound[3]





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added collagen at the time of application. Theraskin also contains the fully developed ECM at the time of implantation, further aiding in the progression of the wound-healing cascade.[2]

Keratinocytes are also supplied with Apligraf and Theraskin, and these cells further aid in wound closure by enhancing migration across the ECM to encourage epithelialization. Because the wound bed is not completely uniform, the needs from one microscopic region to the next may vary. Consequently, the delivery of more viable cells and more collagen is necessary, in order to address the needs of each part of the wound.

Summary

Living cell therapy for the treatment of wounds represents a paradigm shift in the way that we treat diabetic foot ulcers. In the paper by Sheehan, et al.,[4] the author pointed out that wounds that do not show significant progression in healing during the first four weeks of treatment have less than a 50% chance of being fully closed by week 12. This study served as a reminder that there is a need to continually modify the way that we treat these complex wounds. Living cell therapy gives the clinician a convenient way to add many different ingredients to a wound in order to stimulate the healing process.

There are many options for biologic materials to treat wounds. In addition to Apligraf, Dermagraft, and Theraskin, there are a variety of collagen products, mesenchymal stems cells, cell sprays, exogenous growth factors, platelet-rich plasma, hyperbaric oxygen, and even mechanical stimulation with techniques such as shock wave. But among all of these therapies, living cell therapy is unique because it gives the clinician a microscopic tool to potentially produce precisely what the wound needs, in situ.

In this paper, three living cell treatments were discussed, and each acts in a unique way to produce excellent results. Theraskin is the most like natural skin, because it is living skin. Although it has been cleaned and cryopreserved, it provides the most complex array of living cells and collagen, and has been proven to be an excellent product for the treatment of chronic wounds.[5]

Apligraf provides two of the most critical cell types needed, along with bovine collagen, and has a long track record of success.[6] Dermagraft focuses primarily on fibroblasts, but provides an easy system for delivery of cells on a regular basis.[7]

Regardless of the system you choose, the added benefits of living cells are undeniable. These treatments have been shown to be safe, effective, and most importantly, can provide precisely what the wound bed needs most. **PM**

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