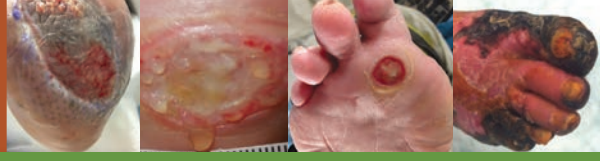


# When One Size Doesn't Fit All

Here's how to select an advanced biologic for treatment of chronic wounds.

BY ADAM LANDSMAN, DPM, PHD

As recently as 15 years ago, our choices for "advanced biologics" for stimulated wound care were limited to different types of collagen and therapy derived from donated fibroblasts and keratinocytes. Today, we have advanced to a whole new era of biologics where the list includes nearly every component of the wound bed and surrounding skin, as well as the addition of materials not normally associated with skin. Interestingly, when trying to compare treatment options, the value of the individual components for a given wound seems to be largely ignored in the shadow of studies that focus simply on healing rates. The truth of the matter is that nothing will ever work 100% of the time, because the needs of each wound are unique. In fact, one should be suspicious of any product that works more than 75-80% of the time, because it just seems so unlikely that one product could supply everything needed by every wound. In short, the magic bullet does not exist.

In order to gain a better understanding of the value of various advanced biologics, it is worth briefly reviewing the role that many of these materials play in the healing process (Figure 1).

**Fibroblasts**

The primary role is to form collagen, but they also produce growth factors.

**Keratinocytes**

Keratinocytes play a role in chemotaxis by producing growth factors. Keratinocytes also stimulate the migration of epithelial cells across the collagen scaffold.

**The truth of the matter is that nothing will ever work 100% of the time, because the needs of each wound are unique.**

**Epithelial Cells**

Normally, these are the primary structure of living skin. Although donated epithelial cells may help to produce growth factors, only autologous epithelial cells will survive, and ultimately close the wound. For

*Continued on page 86*



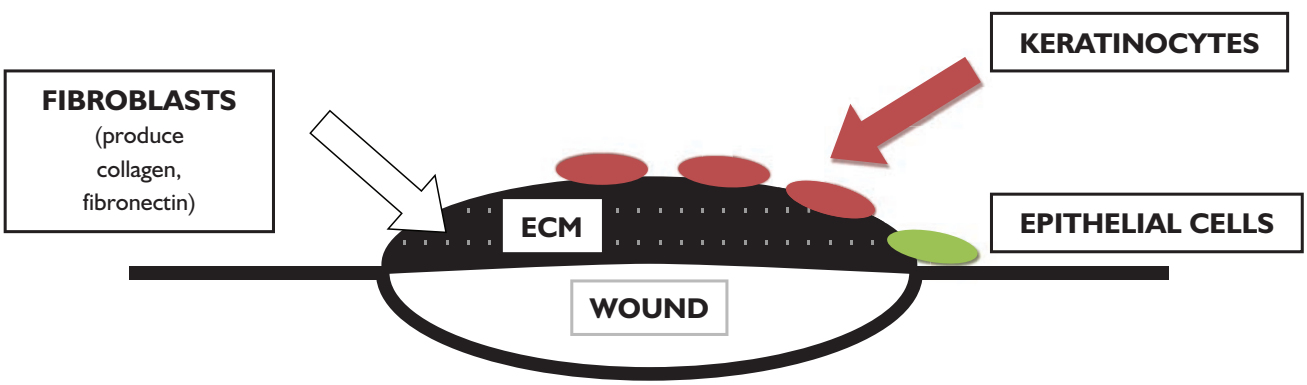
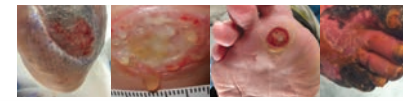



Figure 1: Fibroblasts produce collagen and fibronectin which help form the extracellular matrix (ECM). Keratinocytes will prime the newly formed ECM to facilitate migration of epithelial cells across the ECM surface, aided by hyaluronic acid.



*One Size* *(from page 85)*

this reason, the goal of most treatments is to stimulate epithelial proliferation and migration across the wound surface.

**Growth Factors**

Although PDGF and TGF-b are the ones most commonly linked to angiogenesis, mitogenesis, and chemotaxis, there are numerous other growth factors that play a role in wound healing, and we will revisit this.

**Hyaluronic Acid**

When one considers collagen, usually a "bird's nest" of interwoven fibers is visualized. However, hyaluronic acid fills the space between these fibers, and is equally critical in stimulating cellular migration across the wound surface.

**Collagen**

Collagen is the most fundamental component of the extracellular matrix. Along with hyaluronic acid, it forms the basis for the structure of skin. In its pure form, collagen is typically non-immunogenic and can be incorporated into the wound bed. However, there are numerous types of collagen, and the type must match the structure of the skin.

***What Does a Wound Need to Heal?***

When a wound fails to heal, the failure can usually be attributed to a host of problems. Most commonly, with the diabetic foot, the cause of a chronic wound is multifactorial (Table 1).

One of the most widely cited studies on wound healing[1] suggested that under normal circumstances, a wound should have a significant progression of healing during the first 4 to 8 weeks of treatment. If this progression does not occur, then some action is warranted to stimulate the process. The need to enhance wound healing has led to numerous new biologically active products. Because we cannot simply examine a wound to easily measure the presence of fibroblasts or the level of growth factors, we have to rely on our clinical experiences to determine what is actually needed. Once the decision has been made to use an advanced biologic product, the clinician must have an understanding of the relative contributions of a given product, to judge if this is the proper course of treatment.

TABLE 1:

**Factors Which Prevent Chronic Wounds from Healing**

- Poor vascular supply
- Elevated mechanical pressures, either from fat pad atrophy, biomechanical abnormalities, or foot deformity
- Poor glycemic control
- Diminished albumin levels
- Infections, including the presence of biofilms
- Neuropathy (sensory, motor, autonomic)
- Unrecognized foot trauma and the presence of foreign bodies
- Diminished growth factors, collagen, and/or hyaluronic acid
- Elevated Matrix Metalloprotease (MMP) levels

Many of the biologic products are composed entirely of collagen or are collagen-based. When choosing a collagen, there are many different types available and many sources. Once decellularized, collagens are essentially non-immunogenic, so exogenous collagen can be incorporated directly into the wound bed if conditions are good. This means that type 1 collagen, regardless of the source (human, bovine, porcine, etc.), can be used for the treatment of a human wound and be incorporated into the wound bed. However, the type of collagen makes a difference, as does the manner in which it was prepared.

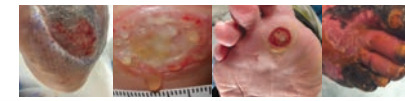
When collagen is prepared for use on a chronic wound, it may be subjected to a decellularization process, as well as a sterilization process. These two processes may alter the characteristics of the collagen by introducing chemicals, and possibly gamma irradiation as part of the cleansing process. As a result, the collagen may be slightly immunogenic, and also may be cross-linked. Although cross-linking adds strength, it progressively blocks neo-vascularization of the implant. The process of collagen integration is highly dependent on this proliferation of vascular tissue within the graft, and may ultimately lead to sloughing if the body perceives the graft as a foreign material.

The type of collagen is also critical. Type 1 collagen is the primary type found in skin, and is certainly important if your goal is to close the wound. Type 3, which is a precursor to other types of collagen, is also critical. Type 4 collagen is the dominant variety found in amniotic and placental tissues and is not readily incorporated into wounds. This is not to say that biologic wound treatments made from amniotic and placental tissue have no value, but rather to point out that these products work by a different mechanism rather than serving as a source of collagen.

Among the variety of collagen products available, some are foamed and, therefore, should be applied on a daily, or perhaps weekly, basis due to their relatively low density. Fractionated collagen has the added benefit of shorter polymer chains, with a proportionately higher number of attachable ends, facilitating attachment to the wound bed at these ends. Collagen is also available as a hybrid product, in which the fragments are bound to a silicone sheet to improve the handling characteristics while helping to retain moisture in the wound bed.

Collagen found in sheets, whether fenestrated or solid, is dense, and can be stitched on to the surface of the wound to be gradually incorpo-

*Continued on page 88*



*One Size* *(from page 86)*

rated, while thin, laminated collagen, such as that made from porcine intestine, is frequently cross-linked for strength, making it more of a biologic dressing under which a scab can form.

Regardless of the type of collagen selected, collagen is a strong chemo-attractant, and will act to trigger angiogenesis, mitogenesis, and chemotaxis in the wound bed. Additionally, some collagen products are rich with growth factors, further enhancing these processes.

Amniotic-derived collagen products are primarily Type 4 collagen and, therefore will add minimal collagen to the wound base. Structurally, they are sometimes difficult to handle due to their relative thinness. Although these products are rich in cytokines, the mix of cytokines is different from the ones normally associated with wound healing. For example, there are limited numbers of the most common ones—PDGF, for example. However, they also provide commonly found growth factors, and sometimes it is these less common cytokines that may help to stimulate the wound to heal. For example, tIMP is a potent anti-inflammatory and an excellent stimulant of angiogenesis. As a result, wounds tend to develop granulation tissue rapidly, while showing diminished reddening following treatment.

Hyaluronic acid is found in the extracellular matrix, between and around the strands of collagen, and plays a role as a chemo-attractant as well. But it is also a potent anti-inflammatory, and has the added advantage of blocking MMP (matrix metalloprotease) production. In the esterified form, hyaluronic acid can last up to two weeks in a wound, providing a longer-lasting solution for chronic wounds associated with elevated MMPs and a poorly vascularized wound bed.

### *What Do Living Cells Bring to the Wound?*

Although living cell therapy began with the advent of autologous skin grafts, such as the split thickness skin graft and the isolation of platelet-rich plasma preparations, the availability of allogeneic living cells and growth factors (i.e., recombinant PDGF) in the late 1990's really launched the use of advanced biologics for wound care.

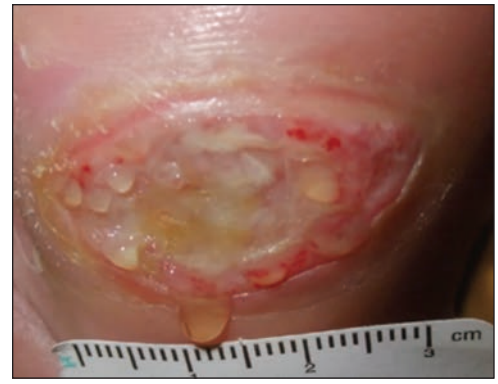


Figure 2: Wound with Slough

The primary difference regarding the treatment of wounds between autologous and allogeneic cells is that it is possible for autologous cells to be incorporated into the wound bed, and possibly even proliferate. Allogeneic cells will never become incorporated into the wound bed. It does not matter if you are considering amniotic tissue, umbilical cord tissue, stem cells, cultured fibroblasts, keratinocytes, or cryopreserved split thickness human skin allografts. Regardless of the source, allogeneic cells are eventually recognized as "non-self", and are destroyed by the host recipient.

**When it comes to biologics, one size does not fit all.**

Since allogeneic cells are never incorporated into the wound bed, what is the benefit of adding them? The truth is that the living cells do serve a significant purpose before their destruction. Before the body recognizes them as foreign, fibroblasts produce collagen and growth factors that are not immunogenic. Similarly, keratinocytes are also producing growth factors that are non-immunogenic and will help to stimulate angiogenesis, mitogenesis, and chemotaxis. Allogeneic stem cells such as those found in amniotic membrane products will act as potent chemo-attractants, thereby stimulating angiogenes, and will produce cytokines such as tIMP to reduce inflammation. But these cells do not ever differentiate, and become epithelial cells that the host can incorporate into the wound bed.

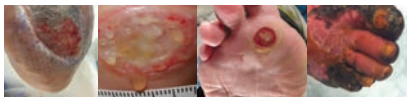
### *Planning Wound Closure with Biologic Products*

When it comes to biologics, one size does not fit all. In a broad sense, one can divide biologics into two categories: products that provide the materials normally found in skin and in the wound bed, and products that provide materials normally not found in the wound bed. Traditional products such as collagen (type 1 and 3), growth factors such as PDGF and living cell therapies, including fibroblasts and keratinocytes, would all fall into this first category. If you have a wound that is deficient in one area, say fibroblasts, then it makes the most sense to select a product that delivers the maximum amount of what you need. In this way, the supply needs can be satisfied.

Alternatively, if a wound has had collagen, growth factors, and living fibroblasts added and not progressed, then it is worth considering the addition of materials that are not normally very plentiful in the wound bed. This is where the new array of amniotic products comes in. The types of collagen and living cells which are abundant in these products are not going to be directly incorporated into the wound bed, but will potentially stimulate the wound in other ways. By acting as a chemo-attractant, stimulator of angiogenesis, and anti-inflammatory, these materials provide a new approach to stimulate wound closure.

Regardless of the biologic chosen, it must be applied in an optimal fashion. It is important to remember that in most cases, the wound bed is a hostile environment that is full of bacteria and inflammatory enzymes, with poor reg-

*Continued on page 89*



*One Size* *(from page 88)*

ulation of moisture and potentially diminished blood supply. Prior to application, it is necessary to prepare the wound bed in order to achieve optimal results. (Figure 2)

**Biofilms**

Biofilms are common to most chronic wounds, and are always a detrimental element. It is estimated that as many as 80% of chronic wounds have biofilms on their surface. Removal of a biofilm can be achieved by wound debridement, which may include direct curettage of the wound surface. However, wound surfaces are rarely smooth and may have tunneling or overhanging margins that prevent full mechanical debridement. There are a variety of topical agents which can be used to enhance the removal process. Products containing ionic silver, detergents, and sodium hypochlorite are proven to disrupt and loosen biofilms, while common agents such as saline, isopropyl alcohol, and providone iodine do little to destroy the biofilm.

**MMPs**

MMPs act as a naturally occurring debridement agent and are extremely beneficial in low quantities. Chronic wounds may exhibit excessive levels, resulting in a continuous destruction and inflammation over the surface of the wound. Although the transition from beneficial to excessive levels is poorly defined, there is a general perception that wounds with apparent slough and periwound erythema generally have elevated MMPs (and biofilms). MMPs can be effectively reduced with dilution, particularly if you are using a solution that can reduce biofilms. It can also be reduced with esterified hyaluronic acid agents (HYAFF) that block production, or with collagen products which are attacked by the MMPs to essentially burn them out, while stimulating granulation tissue formation[2]. This sort of sacrificial collagen strategy works especially well with foamed collagens that are reapplied daily, or every other day, to bring a fresh supply of collagen on a regular basis.

**Vascular Supply**

Probably no wound expert would be surprised that wounds with poor blood supply don't readily heal. It is important to utilize the tools available to enhance wound bed vascularity when necessary. Negative pressure wound therapy is widely accepted to stimulate the formation of granulation tissue, and may be considered prior to, or in conjunction with, other biologic treatments. Some biologic products also enhance granulation tissue formation through the stimulation of angiogenesis.

***Conclusions***

Our access to advanced biologics has brought many new options for clinicians. Historically, the goal was to determine what the wound was missing and try to replace those components. However, new biologics bring new opportunities to introduce a variety of cytokines, collagen, and other materials that are not normally associated with skin. In this article, some fundamental differences in what these biologics can do have been illustrated as well as demonstrating that each wound may benefit from many different approaches.

Wound preparation is also critical, and the use of an expensive biologic on a wound with excessive biofilms, MMPs, or poor vascularity will likely fail. The use of debridement, specialized solutions, and even "sacrificial collagen" may be beneficial as a pre-treatment before applying a living cell product.

In the future, advancement in wound diagnostic techniques, such as rapid MMP and bacteria test kits, as well as simple ways to assess wound bed vascularity (e.g., near infra-red spectrometry) will provide better guidance to clinicians who are trying to determine exactly what a wound needs to stimulate healing. **PM**

**References**

[1] Sheehan P, Jones P, Giurini JM, Caselli A, Veves A. ; Plast Reconstr Surg. 2006 Jun;117(7 Suppl):239S-244S.

[2] Motolese A, Vignati F, Brambilla R, Cerati M, Passi A. Interaction between a regenerative matrix and wound bed in nonhealing ulcers: results with 16 cases. Biomed Res Int. 2013; 2013:849321.

**Dr. Landsman** is Chief, Division of Podiatric Surgery, Cambridge Health Alliance, and Assistant Professor of Surgery, Harvard Medical School.