CHAPTER **32**

REGENERATIVE MATERIALS THAT FACILITATE WOUND HEALING

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The treatment of problematic and chronic wounds has evolved over the last three decades, progressing from the introduction of the first simple occlusive dressings (1, 2) to drugs and biological materials. The addition of regenerative materials as well as biological matrices has been a more recent addition to treatment modalities for wounds that do not respond to standard and more conventional approaches for wound care. The rapid development and growth of cellular and acellular tissue replacements, matrices, and engineered products designed specifically for use in problematic wounds occurred subsequent to the introduction of living cell equivalents over a decade ago (3, 4). Since then, numerous acellular materials have been introduced and studied for use in tissue repair (5).

Currently, the variety and selection of regenerative materials, more recently referred to as biomodulators is so large that it has become necessary to classify them as either drugs, cellular, or acellular products. This chapter will focus on the use of true matrix replacements including living cell and acellular wound materials. Applications of these types of products may extend beyond wound healing as is applicable to ulcers, to include tendon repair, hernia repair, tissue augmentation, and other internal uses. It is the intent of the authors to discuss only the use in wound healing as it applies to acute, chronic, and problematic wounds especially in relation to wounds of the foot and ankle.

It is important to recognize the primary role of regenerative products is not to act as autologous skin graft, rather they are designed to provide a means of dynamic interaction in the wound bed, thereby assisting and promoting tissue regeneration and wound closure. The term that best describes this process, biomodulation, was first used during an International Consensus on Acellular Matrices for the Treatment of Wounds (6).

THE EXTRACELLULAR MATRIX

The extracellular matrix (ECM) is a key component of the tissue repair process. Its composition includes fibronectin, elastin, collagen, proteoglycans, and hyaluronic acid. The ECM is best compared to the framework or skeletal structure of a building where all other components are added to the basic foundation and framework. Growth factors, fibroblasts, and other cellular components depend on the ECM for on-going activity and tissue regeneration. The absence of an adequate ECM may significantly impair the natural sequence of tissue repair. It is specifically the need for the presence of a matrix and regeneration in a wound bed, that has led to the development of regenerative products with a three-dimensional matrix (with or without a living cell component) that will provide the framework for the repair process.

The majority of currently available materials are either animal or human derived processed tissue, collagen, or hyaluronic based dressings, synthetic products, or chemical constructs. Living cells can also be used in conjunction with synthetic or collagen based constructs. Due to the source of materials, many of these products are classified as biological (animal, human or plant derived) by the Food and Drug Administration (FDA) (7).

Regardless of the manufacturing process, the tissue replacements and regenerative matrices are not anti-microbial agents. When placed in tissue defects that are not sterile or surgical uncontaminated wounds, bacterial growth becomes a barrier to effectiveness and outcomes. Aggressive debridement is needed to remove all nonviable and contaminated tissue. High levels of bacterial burden are known to impair the repair process, increase protease activity, and lead to further tissue breakdown (8, 9). The majority of regenerative materials are protein based; therefore they are sensitive to protease levels, which may contribute to rapid degradation. Bacteria may also proliferate in a matrix and contribute to wound infection and further elevation of matrix metalloproteases. Prior to application of any of the products discussed in this section, bacterial presence must be significantly reduced or eliminated. The optimal environment for application of a wound matrix or regenerative product is in the surgical setting, where excisional and full-thickness debridement may be performed without difficulty. Post application, protection from outside contamination, antibiotic use, and topical antimicrobials should always be considered to prevent new

colonization or proliferation. Finally, when choosing to use any newer technology, which inevitably comes with a significantly higher cost, determine if there are sufficient data to support the use of the product over more conventional modalities and whether less expensive conventional treatments may provide similar results and time to wound closure.

LIVING CELL PRODUCTS

Cellular-derived wound healing products contain living cells such as fibroblasts, keratinocytes, or stem cells that are commonly embedded within a collagen or polyglactin matrix. Living cell products are designed to simulate the functional and biological properties of human skin by providing a mechanical barrier to infection, encouraging extracellular matrix formation, and stimulating keratinocyte growth and differentiation. Living cell products are indicated for burn wounds, epidermolysis bullosa (EB), and most chronic wounds including diabetic, venous and pressure wounds.

Only a relatively few live cell products are approved for clinical use. It is important for the clinician to understand the indications for each product and carefully evaluate any potential barriers to healing such as infection, comorbidities, or patient noncompliance in order to provide the best possible clinical outcome. Wounds of different etiology, depth, age, and surface area must be treated differently and have dissimilar clinical prognoses. The use of adjunctive therapies such as compression, offloading, and antibiotics should always be considered where appropriate to maximize healing success. As living cell products are particularly sensitive to bacteria and proteases, aggressive debridement in the presence of adequate blood flow and the absence of any contraindication to sharp surgical debridement is important.

Normal wound healing requires a timely cellular response to injury by activation of keratinocytes, fibroblasts, endothelial cells, macrophages, and platelets with resultant intercellular signaling through the coordinated release of growth factors and cytokines (10). The vast majority of chronic wounds fall into one of three categories: pressure sores, diabetic ulcers, and venous ulcers. Prolonged chronic wounds have been shown to be deficient in growth factors (EGF, KGF, PDGF and IGF) and display decreased keratinocyte and fibroblast migration, increased reactive oxygen species, increased tissue proteases, and microbial contamination (11). Normal dermal fibroblasts synthesize and deposit critical extracellular components as well as secrete key growth factors important for intercellular signaling and repair. Fibroblasts from chronic wounds show pathologic changes in morphology, growth and gene expression and have decreased or nonexistent replicative and functional ability (12). Keratinocytes are similarly dysfunctional, losing the ability to migrate from the wound edges and re-epithelialize the wound surface (13). Eventually these key cells become senescent and lose the capacity to react to growth factors that would normally stimulate a healing response (14). Wound healing in the diabetic patient is particularly challenging, as diabetic ulcers are notoriously slow to heal and prone to more serious complications such as osteomyelitis and amputation (15-17). There are over 100 known physiologic factors that contribute to wound healing deficiencies in the diabetic patient including derangement of cellular systems responsible for growth factor function, angiogenic response, macrophage function, collagen formation, epidermal barrier function, and granulation tissue formation (18-20). Regardless of the specific etiology, it is paramount to restore the continuity and integrity of the damaged skin in a timely fashion in order to minimize further morbidity and prevent complications. Regenerative live cell products are designed to mimic the inherent cellular properties of the skin and provide temporary supplementation of critical functions lost by the chronic wound such as keratinocyte and fibroblast proliferation and differentiation, extracellular matrix synthesis and eventual re-epithelialization. The clinician needs to differentiate between patients with normal and active cell responses versus those with impaired cellular activity, to assist with the choice of a living cell versus an acellular product.

Apligraf

Apligraf (Organogenesis, Inc.) is a bilayered skin equivalent designed to replicate the normal skin's epidermis and dermis. The epidermal equivalent layer consists of a neonatal keratinocyte layer that is exposed to oxygen during the manufacturing process giving rise to a stratified monolayer similar to the stratum corneum. The dermal equivalent layer contains neonatal fibroblasts impregnated on an extracellular collagen matrix composed of both bovine and human type I collagen. It is void of antigenic cells such as Langerhans cells, melanocytes, lymphocytes, macrophages, hair follicles, blood vessels, or sweat glands. Although the mechanism of action is still not fully understood, it is believed that Apligraf creates a microenvironment that provides a physical and biological barrier against wound infection and also produces a variety of metalloproteinases (MMPs), cytokines, and growth factors responsible for keratinocyte migration and extracellular matrix formation (21, 22).

Apligraf is approved by the FDA for chronic venous ulcers of greater than 1 month duration and for diabetic

ulcers of more than 3 weeks duration. It is supplied as a circular disk that is 7.5 cmin diameter and 0.75 mm thick. It has a shelf life of 10 days and must be stored at 20-23°C until use. Apligraf may be applied every 4 to 6 weeks depending on the wound type, location, and clinician preference. As with most graft applications, wound bed preparation is critical and must involve proper debridement and control of edema and infection. Apligraf can be meshed or slit to facilitate drainage and is laid flat directly over the wound bed with the dermal side (glossy side) down. The graft should overlap the wound margin by 2-3 mm and care should be taken to smooth any wrinkles or air pockets. The graft is secured in place with staples or adhesive strips and protected with a soft primary dressing. Application of the product to the plantar diabetic foot requires special off-loading precautions to prevent disruption with ambulation. When applied to venous ulcers, compression is still required to address venous return. Compression needs to be applied carefully to prevent product disruption.

In 2000, Falanga et al published a prospective, randomized study of 214 patients with chronic venous leg ulcers treated with Apligraf with compression therapy versus compression therapy alone. They found that those patients treated with Apligraf were 3 times more likely to heal wounds older than 1 year ($P = 0.008$) and 2 times more likely to attain complete wound healing by 24 weeks ($P = 0.002$) (23). Veves et al studied 208 patients in a multicenter, randomized, controlled trial in 2001 that compared Apligraf with moist gauze dressings for the treatment of diabetic foot ulcers (DFU). At 12 weeks, 56% of patients treated with Apligraf had complete wound healing versus 38% in the control group ($P = 0.004$). The Apligraf-treated patients also had a faster median wound closure time of 65 days versus 90 days ($P = 0.003$) (24). Apligraf has also been used in the treatment of EB. Fivenson et al published a small study of 9 patients with 96 sites of skin loss, of which, 90% to 100% healing was observed by 5 to 7 days, with clinically normal-appearing skin present by days 10 to 14. Falabella et al also reported success in treating 69 acute EB wounds with no adverse events related to the application of Apligraf (25).

Dermagraft

Dermagraft (Advanced Biohealing, Inc.) is a cryopreserved single-layered dermal substitue containing human-derived neonatal fibroblasts cultured on a bioresorbable polyglactin 910 scaffold. It stimulates the ingrowth of fibrovascular and epithelial tissue by depositing extra-cellular matrix components such as collagens, vitronectin, glycosaminoglycans and also secretes a variety of cytokines and growth factors including vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), insulin-like growth factor (IGF-1) and granulocyte/macrophage colony-stimulating factor (GM-CSF). The fibroblasts continue to secrete growth factors and recruit host cells until fibrovascular ingrowth gradually replaces the donor cells and tissue. Dermagraft is void of antigenic cells and does not appear to stimulate rejection.

Dermagraft is primarily indicated and approved for the treatment of full-thickness diabetic foot ulcers of more than 6 weeks duration that are not overlying bone, tendon, muscle, or joint capsule. Dermagraft is cryopreserved and must be stored at -70 to -80°C until ready for use. It is supplied in a clear bag containing one piece approximately 5 cm x 7.5 cm. The graft must be thawed by submerging in 34-37°C water for approximately 2 minutes and can be held aside in saline for up to 30 minutes. The graft is laid flat on the wound bed (either side of the graft may be placed down) and trimmed to the approximate circumference of the wound margins. Care should be taken to smooth out any wrinkles or air pockets to maximize surface area contact. The graft is secured in place with staples or adhesive strips and a soft primary dressing should be applied directly over the graft. Control of edema or proper offloading is accomplished with an appropriate secondary dressing. The primary dressing should be left in place for a minimum of 72 hours. Dermagraft can be applied weekly for a total of 8 applications over a 12-week period. When applying Dermagraft to the plantar diabetic foot, off-loading and removal of all pressure from the wound site is imperative in preventing disruption of the material when ambulating.

Dermagraft was approved by the FDA in September 2001. The pivotal Dermagraft study was a multi-center randomized controlled study of 314 patients published by Marston et al that compared Dermagraft with conventional wound care methods over 12 weeks. Patients included in the final analysis had ulcers of greater than 6 weeks duration. The results showed that 28% of patients treated with Dermagraft achieved complete healing versus 14% in the control group ($P = 0.035$). Wounds were 1.6 to 1.7 times more likely to heal in the Dermagraft group and the median percentage of wound closure was 91% versus 78% in the control group ($P = 0.44$) The incidence of ulcer-related adverse events was also lower in the Dermagraft group (19%) when compared with the controls (32%; $P = 0.007$) (26). Other non-FDA approved uses included venous ulcers, fasciotomy wounds, buccal fat pad donor site healing, pediatric post-surgical abdominal wound healing, and vestibuloplasty (27).

Cultured Epidermal Autograft

A Cultured Epidermal Autograft (CEA) is a single-layer epidermal substitute comprised of the patient's own keratinocytes that are cultured ex vivo together with mouse fibroblasts to form a thin sheet of skin (28). The entire total body surface area of a human (1.8 m^2) can be produced in just 4 weeks, although the minimum required preparation time for normal-sized wounds is 16 days. CEAs must be used in conjunction with a dermal substitute, which makes it particularly fragile. It is currently approved for full-thickness burns of total body surface area greater than 30% and large congenital nevus excisions, although some success has been reported with treatment of leg ulcers. (29, 30). Although CEAs have shown some limited success with burn wounds (31), the majority of the literature concludes that it is generally unpredictable and inconsistent and should be used as an adjunct to conventional burn wound coverage with split-thickness autografts (32). Various improvements to cultured epidermal autografts are currently being studied including a hyaluronic acid membrane carrier and a "spray-on" application (33, 34).

The Future

Pilot studies are underway for the development of new live-cell regenerative wound care products. One such possibility is the use of multipotent adult stem cells, which have shown promise in accelerating wound repair and reconstituting the wound bed. Although considerable focus has been placed on bone marrow-derived mesenchymal stem cells, other types of stem cells are being studied including those derived from hair follicles and adipose tissue (46-51). Currently there are no FDA-approved products available and randomized clinical trials are still needed.

ACELLULAR MATRICES

Acellular matrices are approved for use in most chronic wounds including diabetic, venous and pressure ulcers, surgically dehisced wounds, acute and chronic wounds. They may be classified as allografts (human tissue), xenografts (animal derived), or chemical constructs that may contain animal derived collagen in additional to chemical components. There are a large number and variety of acellular products. The increased interest in and use of regenerative materials have resulted in an on-going stream of number products being introduced onto the market on a regular basis.

Reviewing the list of acellular materials provides an insight into the variability of materials, structure, and components featured in each type of matrix. It becomes important for the clinician to understand that not all products will function, integrate, or respond in the same manner. Ideally, one would like a material that is most similar to natural or native dermal matrices thereby allowing cells to integrate and divide as they would in a natural host. The function of the matrix is also determined by its physical placement and location, e.g., an acellular matrix is placed in a large defect to allow for cell migration into the material while further acting as a chemoattractant. The final anticipated result is rapid granulation leading to wound closure. The quality of regenerated host tissue with its resulting tensile strength, turgor, and degree of scarring will also be determined by the speed and manner in which wound repair occurred. In summary, while these materials may act to promote angiogenesis, provide a framework for cell migration and integration, act as chemoattractants, bind proteases, contain growth factors to promote healing, and have receptors to bind fibroblasts, they still may not necessarily function as expected. To promote appropriate product selection, the clinician must have a basic understanding of the differences between products and what to select for the type of wound they are treating. Following is a brief review of primary considerations.

Sterilization.

Regenerative matrices are either clean processed or fully sterile. This has also been described as aseptically cleaned versus terminally sterilized. Aseptic cleansing does not guarantee the removal of all viral contamination and does carry a risk of hepatitis or human immunodeficiency virus transmission. Cadaver derived tissue may be either aseptically cleaned or terminally sterilized depending on the manufacturer. The disadvantage of the more common terminal sterilization techniques is that they may damage and change the collagen structure of the product rendering it more susceptible to rapid degradation (35). High levels of matrix metalloproteinases in chronic wounds associated with inflammation, high levels of bacterial burden, and repetitive trauma, will more easily degrade a product with denatured collagen or one without heavy cross-linkage.

Structure

Matrices may be of human, animal, or synthetic origin. Careful review of the product literature will prevent selection of a product that may be problematic to wound repair. Human tissue is close to the natural matrix but may not be terminally sterilized or structurally strong. Cross linkage may be weak and rapid degradation is likely to occur in the chronic wound environment. Transmission of virus, as previously mentioned is a serious consideration. Animal products, particular certain porcine materials, may contain remaining host DNA fragments, which are known to induce a higher host inflammatory response as well as increased presence of giant cells (36-38). The increased inflammatory

reaction is of particular relevance if the material is being considered as an implant versus a topical biologically active cover.

The rate of degradation in a chronic wound, while influenced by the aforementioned factors, is further affected by the degree and type of cross linkage. Not all products are cross-linked in a similar fashion. The manufacturing process, sterilization, and the physical characteristics of the material used determines the resulting cross linkage of a product. Non-cross linked materials are likely to be rapidly absorbed while heavily cross-linked will remain more resistant to breakdown. Even cross-linked materials vary in whether the bonds are rigid or flexible. Cell migration is facilitated by the absence of cross-linking or flexible links versus rigid cross-linking. Chemical or synthetic products are sterile, non-cross linked and rapidly reabsorbed (39,40).

Ultimately, the choice of which material to use will be based on the wound etiology, wound characteristics and desired outcome. A clean full thickness wound not requiring extensive debridement that is to undergo a split thickness or other graft may benefit from a chemical construct or acellular graft that will allow rapid cell migration. The goal with a wound where autologous grafting is the endpoint, is rapid cell integration and resulting granulation thereby decreasing the time one must wait before a wound bed is graft ready. In such cases, expediting granulation and decreasing time to a graft-ready wound bed, is important in decreasing the time to surgery intended to fully close a wound, particularly in the high risk and immuno-compromised patients. Heavy or rigid cross-linked materials would not be desirable in this scenario. Granulation appears to be further expedited through the use of Negative Pressure Wound Therapy (41).

The selection of a more cross linked product would be appropriate in wounds where inflammation is high as is the case in patients with vasculitis and other autoimmune disease.

An additional and important consideration prior to application, is the source of the product. Acellular materials may be human or animal derived (bovine, porcine, equine). Allergies to animal products as well as cultural issues must be considered

STANDARD CARE VERSUS ADVANCED TECHNOLOGIES: CONSIDERATIONS FOR USE

Advanced technologies, including regenerative materials, are associated with a higher financial burden when compared to conventional dressings and approaches to wound care. Regenerative tissues and matrices may sound appealing but require scientific and clinical evidence to justify their use in the current financially burdened medical environment. Individuals with wounds that are progressing to closure, without factors impairing the normal repair process and with acute wounds may be expected to respond well to conventional dressings and treatments. The majority of patients are still best treated with less expensive and basic dressings and wound care.

Regenerative materials and wound matrices are excellent options in patients unable to effectively or rapidly generate a wound matrix. The matrix is needed as a scaffold to support cell ingrowths, cell differentiation, binding of cells to receptors, chemoattraction of cells and other growth factors, angiogenesis, and wound bed granulation. Regenerative materials may support some or all of these properties.

Underlying disease and wound etiology must be addressed if successful outcomes are to be expected. One must keep in mind that regenerative tissues are designed to address the wound defect and repair process, not the disease state. Diabetes control, adequate vascular supply, control of infection, bacterial burden, and medical attention to the patient's primary disease states must be addressed prior to application of any of the materials. Additional factors to consider prior to applying a regenerative material include patient eligibility, vascular status, ability to undergo aggressive debridement, healing potential, ability to maintain sterility at wound site, wound size, wound drainage, periwound edema, level of patient activity and ambulation and ability to control patient's disease states. A final consideration is being able to secure the product, whether with sutures, staples or adhesive strips. Products that are not in full contact with the wound bed, allow for disruption of the material, accumulation of exudate, and an environment promoting bacterial growth.

What are the advantages of regenerative materials? These include creating an environment conducive to wound closure, suppling a matrix in a defect where one is not present, expediting granulation, facilitating cell migration into a wound bed, increasing angiogenesis, chemoattractant properties, and reduced time to wound closure (6, 42-44). What are the disadvantages? These include high short term costs (long term cost of care may be reduced and considered an advantage), high potential for product contamination and infection, need for aggressive wound bed preparation, high failure rate with inappropriate handling, and need for a higher level of education and training of the clinician. Use of antimicrobial agents or secondary dressings should be considered to reduce risk of contamination progressing to a true clinical infection.

Finally, consider whether the chosen product is being

used as a true matrix or scaffold, which is to remain in the wound until full closure or whether it is to act as a biological cover. Know what to expect when applying the product. A deep defect may require a material that will allow rapid cell integration, rapid product degradation, and coverage with secondary dressings. A wound that is not closing due to reduced cell activity or presence of high levels of matrix metalloproteinases may benefit from a regenerative tissue being used as a biological modulating cover to alter the wound environment to one that is favorable rather than destructive. The chronic wound that does not progress to closure may be one that has the potential from a cellular level but not from an environmental one. Controlling lifestyle, activities and compliance is especially challenging in the chronic wound patient. Off-loading may be particularly difficult with the ambulatory diabetic patient.

PAIRING PRODUCT WITH A WOUND

Variability between wounds, even those of a similar etiology, is difficult as all wounds vary in depth, exudate, bacterial burden, location, and presentation. Only general recommendations may be made concerning the appropriate choice of product. The following general rules provide guidelines, however individual variation must be considered. Wounds associated with high levels of inflammation, including vasculitic and inflammatory ulcers will respond better to products that are highly cross linked as this reduces the rate of degradation.

A flexible cross linkage will allow cells to migrate into the matrix with less difficulty. Where rapid granulation is desired, as with wounds being prepared for grafting, a chemical construct without cross linkage is more appropriate as cells will migrate without difficult, the product will degrade rapidly, and a graft may be placed over the resulting healthy wound bed. In deep defects particularly where negative pressure is used, a product with less cross linkage is preferred. One should choose a product that can be easily meshed to allow for exudate to escape from the wound and not accumulate under the product. Materials that require weekly application due to associated rapid degradation, are more suited for the outpatient setting although a surgeon may choose to use these on more superficial exposed areas, which are not closed by primary intention in the operating room. Wounds that are secondary to trauma but present with a clean base, may be debrided and covered with a xenograft, which is left intact until complete healing occurs.

A few import recommendations include: debridement of all non-viable tissue, aggressive cleansing to remove bacterial burden, use of postoperative antibiotics for 10 days based on preoperative or intra-operative cultures (in the presence of bone infection the product should not be applied), coverage with a secondary antimicrobial dressing followed by bolstered gauze and wraps, pressure reduction or relief at the surgical site, and minimal disruption of the dressings for one week periods. Manufacturers cannot recommend the use of antibiotics however, the clinician may consider placing the allografts and certain xenografts in an antibiotic solution (based on suspected organisms) during the intra-operative procedure, for up to 20 minutes prior to application. Although there is no evidence to support this practice, any bacteria introduced on the wound surface or product is reasonably addressed by this treatment. Bacteria remaining deep in the tissue may still, however, contribute to infection. When graft materials become malodorous, overly moist or separated, they will need to be removed, as these are indicators of bacterial overgrowth.

The material presented has focused on the use of acellular matrices that assist with tissue generation for use in open wounds of all etiologies. These same materials may be used for tendon repair and other orthopedic use (45) as well as for tissue augmentation. The reader is referred to the medical literature for information on use beyond wound repair. Growth factors may also be considered regenerative products. Becaplerim is an FDA approved growth factor for the treatment of diabetic foot ulcers (46). Although growth factors are considered regenerative, they are classified as drugs and not devices or biological agents. Therefore a discussion on their use has not been included. Stem cells are also considered regenerative and are being explored for use in chronic wounds (47-51). Stem cells are considered a new area of clinical research and materials that may, in the future, be yet an additional form of treatment for problematic wounds.

CONCLUSION

Regenerative materials, which include but are not limited to growth factors, cell products, collagen based dressings, chemical constructs and acellular allografts, and xenografts are all designed to assist with the repair process when it is not occurring in an orderly and expected manner. Use of all the products discussed may be considered when the wound repair process is delayed, inhibited, or not progressing. Regenerative materials may be a primary choice when used in the surgical patient where postoperative complications may be expected if the wound is not covered with an interactive dressing. Careful patient selection, aggressive

wound bed preparation and close follow-up is recommended to ensure optimal results. Finally the clinician or surgeon selecting these products needs a good understanding of differences between products, indications for use and appropriate application techniques. When used as directed and when recommended, regenerative products may assist with expediting closure of recalcitrant wounds.

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