CHAPTER **48**

BIOENGINEERED SKIN SUBSTITUTES

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INTRODUCTION

Diabetic foot ulcers are a significant cause of nontraumatic amputations and morbidity. Close to 350 million people have diabetes worldwide (1). Based on the rule of 15, 15% of diabetic patients develop ulcers, 15% of ulcers develop osteomyelitis, and 15% of ulcers result in amputation (2). A combination of factors have been shown to cause foot ulcers such as lack of feeling, peripheral arterial disease (PAD), lower extremity deformities, trauma and even improper wound-healing modalities. Another major factorial concern is the economic cost for diabetic foot care. Based on the American Diabetic Association 2011 fact sheet, the total cost of diagnosed diabetic foot care yearly is $174 billion; which is broken down to $116 billion for direct medical cost and close to $60 billion indirect costs such as disability, work loss, and premature mortality (3). Diabetic ulcers may be reduced and even prevented with routine blood testing, cessation of tobacco use, physical activity, weight control and routine podiatric visits. However, once a foot ulcer has evolved, it is important to have the proper foot care with the appropriate wound agent, dressings, and even footwear modification.

A chronic wound, unlike an acute healing wound, does not follow the orderly healing phases. In fact the inflammation phase is prolonged and the proliferation phase is altered and discontinuous (4). The prime defects in a chronic ulcer consist of a surplus of inflammatory cytokines, which can lead to risk of infection due to protein degradation. Increase in protein degradation therefore leads to inhibition of angiogenesis and cell recruitment (2). Inhibition of essential growth factors create an environment for bacteria to colonize and overgrow, and since the proliferation phase is intermittent in a chronic wound, the aged fibroblasts do not have the same quality to activate healing (2). Yet when it comes to diabetic wounds, it is very important to determine the factor or factors that are impeding wound healing.

Hyperglycemia is a major factor affecting and prolonging the inflammatory phase. It affects the function of fibroblasts and delays neutrophil chemotaxis due to the oxidative stress caused by the alteration of glucose and FFA metabolism (4). Prolonging the inflammatory phase leads to fibrosis and diminished joint range of motion, which are commonly seen in diabetic patients (2). Decreased range of motion increases foot pressures resulting in a greater chance of unconscious injury.

The main goal for treating foot ulcers is to obtain adequate information on prevention of further complications that may lead to lower extremity amputation, decrease morbidity, and of course foot ulcer recurrence. When approaching a foot ulcer, it is very critical to perform an ulcer evaluation. The evaluation should reveal the etiology of the ulcer therefore performing the proper management.

When facilitating healthy granular tissue to a wound, debridement plays an important role in healing (5). Debridement of ulcers is categorized as enzymatic, mechanical, surgical, and even maggot debridement. Deciding on the appropriate technique is dependent on the question, "Which method of debridement will accomplish faster and less painful healing to the wound?" Maggot debridement may be an alternative for mechanical debridement. Maggots secrete proteolytic enzymes such as allantoin, sulfhydryl radicals, and growth stimulating factors that are effective in removing necrotic and infected tissue (5). The literature states maggot therapy is more effective in removing nonvitalized tissue in a more timely fashion compared to hydrogel application. It has also shown an 80% success rate compared to a less than 50% success rate with conservative debridement (5). Maggots may be used in acute and chronic ischemic leg wounds, osteomyelitis, and diabetic foot wounds. Overall, the cost for therapy is less than conservative treatment; contributing factors include fewer clinical visits, fewer dressing materials, and fewer nursing cost. Disadvantages with this type of therapy include the inability to treat Pseudomonal infections as well as a dehydrated wound bed (5).

Other modalities of treatment may also be implicated with debridement. A more novel approach, extracorporeal shock wave therapy (ESWT) has been effectively used for the management of diabetic ulcers. Many studies can support a significant difference of healing time and rate, and index of re-epithelialization (6). They also report no adverse

effects, a well-tolerated procedure, antimicrobial effects, and no anesthesia involvement, which is very cost effective (7). ESWT has also been compared to hyperbaric therapy. Clinical results showed completely healed ulcers in 57% and 25%, respectively; ≥ 50% improved ulcers in 32% and 15%; and no ulcers worsened (8). Some insurance providers will not cover this type of modality. However, it is far more cost-effective when compared to surgical intervention.

Ulcers greater than four centimeters increased the likelihood for limb amputation especially when the ulcer is located at the heel (9). A heel ulcer is more likely to develop into osteomyelitis due to the contributing factors of extended period of weight bearing, PAD, and heel fat pad atrophy. A limb salvage algorithm has been supported by multiple studies in the literature revealing as high as a 76% limb salvage rate after 2 years (9), aggressive heel resection on revascularized patients, negative pressure wound therapy (NPWT) and split-thickness graft and or platelet derived growth factor (PDGF) agents. Even though this type of algorithm seems costly, statistics show that by using this algorithm, cost was estimated to be $28,000 compared to the cost of a lower extremity amputation, which including postoperative care is $66,000 (9).

CURRENT AND NEW TRENDS

One of the most commonly utilized modalities of treatment, skin substitutes, have many different names: bioengineered skin equivalents, tissue-engineered skin (constructs), biological skin substitutes, bioengineered skin substitutes, skin substitute bioconstructs, living skin replacements, and bioengineered alternative tissue (12). Despite the variable nomenclature, they all serve to perform the same purpose. From a clinical standpoint, all bioengineered skin equivalents need to comply with three major requirements: they must be safe for the patient, be clinically effective, and be convenient in handling and application (12). The literature has defined the "ideal skin substitute" as having many different qualities (13). Ideally they must not be toxic, immunogenic, or cause excessive inflammation. They should also have low or no level of transmissible disease risk. The ideal graft is biodegradable, repairable, and able to support reconstruction of normal tissue. It should provide pain relief, prevent fluid and heat loss, and protect the wound from infection. A few other "ideal" qualities to look for in a skin substitute are cost-effectiveness, whether or not it is readily available, user-friendly, and whether or not it has a long shelf life (13).

The different classifications of currently available skin-substitute products can be broken down by a number of different traits. Anatomical structure may be defined as whether the skin substitute is comprised of primarily epidermal, dermal, or dermo-epidermal tissues (otherwise known as a composite). Also, they may be further broken down by the duration of wound coverage, including permanent, semi-permanent, or temporary. Of further significance is whether or not the biomaterial is mainly biological (autologous, allogeneic, or xenogeneic) or synthetic (biodegradable or nonbiodegradable). Finally, it is important to note whether or not the skin substitute is comprised of cellular components, or if it is acellular (12).

The class of bioengineered skin equivalents that are a composite of dermis and epidermis are known as the dermoepidermal skin substitutes. These substitutes aim to mimic the histological structure of normal skin, and by doing so aim to provide some functional similarities to human skin (12). They are the most advanced products histologically, which in turn makes them the most expensive (14). The majority of them are based on allogeneic skin cells incorporated into a dermal scaffold (12). They act primarily as temporary biologically active wound dressings, not only by providing mechanical coverage of the wound, but also by inducing a number of growth factors, and cytokines, etc. These are temporary grafts, and must be removed after a period of time indicated for the specific graft used due to keratinocyte rejection versus fibroblast tolerance, which basically illustrates the difference of HLA antigen on fibroblasts and keratinocytes. This leads to the rejection of the graft in time, and is the reason that these grafts cannot be used for primary and permanent skin closure (12).

One of the most well-known and commonly used skin equivalents is Apligraf. Composed of cultured keratinocytes and fibroblasts, it is composed of an allogeneic cell source (neonatal keratinocytes and neonatal fibroblasts), and a xenogeneic scaffold source (bovine collagen). It is considered a temporary bioactive dressing because the allogeneic cells of the construct do not survive after one to two months in vivo (15). The Apligraf graft delivers ECM components to the wound as well as cytokines and growth factors (IF alpha and beta, PDGF, IL-1, IL-6, IL-8) (16). Apligraf may be used for the treatment of diabetic ulcers that have not properly responded to at least 4 weeks of conventional wound care. It may also be used for venous ulcers with wounds extending through the dermis. The caveat is that there may not be any exposed tendon, bone, muscle, or joint capsule. Although it does has a short shelf life (only 5 days) and requires delicate handling, it is reported to be the most clinically successful product in its category, showing a 25% improvement in ulcer treatment when compared to conventional treatments (17). Usually, Apligraf applications will resorb in 7-14 days. It is relatively expensive, with a cost of $28 per cm2.

Tissue Tech autograft is a complex composite construct that manages to combine two separate tissue-engineered products that are applied consecutively to the wound bed (12). The two separate constructs are supplied by Hyalograft 3D (dermal replacement layer), and Laserskin (epidermal replacement layer). This combined graft is based on autologous keratinocytes and fibroblasts that are grown on hyaluronic acid membranes (12). The cultured graft portions are then placed in consecutive applications of Hyalograft on day 16 and Laserskin on day 23. This is the one of the few permanently applicable composite bioengineered skin equivalents on the market.

Epidermal substitutes are another subcategory of bioengineered skin equivalents. One of the most significant steps in production of epidermal substitutes is the specific isolation of keratinocytes from a donor, and then the in vitro culturing of those keratinocytes. A skin biopsy of 2-5 cm^2 is required to begin a culture growth of the autologous cells, after which a lengthy process is used to perform the process of keratinocyte isolation (12). Cultured epithelial autografts (CEA), the end-product of the process of keratinocyte isolation and culturing, are qualified by their clonal cellular composition: holoclones, meroclones, or paraclones (12). Holoclones are formed by basal keratinocytes, have the highest proliferative potential, and are ultimately essential for the long-term survival of the graft (12). On the opposite end of the spectrum, paraclones have the lowest proliferative potential, and are only able to replicate a few times before senescence. Therefore, they are not ideal for use in wound closure. The production of keratinocyte processing and expansion only takes approximately three to four weeks to produce a CEA large enough to cover the entire body surface, which amazingly all begins with a 3 cm^2 skin biopsy (18).

Epidermal substitutes, and specifically confluent CEAs, are not without their disadvantages. They have highly variable "take" rates (15-85%), long culture time, graft friability, and difficult handling processes (19). Due to various mechanical and processing differences, confluent CEAs are less than ideal when compared to subconfluent keratinocytes, which are inhibited from growing to full confluence in vivo. Along with the combination of various unique delivery systems, subconfluent keratinocytes have helped counteract some of these disadvantages (13).

MySkin is a product that uses subconfluent keratinocytes harvested from autologous tissue (12).These keratinocytes are grown on a silicone support layer, which allows overall easier handling and application and decreased culture time. Specific indications for MySkin include treatment of neuropathic, pressure, and diabetic foot ulcers (12). Bioseed-S is another subconfluent autologous keratinocyte product using a fibrin sealant, and is mainly indicated in chronic venous leg ulcers (12).

CellSpray is a unique product that aims to harvest subconfluent keratinocytes in their most active state and then applying those to the wound bed by spraying. This process in turn results in decreased culture time and earlier wound coverage. However, this type of application is limited to burns, partial-thickness, and graft donor site wounds (12).

The previously discussed epidermal substitutes are great for grafting purposes in that they provide permanent wound closure. However, their "take" rates with isolated grafting are inconsistent, and it is generally accepted that combination grafting with a dermal substitute is necessary for proper healing of full-thickness wounds. Dermal substitutes are generally acellular and are made of either allogeneic, xenogeneic, or synthetic materials. From a clinical standpoint, these grafts are much easier to obtain a license for clinical application and have easier handling and "take" rates than isolated epidermal grafts (12).

One of the most commonly encountered dermal substitute products is GraftJacket. It is 0.4-0.8 mm thick, and comprised of freeze-dried acellular dermal matrix with a preserved basement membrane harvested from cadaveric human dermal tissue. It has been successful in the use of tendon repair as well as various other lower extremity wounds (20).

OASIS is another dermal substitute, but is unique in that it is xenogenic. It is produced from porcine small intestine submucosa and used for closure of acute, chronic, and burn wounds (12). One positive quality of OASIS is that it is decellularized to prevent immunological responses (12). Evaluation of OASIS in vivo on rodent full-thickness wounds showed that it contributed to minimization of contraction (21).

Integra consists of bovine type I collagen and shark chondroitin-6-sulfate glycosaminoglycan, all of which is bonded to a silicone sheet (22). The first 15-20 days post-application of the graft includes a process of neodermis formation and vascularization of the wound, and afterwards the silicone layer of the graft is peeled off and can be closed permanently with a split-thickness skin graft (12). It can be used for chronic ulcer treatment as well as full-thickness nonthermal skin wound management (23). It has many advantages as a whole, including long shelf life, simple handling, and low immunogenic response (12). However, it cannot be used on infected wounds, and the vascularization phase of 10-14 days is relatively long. Also, the prospect of performing a split-thickness skin graft

afterwards is an added burden in the healing process.

Dermagraft is a cryopreserved material composed of polyglactin mesh seeded with living cultured neonatal fibroblasts from foreskin (12). The graft self-hydrolyzes within 20-30 days, all the while producing growth factors and ECM components (12). It is indicated and licensed for chronic diabetic foot ulcers. The disadvantages are that it includes a necessity for multiple applications as well the fact that it is a higher cost skin-substitute.

One of the newer skin-substitute products, Primatrix, is a bioactive and regenerative extraceullular matrix comprised of fetal bovine dermis composed of type I and III collagen (24). A wide variety of indications as well as a long shelf life (3 years), room temperature storage, and a quick hydration period (60 seconds) make this a very favorable skin-substitute product. Also, it is available in a large variety of sizes, from 4x4 cm to as large as 20x25 cm (24). Karr et al showed that Primatrix, when compared to Apligraf, was superior in that it healed wounds faster than Apligraf, despite being used on wounds averaging larger sizes (24).

CONCLUSION

The expanding field of bioengineered skin substitutes continues to grow and is ongoing. Many of the products that were briefly discussed are showing an increase in use and the advancements that are being made offer new treatment options for chronic and difficult wounds. Cost and patient selection remain the limiting factors and should be considered when choosing a skin substitute. While an ideal graft is yet to be seen, many of the future perspectives are currently being researched.

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