REVIEW ARTICLE

An overview of factors maximizing successful split-thickness skin grafting in diabetic wounds

Ryan J. Donegan, DPM1*, Brian M. Schmidt, DPM1 and Peter A. Blume, DPM, FACFAS2

1Section of Podiatric Surgery, Department of Orthopedics and Rehabilitation, Yale New Haven Hospital, New Haven, CT, USA; 2Orthopedics and Rehabilitation and Anesthesia, Yale School of Medicine, New Haven, CT, USA

Open wounds, from ulcerations or slow healing, are one of the comorbidities in diabetic patients that can lead to amputation. Therefore, an optimal way to close and heal wounds quickly in diabetic patients is required. Split-thickness skin grafts (STSG) offer a quick method of wound closure for diabetic patients. This article review will look at causes of failure in STSG, and ways to optimize success.

Keywords: biofilm; chronic; bioengineered alternative tissue; debridement; closure

*Correspondence to: Ryan J. Donegan, 508 Blake St, New Haven, CT 06515, USA, Email: ryan.j.donegan@gmail.com

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Diabetes mellitus has a global impact. The International Diabetes Federation has estimated that globally 382 million people were affected by diabetes mellitus in 2013, projected to increase to 592 million people by the year 2035 (1). As a result, there will be an increase in the total number of cases of foot ulcers, occurring in up to 4% of patients with diabetes mellitus (2). Ulceration contributes the greatest cause of non-traumatic minor and major amputations of the lower limb, with diabetic foot ulcer patients having 25 times higher risk than the rest of the population (3). The development of diabetic foot ulcerations is multifactorial, generally due to peripheral vascular disease, peripheral neuropathy, and immunopathy, affecting approximately 15% of the diabetic population at some point during their life (4, 5). This triad of vasculopathy, neuropathy, and immunopathy not only leads to pedal ulcerations but also increases the susceptibility to soft tissue and osseous infections which can ultimately lead to amputation, loss of limb, and life. Therefore, the restoration of an intact skin barrier is of utmost importance to prevent a portal of entry for infection. Ideally, this is accomplished in a manner that minimizes wound contraction to maintain function, and minimize cosmetic disfigurement. Split-thickness skin grafts (STSG) currently represent the most rapid, effective method of reconstructing large skin defects (6, 7), granulating tissue beds, tissue loss across joints in areas where contraction will cause deformity, and where epithelialization alone will produce an unstable wound cover (8, 9).

For both chronic and acute wounds, STSG offer a rapid and effective way to provide closure and healing. Ideal conditions for successful STSG include red granulation tissue dominating the wound bed, no visible tendon or bone, no discernible sloughing or exudate in wound, no residual necrotic tissue, no local signs of soft-tissue infection, no systemic signs of infection, and no severe peripheral arterial disease (ankle-brachial index > 0.9 or distal pulses present) (6).

Ramanujam et al. (10) showed that diabetic patients without comorbidities had no significant difference in healing times compared to non-diabetic patients for STSG; however, compared to diabetic patients with comorbidities, there was significant difference. Overall, healing time is 1.99 weeks longer for diabetic patients than for non-diabetic patients. Compared to patients without diabetes mellitus, diabetic patients experience a 5.15 times higher risk of postoperative complications after STSG. These complications include wound dehiscence, infection, and the need for revisional surgery. Diabetic patients with comorbidities are at a significantly higher risk for delayed healing from STSG compared to diabetic patients without comorbidities and non-diabetic patients. For diabetic patients, the presence of any preexisting comorbidity, history of amputation, or trauma is negatively associated with the successful outcome of STSG. Furthermore, duration of diabetes, hemoglobin A1c level, chronic kidney disease, blood urea nitrogen level, and creatinine concentration represent modifiable characteristics that
need to be addressed when selecting patients for STSG of diabetic foot wounds. Therefore, it is suggested that a comprehensive medical and surgical approach is imperative to maximize STSG success rate in diabetic patients (11–13).

Wound bed preparation
Successful incorporation of STSG requires vascularized granulation tissue. Given the high prevalence of peripheral vascular disease in the diabetic population, it is important to identify the need for co-management of vascular surgeons.

Peripheral neuropathy plays a role in the etiology of over 80% of diabetic foot lesions (14, 15), but inadequate perfusion always results in non-healing wounds (16, 17). Lower extremity ischemia secondary to peripheral vascular disease reduces the pedal supply of oxygen, nutrients, and soluble mediators that are involved in the repair process (18). This lack of arterial blood flow decreases tissue resilience, leads to rapid death of tissue, and impedes wound healing. Clinical indications of vascular disease include diminished or absent pulses, pallor on elevation, rubor on dependency, sluggish refilling of the toe capillaries, and thickened nails or absence of toe hair. It is important to realize that palpable pedal pulses do not guarantee that there is no possibility of limb-threatening ischemia. Upon wound bed debridement and preparation, there should be prompt signs of healing, including the development of wound granulation within several days; otherwise a low threshold for non-invasive vascular studies and arteriography should be undertaken for these patients.

Options for restoring adequate perfusion in the presence of a simple occlusion include surgical bypass to the dorsalis pedis artery, posterior tibialis artery, and even the more distal tarsal arteries. Endovascular techniques, such as percutaneous transluminal angioplasty (PTA) and cryoplasty, play an important role in patients considered poor or non-candidates for surgical revascularization secondary to comorbidities such as coronary artery disease, uncontrolled hypertension, diabetes mellitus, or inadequate target vessel. For many patients, PTA can be the sole means of treatment for peripheral artery disease. PTA, especially in the arteries below the knee, has made significant progress in recent years. There are some new appliances being used in the PTA of peripheral arteries in treating diabetic foot, such as intravascular ultrasound ablation, cutting balloons, drug-eluting balloons, and other special micro balloons and stents. Basco et al. (19) reported on 126 lesions treated in 88 patients who underwent lower extremity revascularization using cryoplasty. Technical success rate was 97%. Limb salvage rates were 75 and 63% for patients with critical limb ischemia after 1 and 3 years, respectively. Siracuse et al. (20) reported their results in treating 221 patients with below-knee popliteal artery lesions. Treatment included PTA with or without a stent, atherectomy with or without PTA/stent, and stenting with PTA and atherectomy. Sixty-five percent had no restenosis at 1 year, and had lower short-term restenosis in diabetic patients compared with non-diabetic patients. They concluded that diabetic patients benefit most from atherectomy with PTA, and statin use is protective against restenosis and mortality, and should be the standard of care in peripheral endovascular interventions. Wu et al. (21) performed a meta-analysis investigating PTA versus primary stenting, to determine which procedure is more beneficial for treating infrapopliteal arterial disease. From the prospective randomized trials included, they found 1-year outcomes did not show any significant differences between the PTA and the primary stenting groups, both having the same 1-year benefit. They concluded that there was insufficient evidence to support the superiority of either method. Jens et al. (22) randomized controlled trials comparing either balloon angioplasty or drug-eluting balloon with optional bailout stenting, or primary stenting using a bare stent or drug-eluting stent to one another in critical limb ischemia patients with below-the-knee arterial lesions. Bare stent versus balloon angioplasty and drug-eluting stent versus balloon angioplasty trials showed low-quality evidence of equal efficacy. One trial, comparing drug-eluting balloon with balloon angioplasty, showed moderate-quality evidence of improved wound healing and binary restenosis in diabetic patients after 12 months; amputation and death rate did not differ significantly. For drug-eluting stent versus bare stent, most trials showed equal efficacy between strategies. They concluded that balloon angioplasty with optional bailout stenting using bare stent should remain the preferred strategy in treating below-the-knee arterial lesions.

Newer alternatives to bypass include processed lipoaspirate cells autologous transplantation, lipo-prostaglandin E1, granulocyte colony-stimulating factor, De Marco formula, low-dose urokinase, and heparin-induced extracorporeal low-density lipoprotein precipitation, which directly removes fibrinogen levels from the cardiovascular system and improves microvascular circulation (23).

Bioengineered alternative tissues
Pedal wounds with exposed bone, ligament, and tendon pose additional challenges as direct placement of STSG have high rates of failure as these structures do not provide adequate vascular wound bed to allow take and nourishment of skin graft. The other complication of STSG over these structures is adhesions, limiting function and resulting in breakdown. Tendon-exposed wounds should be closed quickly to prevent tendon desiccation and resultant functional disability, but granulation tissue over tendon
and ligament is difficult (24). Inducing granulation tissue over such structures requires additional wound bed preparation. Successful options include bioengineered alternative tissues (BAT), allografts, and negative-pressure wound therapy (NPWT). Since chronic ulcers often lack healthy, vascularized tissue to support artificial dermis, recipient bed preparation for dermis implantation via NPWT or allografting may be necessary. NPWT promotes granulation growth, while allografting stimulates angiogenesis. In a series of 20 patients, Egemen et al. (25) applied NPWT to the wound bed prior to grafting. The vacuum was set to a continuous negative pressure of 125 mm Hg, with dressings changed on alternate days. Adequate granulation tissue formation was achieved, allowing for direct application of meshed STSG.

BAT are products derived from human, animal, and synthetic tissues that have been manufactured, cleaned, or otherwise altered. BAT can be categorized as dermoinductive or dermoconductive. Dermoinductive products contain viable cells, including fibroblasts and keratinocytes, which are delivered to the non-healing wound site with the goal of activating senescent cells in the chronic diabetic wound by releasing cytokines and growth factors that are produced in the grafted cells. Dermoinductive products should be reserved for more superficial wounds. This includes products such as Apligraf (Organogenesis, Canton, MA) and Dermagraft (Advanced Biohealing, San Diego, CA), which have both demonstrated clinical efficacy (26, 27). In contrast, dermoconductive products provide an organized scaffold to facilitate cell migration of fibroblasts and serve as a template for the formation of neodermis, which is histologically similar in appearance and structure to normal dermis. This provides a durable dermal layer necessary for granulation tissue formation, allowing a skin graft to be placed over the neodermis for definitive wound closure. Examples of this type of tissue include Integra Bilayered tissue (Integra LifeSciences, Plainsboro, NJ) and hMatrix (Bacterin, Belgrade, MT). Thorough debridement must be performed before application of dermoconductive products to remove biofilm and necrotic tissue. This category of products should be reserved for deeper wounds with exposed fascia, tendon, or bone (28). The failure rate of skin grafting on neodermis is 7%, which is lower than that of direct skin grafting on tendons with or without granulation tissue (29). As the neodermis matures, it appears golden yellow, which indicates its readiness for skin grafting, usually occurring between 16 and 28 days after artificial dermis placement. Shores et al. (30) placed Integra Bilayer Matrix Wound Dressing directly over exposed tendons with a subsequent STSG several weeks later in 42 patients. STSG was applied after generation of highly vascularized neodermis, on average 35.3 days after the initial placement of Integra. The size of the tissue defect including the area of tendon exposure ranged from 4 cm² to 336 cm² with an average of 65.1 cm². Average STSG thickness was 0.011 inches. There was 92.5% take in all skin grafts, with all patients exhibiting durable skin coverage at the end of their follow-up period. With physical and occupational therapy, patients were able to attain an average range of motion in their skin grafted joints of the lower extremity that was 90.6% compared to their contralateral side. Yeong et al. (24) reported on 23 patients with 33 wounds, in which artificial dermis was used to prepare tendon-exposed wounds for STSG, 11 of which were chronic ulcers. Thirty-nine percent of the patients had underlying diabetes mellitus, and 55% of the wounds were found in the lower extremities. The mean area of artificial dermis implantation was 67 cm², with mean duration from artificial dermis implantation to STSG 21 days. Overall success rate was 82%, with 63% in the chronic ulcer group. Silverstein (31) also described the use of this technique to close tissue defects of five diabetic feet. All (100%) wounds healed with complete coverage of exposed bone, tendon, cartilage, and fascia.

Some clinicians have also suggested the use of NPWT on meshed Integra to speed rates and success of revascularization. Much like STSG, hematoma and shearing forces can interrupt healing and lead to loss of artificial dermis. Helgeson et al. (32) immobilized Integra for 5 days. As soon as the implanted artificial dermis changed to a golden yellow color indicating formation of the neodermis, the outer silicone layer was removed and STSG were applied. Helgeson harvested skin grafts at least 0.008 inches thick for grafting on the neodermis. When exposed tendon or bone is not present in the wound bed, and adequate vascular supply is present, an algorithmic approach to wound healing can be applied to maximize STSG success, focusing on local wound conditions and their management.

**Tissue management**

Necrotic tissues in a wound should be removed as it prevents proper assessment of the wound bed, and also can be a source of bacterial growth. Of note, bacterial colonies can produce unwanted metalloproteinases that negatively affect extracellular matrix (ECM) components during the healing process, and form biofilm in wound beds. Biofilm is bacterial colonization of the wound surface that is highly resistant to antibiotic treatment, including standard treatments such as systemic antibiotics (33–37). This resistance is partially due to the low metabolic rate of these colonies, which directly impacts the mechanism of action of commonly used oral or parenteral antibiotics as well as the polymicrobial nature of the biofilm (38–42). In addition, these colonies attach to the surface of wounds and surround themselves in a relatively protected microenvironment consisting of an
exopolysaccharide matrix (33, 43–47). Biofilms show increased resistance to antimicrobial, immunological, predatory, and chemical attack (48–50). Once established, biofilms are highly resistant to removal and eradication (51). The reason acute wounds progress through stages of healing, while chronic wounds appear to stall in the inflammatory stage, is likely because of persistent colonization by bacteria (52), leading to persistent inflammatory responses with abnormal cytokine and matrix metalloproteinase levels (53, 54) (Fig. 1).

James et al. (55) reported the presence of biofilms in 60% of chronic wounds, defined as open for 30 days, versus 6% of acute wounds (55). Unlike an infection, mature biofilm develops within 10 hours and persists indefinitely while the wound remains open (56). Once matured beyond this (48 hours), biofilm becomes increasingly resistant to antibiotics (51). Six features have been used to indicate the presence of bacterial biofilm in human chronic wounds (57). These include indicators such as a pale wound bed, friable granulation tissue, yellow discharge, necrotic tissue, a clear slime, and a putrid smell. At present, unless the wound is heavily populated, tissue biopsies or swabs are required combined with microscopic identification techniques to confirm the presence of a wound biofilm. Scanning electron microscopy (SEM) has also been used to show the presence of biofilms within wound tissue (33). In diabetic foot ulcers, diagnosis is generally based on clinical signs and symptoms of inflammation. Clinically seen will be wound bed color change, friable granulation tissue, abnormal odor, increased serous exudate, and pain at wound site with infection.

Inflammation and infection control

Studies have shown that many commercial topical agents and wound dressings are ineffective against biofilm infections (58). Instead, thorough debridement and systemic antibiotics, where antibiotic treatment is tailored specifically to each wound infection, together with a rotating topical antiseptic for the extremely recalcitrant wounds is required (59).

Virtually all chronic diabetic wounds contain bacteria, ranging from contamination, colonization, and critical colonization to infection (60). Usually critical colonization and infection stages impede wound healing. The impact of bacteria in a wound depends on three factors: bacterial load, bacterial strain virulence, and capability of host to mount resistance. In diabetic patients, the effect of bacterial loads can be observed even at a lower count or even with the normal skin flora due to a weak immune system and impaired leukocyte function. Infections in diabetic foot ulcers are commonly polymicrobial and contain both aerobic and anaerobic bacteria (61). The most common bacteria observed in chronic wound infections are Staphylococcus aureus (93.5% of ulcers), Enterococcus faecalis (71.7%), Pseudomonas aeruginosa (52.2%), Coagulase-negative Staphylococci (45.7%), Acinetobacter baumannii (13%), and Klebsiella pneumonia (6.5%) (55, 62). Many studies have reported the evidence of antibiotic-resistant isolates in biofilms, in particular methicillin-resistant Staphylococcus aureus (MRSA), vancomycin-resistant Enterococcus (VRE), and multidrug-resistant Acinetobacter baumannii (63, 64).

Preoperative wound swabs are routinely performed to identify subclinical wound bed colonization, as well as specific strains of bacteria, such as Pseudomonas aeruginosa or Staphylococcus aureus, which can have detrimental effects on graft take (65, 66). However, it remains important to know whether a near-sterile recipient is required for successful skin grafting, as not all wounds can be cleared from bacteria, despite prolonged antibiotic administration and sustained wound bed preparation (67). In an analysis by Bosman et al. (6), wound swabs taken immediately before grafting showed that approximately half the wound beds (53%) had been contaminated, the other half (47%) being sterile. MRSA was detected in five cases, and either Pseudomonas aeruginosa or Staphylococcus aureus was detected in 23% of the wounds. Contaminated wounds did not display a lower mean graft take percentage than near-sterile wounds (87% vs. 90%, respectively). Wounds containing either Pseudomonas aeruginosa or Staphylococcus aureus did have inferior outcome (mean take percentage 78.9% vs. 91.3%, respectively) whereas diabetes also appeared to be a deteriorating factor (mean take percentage 83.0% vs. 90.7%) (9, 53, 68–74). They found that although wound cultures showed that positive swab cultures did not impede good graft take, the presence of specific strains, such as Pseudomonas aeruginosa or Staphylococcus aureus, infers suboptimal outcome. They concluded that qualitative instead of quantitative analysis of the wound swab, whereby specific strains of bacteria are identified, is recommended. Wolcott and Rhoads (53) observed that the chronic wounds treated by specifically targeting

![Fig. 1. Biofilm present on wound bed.](image-url)
biofilms, transformed non-healable wounds into healable wounds. When combined with antibiofilm compounds, the use of antibiotics declined 25% during the 4-year study period.

Chronic wounds often exhibit a highly persistent inflammatory phenotype, epitomized by the influx of polymorphonuclear leukocytes (PMNLs) to the wound site, elevated matrix metalloproteinases (MMPs), and an imbalance of several cytokines (69). The continued presence of bacteria in the wound further exacerbates the situation by causing additional infiltration by PMNLs, together with MMP production (51). Diabetic patients exhibit further dysregulated inflammatory and immune responses that predispose them to chronic wound infections. In diabetic chronic wounds, there is a disruption of the balance between ECM synthesis and degradation (70). MMPs regulate extracellular structural proteins and consequent tissue remodeling (71). Biopsies carried out on non-healing ulcers have shown higher presence of MMPs than those carried out on healing wounds. This altered MMP expression in chronic ulcers result in excessive matrix degradation, preventing normal matrix formation and remodeling, leading to formation of chronic wounds (72). The inflammatory stage is extended in the non-healing wounds and this is reflected by the continued presence of neutrophils and elevated MMP. Amato et al. (70) showed that levels of the collagenases, MMP-1 and MMP-8, are over expressed in human non-healing wounds compared to normal healing wounds, and the role of MMP-8 appears to be predominant because of a higher over-expression. This chronic inflammatory condition results in the continual infiltration by poly and mononuclear cells that include neutrophils, PMNLs, macrophages, and foreign body giant cells at the site of injury resulting in a continuous secretion of potent proteases, such as collagenases, gelatinases, and neutrophil elastase into the wound. Nguyen et al. (75) has also shown that diabetic biofilm-containing wounds had significantly less TLR 2, TLR 4, interleukin-1β, and tumor necrosis factor expression than wild-type wounds with biofilm. Whereas both groups had similar bacterial burden and neutrophil infiltration after development of biofilms at 3 days post-wounding, diabetic wounds, however, had significantly less neutrophil oxidative burst activity.

The upregulation of proteolytic enzymes, especially MMPs, causes excessive matrix degradation (76–80). The MMP–tissue inhibitor of metalloproteinase (TIMP) balance is critical for regulating cell-matrix composition. Many studies have documented the important role of this balance in the pathophysiology of chronic wounds (74, 81). Hyperbaric oxygen therapy (HBOT) has been shown to significantly decrease MMP protein levels in ischemic wound tissue, while the level of TIMP significantly increases (73).

**Wound bed debridement**

There is debate over the depth of tissue debridement necessary to remove biofilm, and even after debridement, repopulation of biofilm within 24 hours can occur (82). Therefore, maintenance debridement in a clinic setting may be required to decrease the biofilm load after the initial operative debridement.

Debridement helps to reduce the bacterial burden within the wound, controls ongoing inflammation and malodor, and encourages formation of granulation tissue (60). The molecular and cellular environment of chronic wounds should be converted to resemble that of acute wounds to allow rapid healing, and for this to occur, non-healing wounds may require repeated debridement (83).

There are multiple techniques that can be used for the debridement of necrotic, sloughy, fibrous, and unhealthy tissue. The options for debridement include surgical and mechanical methods. A wound bed may also be prepared by various non-surgical debridement techniques: autolytic debridement facilitated by interactive dressings, larval therapy using sterile maggots, and enzymatic debridement with ointments containing papain, urea, or collagenase mixtures. Autolytic debridement can be slow and can take a long time to be effective (84). Enzymatic debridement finds use when other techniques are not feasible during the initial management of a chronic wound (85). Mechanical (wet-to-dry) debridement damages healthy granulation tissue (86). NPWT can also be a useful adjunct to biofilm reduction. Morykwas et al. (87, 88) and Timmers et al. (89) suggest that negative pressure therapy expedites wound healing through the evacuation of drainage, promotion of angiogenesis, granulation tissue formation, and biofilm reduction. Gabriel et al. (90) demonstrated fewer days of treatment, more rapid wound closure, and fewer hospital days with the use of negative pressure therapy and antimicrobial solution to soak the wound bed.

**Topical therapy**

Antimicrobial treatment guided by superficial wound culture has been challenged repeatedly in the literature (91–94). Slater et al. (41) reported that only 62% of microorganisms identified through swab cultures correlated with deep tissue cultures, and different microorganisms were found in the swab cultures as compared with the deep cultures. Also relevant, superficial wound culture might not accurately reflect the diversity of the bacteria present in the chronic wound enveloped by biofilm. The use of polymerase chain reaction microbial speciation and quantification is increasingly recognized as a more efficient and accurate method of guiding topical biofilm treatment in the chronic diabetic wound (95–98).

There is a growing consensus that systemic antibiotics may be ineffectual in the treatment of biofilm and/or mild infections associated with chronic diabetic wounds, and can aid in the development of antibiotic resistance.
Topical antimicrobials (ointments, creams, and gels) have long been used for the prevention and treatment of localized, mild to moderate, soft-tissue wound infection. Triple antibiotic, gentamicin, iodine-based, and silver-based are some examples of commonly used topical antimicrobials.

Silver-impregnated dressing materials are commonly used because of its purported antimicrobial properties identified through in vitro studies. Beele et al. reported in a small prospective randomized trial that the use of a silver-impregnated dressing decreases the likelihood of conversion from colonized wound to a clinically infected wound as compared with a non-silver-impregnated dressing. In one study, it was observed that 90% of all sessile bacteria within the biofilm progressively died within 24 hours in the presence of silver-containing wound dressings. Thorn et al. investigated the antimicrobial effectiveness of silver- and iodine-containing wound dressings on preformed biofilms of *Pseudomonas aeruginosa* and *Staphylococcus aureus*. It was found that the iodine dressing was more efficacious than the silver dressing on biofilms. In a recent retrospective single-center study, Wolcott and Rhoads evaluated the frequency of complete healing in subjects with a chronic wound in a limb with critical limb ischemia when managed using biofilm-based wound care. In total, 77% of the wounds healed completely and 23% were classified as non-healing.

**Biologic debridement**

Over the past decade, biologic debridement using maggots has become increasingly popular. The maggots are highly selective and rapid, but often need to be combined with other forms of debridement after initial larval application. The larvae of the green butterfly *Lucilia sericata* or *Lucilia cuprina* can be used for biological debridement to digest the necrotic tissue, and they also secrete bactericidal enzymes. This approach is effective in wounds with MRSA and beta hemolytic *Streptococcus*.

**Surgical debridement**

Surgical debridement, the current gold standard against which other forms of therapy are measured, is quick and effective, although expensive as it requires an operating room and many hospital admissions. Surgical debridement is the fastest means of debridement, allowing surgeons to accurately assess the severity and extent of the wound. A drawback of sharp surgical debridement is the non-selective nature of the method, which endangers normal healthy tissues with the risk of accidental removal. In the presence of ischemic ulcers, it is critical that management should aim toward restoring tissue perfusion prior to aggressive wound debridement or aggressive surgery to ensure wound healing.

Hydrosurgery combines both physical and surgical debridement techniques allowing for precise, controlled, and expedited debridement. Hydrosurgery allows for select removal of necrotic tissue, while decreasing debridement times by 39%. Caputo et al. published a random controlled trial comparing hydrosurgery debridement to conventional surgical debridement in patients with diabetic and venous leg ulcers. On average, hydrosurgery debridement was quicker by about 7 minutes per procedure, and required significantly less instruments and sterile saline. The median time for wound closure was similar in both groups. Mosti et al. compared use of hydrosurgery debridement to moist dressings in patients with vascular leg ulcers. The mean time to debride the wound was 5–8 minutes, and the average time to obtain a clean wound was reduced by nearly 5 days compared to wet-to-dry dressings. Hydrosurgery also minimizes the amount of normal tissue that is accidentally removed by surgery, and in most cases the wound bed is ready for immediate skin grafting. Vanwijck et al. reported on 167 wounds treated by hydrosurgery. Of all the debrided wounds, 95% were immediately covered with an autologous split-thickness meshed graft. Hydrosurgery left a smooth wound surface, which allowed immediate skin grafting in the majority of patients. For all but 8 patients, the engraftment was total. Studies have demonstrated that hydrosurgery efficiently reduces the bacterial load of the wound and prevents the diffusion of microbial contamination deeper into the wound.

**Ultrasound therapy**

The use of acoustic energy continues to grow in popularity as a method of biofilm debridement. There are mainly two classified effects of ultrasound on tissue: thermal and...
non-thermal (119). High-intensity ultrasound likely debrides necrotic tissue as a result of cavitation (120). Low-intensity ultrasound is thought to promote wound healing predominantly by acoustic streaming effects such as increased protein synthesis and production of growth factors (121). In addition, low-frequency ultrasound has been reported to have antibacterial effects (122, 123), and enhance fibrinolysis in vitro (124, 125). Ennis et al. (126) compared low-frequency (40 kHz) non-contact ultrasound to placebo in 55 diabetic patients with recalcitrant foot ulcers in a randomized, multicenter, double-blinded study. At 12 weeks, they reported significantly higher healing rates in the treatment group. Kavros et al. (127) published another study on 163 patients with chronic lower extremity wounds. In the retrospective study, they reported significantly higher percentage of wounds healed with low-intensity and low-frequency ultrasound compared to standard care alone. A recent meta-analysis reported significantly improved complete healing rates with low-frequency and high-intensity ultrasound (20 kHz, 50–60W/cm²) compared to sharp debridement at 3 and 5 months (9).

Hyperbaric oxygen therapy
Reported benefits of HBOT are its detrimental effect on bacteria via the production of oxygen free radicals and by enhancing leukocyte activity (128). The reports of antimicrobial effects target anaerobic bacteria by increasing oxygen concentrations in deeper tissues. Chen et al. (129) reported increased limb salvage rates (78.3%) among patients with infected diabetic wounds who underwent greater than 10 HBOT treatments. Although literature on evaluating the effect of HBOT on biofilm reduction or eradication is rare.

STSG management
After harvesting of STSG, to ensure success, seroma/hematoma must be managed, along with prevention of shear forces that would disrupt the plasmotic phase and angiogenesis phase while the STSG is being incorporated on to the wound bed. To achieve all of the above requirements, a uniform pressure over the entire grafted area through a non-adherent, semi-occlusive, absorbent dressing material is required. NPWT finds a role both before split-thickness skin grafting by decreasing bacterial load and assisting with wound bed preparation, as well as after grafting by fixating graft and reducing/eliminating seroma/hematoma (130). Graft take and complication rates have significantly improved because of the preferential use of meshed grafts and the introduction of NPWT (7, 131, 132) (Fig. 3).

Blume et al. (133) compared conventional therapy (CT) dressing, cotton bolster/sterile compressive/stainless steel gauze dressing that is used for at least 5 days, to NPWT using reticulated open cell foam (NPWT/ROCF). One hundred and forty-two patients underwent STSG placement, 79 wounds being diabetic foot ulcers. Grafting area was similar between NPWT/ROCF (45.4 cm²) and CT (47.4 cm²). Mean graft take at the first follow-up was 95% for NPWT/ROCF compared to 86% for CT, with maximum graft take of 96% for NPWT/ROCF compared to 83% for CT. There were significantly fewer repeated STSGs required in the NPWT/ROCF group (3.5%) compared to the CT group (16%). There were fewer complications (seroma/hematoma/infection) to graft failure in the NPWT/ROCF group compared to the CT group. The ROCF dressing conformed to the wound geometry with negative pressure, promoting skin graft adherence while removing exudates and edema from the surrounding tissues. Egemen et al. (25) used standard black polyurethane foam with a continuous negative pressure of 75 mm Hg for both wound bed preparation and management post-application STSG. The mean number of silver-impregnated foam dressing for wound bed preparation was 2.9, with a mean of 2.6 NPWT foam changes after skin grafting. All wounds completely healed without the need for further debridement or re-grafting. (Fig. 4).

There are limitations and shortcomings to this review article. One limitation is that this is not a meta-analysis; not every study included was a rigorous scientific endeavor, and not every study investigating STSG was included.

Conclusion
Graft survival is predicated on several factors: historically, graft failure rates were high and primarily attributed to infection (7), highlighting the importance of biofilm management and eradication; as well preventing shearing, seroma, and hematoma formation beneath the graft with immobilization to allow for the initial take or incorporation, which occurs by diffusion of nutrition.
from the recipient site, termed ‘plasmatic imbibition’. STSGs must be placed on a well-vascularized bed with low bacterial counts to prevent infection. Revascularization generally occurs between days 3 and 5 by reconnection of blood vessels in the graft to recipient site vessels or by ingrowth of vessels from the recipient site into the graft (134). Skin grafts generally will not take on poorly vascularized wound beds, such as bare tendons, cortical bone without peristeum, heavily irradiated areas, or infected wounds. However, virtually any tissue type with a vascular granulating bed is acceptable for grafting (135). NPWT has been shown to provide many aspects of STSG success by promoting granulation tissue, lowering bacterial counts, and removing accumulated fluid, such as hematoma/seroma, both of which reduce the chronic inflammatory process that occurs in chronic wounds such as elevated MMPs.

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