The Use of Cryopreserved Human Skin Allograft for the Treatment of Wounds With Exposed Muscle, Tendon, and Bone

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Abstract: *Background.* Wounds with exposed bone or tendon continue to be a challenge for wound care physicians, and there is little research pertaining to the treatment of these particular wounds with allograft skin. The purpose of this study was to evaluate the effectiveness and safety of a biologically active cryopreserved human skin allograft for treating wounds with exposed bone and/or tendon in the lower extremities. *Methods.* Fifteen patients with 15 wounds at a single hospital-based wound care center were included in the study. Eleven wounds had exposed bone, 1 wound had exposed tendon, and 3 wounds had exposed bone and tendon. Standard treatment principles with adjunctive cadaveric allograft application were performed on all wounds in the study. *Results.* In this study 14/15 (93.3%) of the wounds healed completely. The mean duration of days until coverage of the bone and/or tendon with granulation tissue was 36.14 (5.16 weeks) (range 5-117 days). Mean duration to complete healing of the wound was 133 days (19 weeks) (range 53-311 days). The mean number of grafts applied was 2. There were no adverse events directly related to the graft. Zero major amputations and 1 minor amputation occurred. *Conclusion.* This study found biologically active cryopreserved human skin allografts to be safe and effective in treating difficult wounds with exposed bone and/or tendon. To the authors' knowledge, this is the largest study to date focused on the utilization of allograft skin as an adjunct therapy for lower extremity wounds with exposed tendon and/or bone.

Key words: cryopreserved human skin allograft, wound healing, exposed bone, exposed tendon, lower extremity wounds

Chronic lower extremity wounds are a significant health concern and are a cause of morbidity for patients living with them, whether or not diabetes mellitus is a contributing factor. There is a 0.18%-2.00% prevalence of lower extremity wounds in the adult population worldwide,[1] the etiology of which may be vascular (ie, venous, arterial, or mixed), neurotrophic, lymphatic, malignant, inflammatory, traumatic, or infectious. The majority of lower limb ulcerations are related to venous etiology.[2]

Since the aging population is increasing, as well as the prevalence of diabetes mellitus, there is a subsequent increase in the incidence of chronic

Table 1. Inclusion and exclusion criteria.

Inclusion Criteria	Exclusion Criteria
Wounds with exposed muscle, tendon, and/or bone	Full-thickness wounds without exposed muscle, tendon, and/or bone
Osteomyelitis treated with debridement and intravenous antibiotics	Evidence of acute infection
Patient able to comply with offloading instructions	Use of prior skin substitute or advanced biologic dressing

lower extremity ulceration. An estimated 29.1 million people in the United States have diabetes mellitus,[3] and the estimated lifetime incidence of a diabetic foot ulcer is 25%.[4] Diabetic foot ulcers continue to be a major cause of morbidity and immobility and are a leading cause of nontraumatic lower extremity amputation.

Wound care specialists may utilize advanced biologic agents and skin substitutes for the treatment of chronic, nonhealing wounds. These advanced biologic agents may include bioengineered skin substitutes, cryopreserved skin allografts, cryopreserved placental membranes, acellular collagen, and recombinant growth factors.

Wounds with exposed bone or tendon continue to be a major challenge for wound care physicians. Human skin allografts have traditionally been utilized in the treatment of patients with burns, but have also been used for these difficult wounds with exposed tendon and bone to aid in forming granulation tissue and coverage.[5,6] Snyder et al[5] found that cadaveric allografts prevented desiccation, controlled infection, promoted granulation tissue, and reduced pain in nonhealing ulcers. However, there has been little continued research pertaining to the treatment of wounds with exposed tendon and/or bone with allograft skin over the past several years.

TheraSkin (Soluble Solutions, LLC, Newport News, VA) is a biologically active cryopreserved human skin allograft harvested from tissue donors within 24 hours of death and minimally processed to preserve the components of real human skin. It contains epidermal and dermal layers rich in type I, III, and IV collagen. It also contains a mature extracellular matrix, which is the source of growth factors and cytokines that are necessary in biological wound healing.[7]

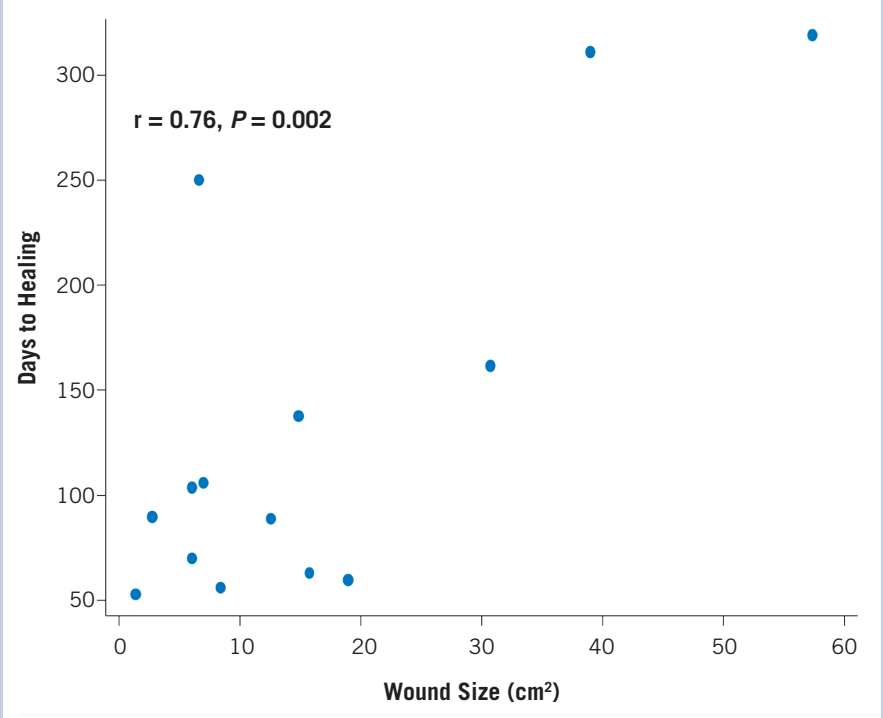

Figure 1. Days to coverage of bone or tendon with granulation tissue as correlated to wound size (r = correlation coefficient; *P* = level of significance).

The primary aim of this study is to evaluate the effectiveness and safety of a biologically active cryopreserved human skin allograft for treating lower extremity wounds with exposed bone and/or tendon. The authors hypothesized that the use of human skin allograft would effectively aid in coverage and healing of these types of wounds.

Methods

A retrospective medical chart review of patients at a

single, hospital-based wound care center from January 2012-November 2014 was performed. Only lower extremity wounds with exposed bone, tendon, or both were included in the study. Wounds without exposed bone and/or tendon, and wounds with prior treatment including the use of skin substitutes or advanced biologics were excluded from the study. See Table 1 for inclusion/exclusion criteria. Fifteen consecutive patients with 15 wounds met the inclusion/exclusion criteria. The data extraction was completed by 1 physician who was educated on proper data collection and was not involved in patient care at the study site during the study period. Data were collected through a standard form in each patient's chart to improve reliability and reproducibility. A second physician not involved in patient care independently reviewed the data and all information was confirmed from participating patients' charts. Eleven wounds had exposed bone, 1 wound had exposed tendon, and 3 wounds had exposed bone and tendon.

Patients were followed by 1 of 2 treating physicians. Standard treatment principles with adjunctive cadaveric allograft application were performed on all wounds in this study. The treating physicians determined necessary debridement and application of the allograft. Following cleansing of the wound, debridement consisted of either sharp or mechanical debridement techniques. Patients were evaluated weekly following the initial application of their graft. If necessary, a second graft was applied at week 2. At week 4, if there was reduction in wound size of 50% or more, no further grafts were applied and standard wound therapy was continued. If the wound did not decrease 50% in size by week 4, additional grafting was performed. The cadaveric allograft was secured and covered with a simple bolster dressing or a negative pressure wound therapy dressing at the time of application based upon wound characteristics and surgeon preference.

Statistical analysis. Relationships of wound size with time to cover and time to heal were evaluated using Pearson's correlations and linear regression. Because these relationships were roughly linear, 2 new variables were calculated that divided each patient's time to cover and time to heal by wound size. Next, time to cover and time to heal variables, unadjusted and normalized to wound size, were compared across patient characteristics using independent *t* tests.

Results

Fifteen consecutive patients met inclusion criteria in this study. Patient age ranged from 26-91 years old. The majority of patients were male (73%), had diabetes mellitus (73%), and had peripheral neuropathy (80%). About half of the patients had peripheral vascular disease (47%). The diagnosis of osteomyelitis was made in 67% of patients who were treated with a prolonged course of IV antibiotics. The mean number of grafts applied was 2. (Table 2).

Mean wound size was 16 cm^2 ± 15 cm^2 (range 1-57cm^2). Fourteen out of 15 wounds (93.3%) completely healed. The mean duration of days until coverage of the bone and/or tendon with granulation tissue was 36.14 days (5.16 weeks) (range 5-117 days). Mean duration to complete healing of the wound was 133 days (19 weeks) (range 53-311 days). Wounds with

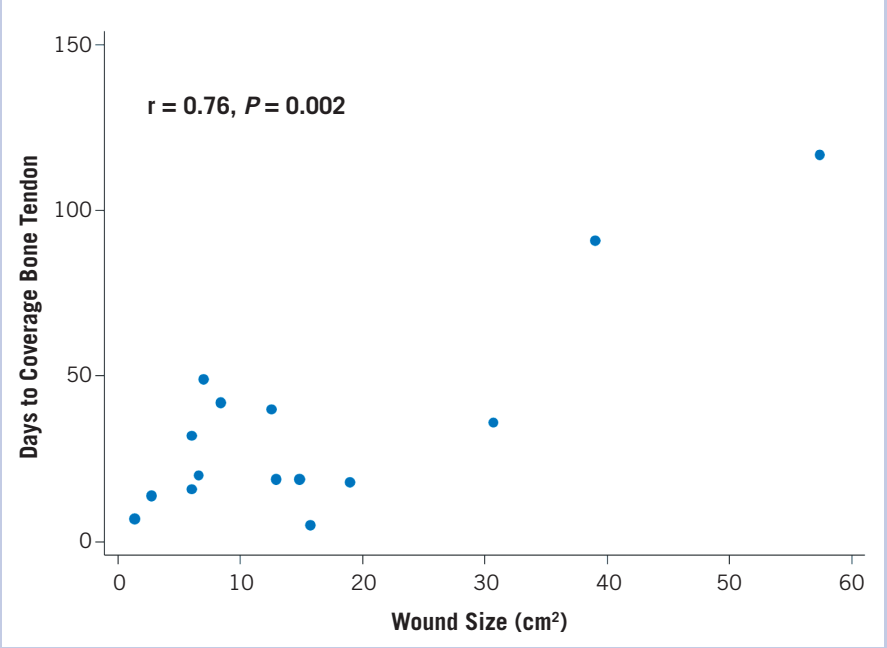

Figure 2. Days to complete healing as correlated to wound size (r = correlation coefficient; *P* = level of significance.) Includes 1 patient who underwent amputation at 97 days.

Table 2. Patient characteristics (n = 15).*	
Characteristic	**Mean ± SD or N (%)**
Age, years	62 ± 22 range: 26-91
Gender Male Female	 11 (73%) 4 (27%)
Diabetes Yes No	 11 (73%) 4 (27%)
Neuropathy Yes No	 12 (80%) 3 (20%)
Peripheral Vascular Disease Yes No	 7 (47%) 8 (53%)
Osteomyelitis Yes No	 10 (67%) 5 (33%)
Hyperbaric Oxygen Therapy Yes No	 3 (20%) 12 (80%)
Vacuum-assisted closure Yes No	 4 (26%) 11 (73%)
Wound size, cm^2	16 ± 15 range: 1-57
Time to cover, days	35 ± 31 range: 5-117
Time to heal, days*	134 ± 93 range: 53-319
*1 patient underwent amputation at 97 days SD = standard deviation	

exposed bone and/or tendon that measured less than 20 cm^2 (12/15, 80%) were covered with granulation tissue within 50 days (Figure 1). Eleven out of 12 (92%) of these wounds were healed within 150 days (Figure 2). A regression equation for days to complete coverage with granulation tissue per cm^2 was identified: 8.03 days + (1.69 x wound size in cm^2). A regression equation for days to complete healing per cm^2 was also identified: 62.03 days + (4.41 x wound size in cm^2).

There was no significant difference in patient characteristics—diabetes mellitus, neuropathy, peripheral vascular disease, osteomyelitis, the use of hyperbaric oxygen therapy (HBOT) or negative pressure wound therapy—and time to coverage per cm^2 or time to heal per cm^2 (Table 3). There were no adverse events directly related to the graft. Zero major amputations and 1 minor amputation occurred.

Discussion

The primary aim of this study was to evaluate the effectiveness and safety of a biologically active cryopreserved human skin allograft for treating lower extremity wounds with exposed bone and/or tendon. The effectiveness of the graft was determined by assessing the number of days required for coverage of tendon and bone with granulation tissue, the number of days required until complete wound closure, and the number of grafts necessary to achieve wound closure. The safety of the graft was assessed by identifying adverse events directly related to the graft during the study.

In this retrospective review of 15 consecutive patients treated with cadaveric allograft, the authors were able to effectively and completely cover all wounds with granulation tissue in an average of 36 days (5 weeks). A linear relationship was discovered between wound size in cm^2 and days to complete granulation tissue coverage of bone or tendon. For example, diabetic ulcers with bone or tendon exposure were completely covered with granular tissue at a rate of 2.69 days per 1 cm^2 of wound size. No adverse events were noted secondary to graft application. Furthermore, 14 of 15 (93%) wounds treated achieved healing. Wounds healed in an average of 133 days (19 weeks). Again, a linear relationship was noted between days to complete wound healing and wound size in cm^2: diabetic ulcers with tendon/bone exposure healed at a rate of 11.7 days per cm^2. One patient did achieve granular coverage of the wound but did not heal. In this case, the authors did observe complete coverage of the wound, but the patient was then lost to follow-up. The patient returned 4 months later and, unfortunately, was admitted to the hospital with a deep bone infection that was ultimately treated with minor amputation which healed without complication. On average, only 2 grafts were necessary to achieve healing. No adverse events were noted, suggesting this is a safe treatment option. As of the date of publication, no recurrence of ulceration has been noted.

Cadaveric human skin allografts have been utilized in complicated wounds. In a retrospective review of 27 patients with 34 nonhealing lower extremity ulcers of various etiologies, Snyder et al[5] utilized glycerin-preserved, frozen, cadaveric split-thickness skin al-

Table 3. Days to complete granulation tissue coverage of bone/tendon per cm^2 and days to complete wound healing per cm^2 by patient characteristics.

	Days to cover	Days to cover per cm^2	Days to heal	Days to heal per cm^2
Diabetes				
Yes	40 ± 10	2.9 ± 0.6	145 ± 94	11.7 ± 8.8
No	22 ± 7	3.6 ± 1.0	105 ± 48	22.0 ± 20
	P = 0.340	*P* = 0.593	*P* = 0.485	*P* = 0.191
Neuropathy				
Yes	37 ± 10	3.1 ± 2.1	137 ± 28	14.2 ± 3.6
No	27 ± 8	3.0 ± 2.0	122 ± 64	15.9 ± 11.0
	P = 0.624	*P* = 0.934	*P* = 0.818	*P* = 0.834
PVD				
Yes	39 ± 10	3.0 ±2.4	134 ± 94	8.5 ± 4.2
No	32 ± 13	3.2 ± 1.8	134 ± 99	19.2 ± 15.5
	P = 0.671	*P* = 0.850	*P* = 0.996	*P* = 0.131
Osteomyelitis				
Yes	40 ± 36	2.7 ± 2.3	147 ± 34	10.6 ± 9.4
No	25 ± 15	3.8 ± 2.7	110 ± 81	21.8 ± 16.3
	P = 0.400	*P* = 0.323	*P* = 0.508	*P* = 0.122
HBOT				
Yes	53 ± 36	3.5 ± 3.0	185 ± 110	10.8 ± 3.
No	31 ± 30	3.0 ± 1.9	120 ± 88	15.7 ± 14.4
	P = 0.280	*P* = 0.680	*P* = 0.300	*P* = 0.585
VAC				
Yes	41 ± 38	3.2 ± 2.8	183 ± 117	16.2 ± 15.1
No	33 ± 30	3.1 ± 1.8	114 ± 80	14.0 ± 12.7
	P = 0.657	*P* = 0.926	*P* = 0.228	*P* = 0.778

PVD: peripheral vascular disease; HBOT: hyperbaric oxygen therapy; VAC: vacuum-assisted closure

lografts as an adjunct for wound management. They found granulation tissue apparent through the graft fenestrations at an average of 13.4 days. In their study, 65% of patients healed via secondary intention while 35% healed after split-thickness skin grafting. Their average healing time was 113.9 days, and no infections occurred while the allograft was in place. Seven of the wounds in that study included exposed tendon and/or bone, and the allograft was found to be particularly beneficial in these wound types.

Biologically active cryopreserved human cadaveric skin allografts have been shown to be an effective treatment for diabetic foot ulcers and venous leg ulcers. In a retrospective study by Landsman et al,[8] 188 ulcers —54 diabetic foot ulcers and 154 venous leg ulcers—closed 60.38% and 60.77% of the time after 12 weeks, and 74.1% and 74.6% after 20 weeks, when treated with cadaveric allografts, respectively. Ulcers in the 12-week group required 2.03 (± 1.47) grafts on average, and the ulcers in the 20-week group required 3.23 (± 2.77) grafts on average to achieve closure. There were 4 wounds with exposed tendon or joint capsule and 1 wound with exposed bone. No adverse events were associated with the graft.

A few studies have compared the effectiveness of cadaveric skin allograft to bioengineered skin substitutes such as Apligraf, a bioengineered skin substitute composed of fibroblasts and keratinocytes on a bovine collagen substrate, and Dermagraft, a human fibroblast-derived skin substitute (both manufactured by Organogenesis, Canton, MA). In a study by DiDomenico et al[9] cadaveric allograft was found to close more wounds at a faster rate when compared to the bioengineered skin substitute. In this prospective cohort study, 66.7% of diabetic wounds closed in 12 weeks with cadaveric allograft whereas 41.3% of diabetic wounds closed in 12 weeks with the bioengineered skin substitute. Wounds with exposed bone and/or tendon were excluded. In a prospective, randomized controlled clinical trial by Sanders et al[10] cadaveric skin allograft more

effectively healed diabetic foot ulcers when compared to the human fibroblast-derived skin substitute. In their study, diabetic foot ulcers were twice as likely to heal after 12 weeks when treated with cadaveric allograft as compared to human fibroblast-derived skin substitute, 63.6% vs 33.3%, respectively. Unfortunately, wound depth, such as exposed bone or tendon, was not discussed.

A couple of recent studies have advocated the use of Integra Bilayer Wound Matrix (Integra LifeSciences, Plainsboro, NJ), a porous matrix of cross-linked bovine tendon collagen and glycosaminoglycan and a silicone layer, on wounds with exposed bone and tendon.[11-13]

This skin substitute consists of 2 layers: a dermal layer composed of a porous matrix of bovine tendon collagen and glycosaminoglycans, and an epidermal layer containing silicone. Clerici et al[14] demonstrated the use of the bilayer wound matrix and subsequent skin grafting for the treatment of 30 diabetic foot wounds with exposed bone and tendon following surgical debridement. They reported an 86.7% healing rate and an average healing time of 74.1 ± 28.9 days following surgical debridement. No major amputations were performed and maximal foot length was preserved. Iorio and colleagues[15] retrospectively studied 80 patients with diabetic foot ulcers who underwent surgical debridement of wounds and application of the bilayer wound matrix in preparation for skin grafting for the coverage of deep wounds with exposed tendon or bone. They reported a 46% limb salvage rate in patients with diabetes with a high risk for amputation and an 83% limb salvage rate in the patients with diabetes who had a low risk for amputation. While these studies suggest the bilayer skin substitute is a viable option for the treatment of deep diabetic foot wounds, both studies routinely applied subsequent split-thickness skin grafting. In the current study, none of the patients required additional split-thickness skin autograft for final coverage.

Other therapies have been studied in the treatment of wounds with exposed bone and/or tendon. Hyperbaric oxygen therapy, for instance, has been suggested as a valuable adjunctive therapy in the treatment of chronic foot ulcers, though controversy remains. Currently, the Undersea and Hyperbaric Medical Society approves HBOT as an adjunctive treatment for diabetic foot ulcers. Also, the Centers for Medicare & Medicaid Services guideline states that patients are eligible for HBOT if they have type 1 or type 2 diabetes mellitus, a lower extremity ulcer caused by diabetes that has failed standard wound therapy, and a wound classification of Wagner grade 3 or higher.

A few studies have reported on wound healing rates for wounds classified as Wagner grade 3 or 4 treated with HBOT. In a recent retrospective study by Oliveira et al,[11] 15/23 (65%) foot wounds classified as Wagner grade 2 or greater healed within 16 weeks following the start of HBOT. A prospective, randomized study by Duzgun et al[12] including 100 patients who had diabetic foot ulcers that were classified as Wagner grade 2 (18%), grade 3 (37%), and grade 4 (45%) compared wound healing rates in wounds treated with HBOT vs standard wound therapy. They demonstrated a 66% healing rate in the HBOT group and a 0% healing rate in the standard wound therapy group. Within the HBOT group, all Wagner grade 2 wounds healed, 68% of Wagner grade 3 wounds healed, and 56% of Wagner grade 4 wounds healed during a mean follow-up of 92 weeks. Unfortunately, a distinction or association between time to healing and wound grade was not discussed. The Hyperbaric Oxygen Therapy in Diabetics with Chronic Foot Ulcers (HODFU) study by Londahl et al[13] was a randomized, single-center, double-blinded, placebo-controlled clinical trial including 94 patients with Wagner grade 2, 3, or 4 foot ulcers. At 1-year follow-up, the investigators found 25/48 (52%) of patients treated with HBOT healed, whereas 12/42 (29%) in the placebo group (P = 0.03) healed. The current study demonstrates 93.3% of wounds healed, and these wounds were healed in less than 1 year.

This study is limited by its retrospective design. Potential selection bias is inherent to a retrospective study. There were no controls and, therefore, no comparative analysis could be performed. The study is also limited by small sample size. However, this is the largest study utilizing human cadaveric skin allograft as an adjunct therapy for lower extremity ulcers with exposed tendon and/or bone. Though wounds were of different etiology — diabetic foot ulcer, traumatic, surgical, and decubitus — this did not appear to change outcomes. Some treatment modalities, such as the use of negative pressure wound therapy vs simple bolster dressing, differed between wounds, but no statistical difference was noted between the treatments.

Conclusion

This study found biologically active cryopreserved human skin allografts to be safe and effective in treating difficult wounds of various etiologies with exposed

bone and/or tendon.To the authors' knowledge, it is the largest study utilizing cadaveric allograft as an adjunct therapy for lower extremity ulcers with exposed tendon and/or bone. In this challenging subset of wounds, 93.3% of the wounds completely healed within an average of 133 days. Granulation tissue within the wound bed was present an average of 36 days from initial graft application, and wounds required an average of 2 grafts. These findings support the use of a biologically active cryopreserved human skin allograft for the treatment of wounds with exposed bone and/or tendon.

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References

1. Spentzouris, G & Labropoulos, N. The evaluation of lower-extremity ulcers. *Semin Intervent Radiol.* 2009;26(4):286-295.
2. Adam, DJ, Naik, J, Hartshorne, T, Bello, M, London, NJ. The diagnosis and management of 689 chronic leg ulcers in a single-visit assessment clinic. *Eur J Vasc Endovasc Surg.* 2003;25(5):462-468.
3. Centers for Disease Control and Prevention. *National Diabetes Statistics Report: Estimates of Diabetes and Its Burden in the United States, 2014.* Atlanta, GA: US Department of Health and Human Services; 2014.
4. Singh, N, Armstrong, D, Lipsky, B. Preventing foot ulcers in patients with diabetes. *JAMA.* 2005; 12;293(2):217-228.
5. Snyder RJ, Simonson DA. Cadaveric allograft as adjunct therapy for nonhealing ulcers. *J Foot Ankle Surg.* 1999;38(2):93-101.
6. Snyder RJ. Treatment of non-healing ulcers with allografts. *Clin Dermatol.* 2005;23(4):388-395.
7. Lin, Q, Rosines E, Clagett J, Taylor BM. Independent study funded by Skin and Wound Allograft Institute, a subsidiary of LifeNet Health, Virginia Beach, VA. Study conducted at Albany Medical Center and the University of Maryland, Institute of Human Virology, January 2010.
8. Landsman AS, Cook J, Cook E, et al. A retrospective clinical study of 188 consecutive patients to examine the effectiveness of a biologically active cryopreserved human skin allograft (Theraskin®) on the treatment of diabetic foot ulcers and venous leg ulcers. *Foot Ankle Spec.* 2011;4(1):29-41.
9. DiDomenico L, Emch KJ, Landsman AR, Landsman A. A prospective comparison of diabetic foot ulcers treated with either a cryopreserved skin allograft or a bioengineered skin substitute. *Wounds.* 2011;23(7):184-189.
10. Sanders L, Landsman AS, Landsman A, et al. A prospective, multicenter, randomized controlled clinical trial comparing a bioengineered skin substitute to a human skin allograft. *Ostomy Wound Manage.* 2014;60(9):26-38.
11. Oliveira N,. Rosa P, Borges L, Dias E, Oliveira F, Cássio I. Treatment of diabetic foot complications with hyperbaric oxygen therapy: A retrospective experience. *Foot Ankle Surg.* 2014;20:140-143.
12. Duzgun AP, Satir HZ, Ozozan O, Saylam B, Kulah B, Coskun F. Effect of hyperbaric oxygen therapy on healing of diabetic foot ulcers. *J Foot Ankle Surg.* 2008;47:515-519.
13. Londahl M, Katzman P, Nilsson A, Hammarlund C. Hyperbaric oxygen therapy facilitates healing of chronic foot ulcers in patients with diabetes. *Diabetes Care.* 2010;33:998-1003.
14. Clerici G, Caminiti M, Curci V, Quarantiello A, Faglia E. The use of a dermal substitute to preserve maximal foot length in diabetic foot wounds with tendon and bone exposure following urgent surgical debridement for acute infection. *Int Wound J.* 2010;7:176-183
15. Iorio ML, Goldstein J, Adams M, Steinberg J, Attinger C. Functional limb salvage in the diabetic patient: the use of a collagen bilayer matrix and risk factors for amputation. *Plast Reconstr Surg.* 2011;127(1):260-267.