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Treatment of Venous Leg Ulcers with Dermagraft®

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Background. *A number of different treatment approaches have been recommended for the treatment of venous ulceration, including local ulcer treatment, compression and drug therapy. Recent advances in tissue engineering have resulted in living tissues being developed for cutaneous wound repair and skin replacement. The aim of this pilot study was to compare the rate of healing of venous ulcers in patients treated with Dermagraft (a human fibroblast-derived dermal replacement) and compression therapy or compression therapy alone.*
Methods. *A total of 18 patients with venous ulceration of the leg were recruited into the pilot study. Ten patients were treated with Dermagraft and compression therapy, and eight patients were treated with compression therapy alone. Healing was assessed by ulcer tracing and computerised planimetry. Skin perfusion was measured by laser Doppler.*
Results. *Five (50%) of the patients treated with Dermagraft and one (12.5%) control patient had healed by the end of the 12-week study period (NS). The total ulcer area rate of healing and linear rate of healing was significantly improved in patients treated with Dermagraft (*$P = 0.001$ *and* $P = 0.006$*, respectively, Mann–Whitney* U*-test). The number of capillaries increased in both the treatment and control group. Peri-ulcer skin perfusion increased by 20% in patients treated with Dermagraft, compared with 4.9% in the control group.*
Conclusion. *The data from this small pilot study suggests that Dermagraft is associated with improved healing of venous ulceration. Following this pilot study, further clinical studies are needed to confirm the validity of these results in 'hard to heal' venous leg ulcers.*

Key Words: Venous leg ulcer; Wound (chronic); Leg ulceration (treatment).

Introduction

Various approaches have been recommended for the treatment of venous ulceration. These include compression therapy, local ulcer treatment, drug therapy and surgery, which are reported in the literature with differing results. Compression bandaging is still the mainstay of venous ulcer treatment, and is frequently used in conjunction with advanced wound dressings[1,2] with 3-month healing rates of between 69 and 74% in patients treated in specialised clinics.[3] The healing rates reported in community treated patients range between 52 and 73%.[4] A variety of prognostic factors also influence the success of compression bandaging, including size and duration of ulcer.[5] Fibrinolytic drugs, intended to treat fibrosis, e.g. stanazolol and pentoxifylline, or drugs which inhibit white cell activation in the microcirculation, e.g. methylxanthines and prostaglandin E1[6,7] are also used to manage venous ulceration. Other pharmacological therapies include zinc, phlebotrophic agents such as hydroxyrutoside and calcium dobesilate, haemorheologic agents such as pentoxifylline and aspirin.[8] Surgical intervention to treat the malfunctioning veins of the lower limb and/or operations involving the ulcer itself, such as skin grafting, have also been recommended.[9,10] In principle, correcting superficial vein insufficiency or perforating vein outflow should be done early. Deep vein reconstruction by stenting, bypass or valvuloplasty should be performed only when all other forms of treatment have failed, because of the major complications associated with these reconstructive procedures. Such complications include deep vein thrombosis (DVT) and pulmonary embolism, as well as ulcer recurrence should the procedures fail.[8] Skin grafting should be reserved for cases in which there is failure to heal after 12 months of properly applied support or compression bandaging, because skin grafting when used as an isolated procedure results in high rates of recurrence.[11]

This study was conducted as part of a PhD thesis.
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The prevalence of venous ulcers in the US is estimated at 500,000–600,000 (i.e. 1.5–2 per 100,000 individuals), increases with age. Estimates of the annual incidence of leg ulcer in the UK and Switzerland are 3.5 and 0.2 per 1000 individuals, respectively.[12] Treatment of venous ulcers can be expensive, leading to a large economic burden on health services in many countries. However, comprehensive studies on the economic costs of venous ulcers are lacking. In a study conducted in the United States, the average total medical cost per patient was $9685 (median: $3036). Home health care, hospitalisations and home dressing changes accounted for 48, 25 and 21% of total costs, respectively.[13] Time absent from work, forced early retirement, loss of functional independence and unquantifiable suffering may be additional factors that contribute to the overall burden of venous ulcers.

Over the past decade, the field of tissue engineering has grown rapidly, and living tissues have become available for cutaneous wound repair and skin replacement. Dermagraft® (Smith and Nephew Plc) is a human fibroblast-derived dermal replacement for wound repair. The origin of the cells is human newborn foreskin.[14,15] It consists of a bio-absorbable three-dimensional scaffold containing growth factors, matrix proteins and glycosaminoglycans, on which fibroblasts are cultured to produce a living, metabolically active dermal tissue.[16] Dermagraft degrades by hydrolysis, leaving the cellular and extracellular components, which act in the wound bed and encourage epithelialisation from the periphery. Dermagraft implants have been clinically studied for over 10 years and like other allogenic dermal implants have not demonstrated rejection.[17] Maintenance of safety, tissue integrity, functionality and viability from product manufacture to end use has been accomplished through innovation in design of both tissue growth and preservation processes. Skin replacement products are the most advanced, and several tissue-engineered care materials have been on the market in the USA and in several other countries for some years. Risks associated with the use of such technologies are low.[18] The dermal layer of skin offers many potential advantages as a therapeutic implant. Fibroblasts do not carry surface antigens (HLA-DR) as do epidermal cells that can result in allograft rejection. Implantation of allogenic dermal tissue does not stimulate an immune response. Kern and colleagues[19] have proposed that interaction of fibroblasts with the fibroblast-derived extracellular matrix is an important modulator of gamma-interferon responsiveness, and that this interaction may play a role in the low immunogenicity of allogeneic fibroblasts grown on scaffolds. This mechanism of action being responsible for the lack of rejection associated with the use of Dermagraft.

Dermagraft was initially introduced for the treatment of chronic wounds, such as diabetic foot ulcers,[20,21] and clinical trials have shown that Dermagraft heals such ulcers more rapidly than conventional therapy alone.[22–28] Although the precise mechanisms by which Dermagraft affects chronic wounds and improves healing are not yet established, a study comparing Dermagraft to saline-moistened gauze reported significantly more chronic ulcers healed in the Dermagraft group than in the control group (71.4% versus 14.3%, $P = 0.003$) at week 12.[29] This study also reported that Dermagraft patients achieved wound closure significantly faster than control patients ($P = 0.004$), and the percent of patients who experienced an infection involving their study wound was also less. These findings were reproduced in a recent study of patients with diabetic foot ulcers of >6 weeks' duration, with 30% (39 of 130) of Dermagraft patients healed compared with 18% (21 of 115) of control patients ($P = 0.023$) after 12 weeks' treatment.[30] The primary objective of treating venous ulceration is to achieve complete healing. Accurate measurement of initial ulcer size and rate of healing is important in the assessment of any treatment regimen. Different methods have been described for the measurement of ulcer size, including measurement of the two maximal perpendicular diameters of the ulcer using a transparent ruler, direct ulcer tracing with digital planimetry and non-invasive, three-dimensional laser imaging.[31–34] Digital planimetry has been shown in clinical studies to be a reliable and valid method for the assessment of wound size and rate of healing.[35,36]

The current pilot study compared the efficacy of Dermagraft when used in combination with four-layer compression bandaging (ProFore®, Smith and Nephew Plc) and four-layer compression bandaging used alone. Using a randomised, prospective, controlled design, the pilot study aimed to confirm whether the addition of Dermagraft to four-layer compression therapy improved venous ulcer healing. Patients presenting with ulcers between 3 and 25 cm^2 and of more than 12 weeks' duration were considered eligible for inclusion. The study also investigated the cutaneous blood flow changes and the histological findings associated with venous leg ulcers treated with Dermagraft.

Patients and Methods

A total of 18 patients were recruited into this single-centre pilot study. The trial protocol was approved by

Leeds General Infirmary's Ethics Committee, and informed consent was obtained from all participants. All patients had chronic venous leg ulcers of more than 12 weeks' duration at the time of presentation, and an ulcer area range of 3–25 cm^2. Venous leg ulcers were selected after clinical examination, duplex finding of venous dysfunction (all patients had evidence of superficial reflux, but no deep venous reflux or evidence of DVT), and by exclusion of other causes of ulceration, especially arterial insufficiency (ankle-brachial pressure index (ABPI) >0.9).

All ulcers entered into the study were clean and the ulcer bed had healthy granulation tissue. Before treatment, ulcers were cleaned with cleansing agents, de-sloughing materials, or occasionally by superficial curettage after application of topical anaesthetics. Protocol-eligible patients were prospectively randomised to treatment (Dermagraft or control) according to a computer-generated code based on the order of admittance to the study.

Patients randomised to the Dermagraft group received Dermagraft at weeks 0, 1, 4 and 8 in addition to four-layer compression bandaging. Control patients were treated with a local non-adherent dressing (Dermanet, Smith and Nephew Plc and four-layer compression bandaging. Four-layer compression bandaging was applied by a trained member of staff. All patients were followed up weekly to 12 weeks on an outpatient basis. Twelve weeks was chosen as the endpoint for the study to mirror other clinical series reported in the literature, and as a period of time in which follow-up was feasible. Complete healing was defined as complete epithelialisation with no exudates drainage.

Assessment of healing

Healing was assessed weekly by means of direct ulcer tracing onto clear plastic sheet and computerised planimetry. Ulcer measurement was performed by a clinician blinded to the treatment group. All ulcers were observed until healed, or until the end of the study at 12 weeks. Healing was calculated by subtracting the final ulcer area from the initial area and dividing by the number of weeks that the patient had been observed to obtain the total area healed per week.

Peri-ulcer cutaneous blood flow measurement

Peri-ulcer skin blood flow (SBF) was measured weekly for 12 weeks by laser Doppler flowmetry, Oxford Array™ (Oxford Optronix, Oxford, England) using the appropriate surface probes. Eight readings from eight different sites were recorded and an average calculated. The first reading at week 0 was considered to represent the initial SBF. The average of the last two readings represented the end-study SBF, regardless of the type of treatment provided.

Histological examination

At the beginning of treatment, an initial 6 mm punch biopsy was taken from each ulcer base. A second biopsy was taken from each ulcer at week 6 if the ulcer had not healed by that time. Biopsies were fixed in formalin, dehydrated and embedded in paraffin wax. Five sections were cut at 4μ and stained with conventional haematoxylin and eosin (H&E). The number of capillaries was counted in the biopsies obtained at weeks 0 and 6. The average number of capillaries was obtained by applying a grid to two different microscopic fields of five H&E stained sections, where two independent investigators counted the capillaries.

Study completion

Patients completed the study if the ulcer had healed, or if they had participated in the study for 12 weeks.

Statistical analysis

The pilot study was not powered for significance due to the constraints of time. The statistical advisor for the study calculated that 132 patients would be needed to achieve significance at the 5% level (personal communication, Mr Mark Airey, University of Leeds). Recruitment of this number of patients was not possible with the constraints on patient numbers in a single-centre study.

Healing outcome was assessed by Fisher's exact test. The percentage reduction in ulcer area was compared between the treatment groups with the Mann–Whitney *U*-test. The total ulcer area rate of healing was compared between treatment groups using a two-sample *t*-test. The total amount of linear healing was calculated for each patient using Gilman's equation[37] as:

$$\frac{\text{Initial area} - \text{final area}}{(\text{Initial perimeter} + \text{final perimeter})/2}$$

This was divided by the number of weeks in the study to give the linear healing per week. Change in SBF and the average number of capillaries (final − initial

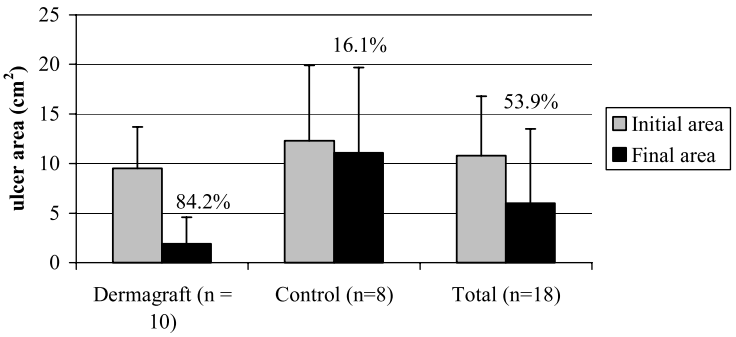


Fig. 1. Mean ulcer area at initial and final assessment.

value) was calculated for each patient and compared between the treatment groups using the Mann–Whitney *U*-test (95% CI).

Results

A total of 18 patients were randomised: 10 were treated with Dermagraft in addition to four-layer compression bandaging and eight with four-layer compression bandaging alone. The patients were well matched at randomisation with respect to demographics and mean ulcer duration (Table 1), there were no significant differences between the two groups.

Five (50%) of the patients treated with Dermagraft and one (12.5%) control patient had healed by the end of the 12-week study. The difference between the two groups was not statistically significant ($P = 0.15$). However, there was a statistically significant difference between the Dermagraft and control groups ($P = 0.002$) with respect to the reduction in ulcer area. Fig. 1 shows the mean ulcer area at initial and final assessment and the percentage reduction in ulcer area. The mean initial ulcer area in the Dermagraft group was 9.5 cm^2 (SD 4.2). The mean initial ulcer area in the control group was 12 cm^2 (SD 7.6). The mean percentage reduction for the Dermagraft-treated patients was 84% (SD 22) and for the control group 16% (SD 43). Fig. 2 shows the mean ulcer area at weeks 0 and 12.

Table 1. Patient demographics

	Dermagraft ($n = 10$)	Control ($n = 8$)
Mean age (y)	58	62
Age range (y)	44–65	54–77
Sex		
Male	6	5
Female	4	3
Mean ulcer duration (weeks)	118.8	120
Ulcer duration (weeks)	12–192	24–288
Mean ulcer area (cm^2)	9.5	12.3
Ulcer area (cm^2)	3.1–17.6	5.4–24.7
ABPI	1.02	1.1

The total ulcer area rate of healing was normally distributed between treatment groups (Table 2). There was a statistically significant difference in the total ulcer rate of healing between Dermagraft and the control groups ($P = 0.001$). The mean total ulcer area rate of healing for Dermagraft was 0.82 cm^2/week (SD 0.33) and for the control group 0.15 cm^2/week (SD 0.39).

The linear rate of healing for both groups is shown in Fig. 3. The mean linear rate of healing for the Dermagraft-treated patients was 0.14 cm/week (SD 0.08), and for control patients 0.033 cm/week (SD 0.085). This difference was statistically significant ($P = 0.006$). As the study was concluded at week 12 no data are available on the maintenance of healing.

Fig. 4 shows the initial and final values for SBF for the two groups. The mean initial SBF for the Dermagraft patients was 125 blood perfusion units (BPU) (SD 40) and for the control patients the mean SBF was 122 BPU (SD 37). Patients treated with Dermagraft had a greater increase (25%) in SBF from initial to final assessment than the control group (8.5%), but this difference between the two groups was not statistically significant ($P = 0.36$). The mean

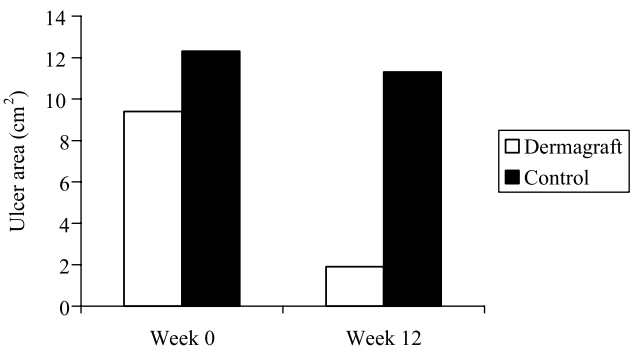


Fig. 2. Mean ulcer area before treatment and at week 12.

Table 2. Total ulcer area rate of healing

Area heal per week (cm^2/week)		Dermagraft ($n = 10$)	Control ($n = 8$)	Total ($n = 18$)
Healed ulcers	Mean	1.01	0.92	1.00
	Median	1.01	0.92	0.97
	SD	0.27	–	0.25
	Minimum	0.750	0.917	0.750
	Maximum	1.420	0.917	1.420
	n	5	1	6
Positively healing (reduced in area)	Mean	0.64	0.18	0.41
	Median	0.64	0.17	0.34
	SD	0.29	0.12	0.32
	Minimum	0.31	0.083	0.083
	Maximum	1.075	0.376	1.075
	n	5	5	10
Non-healing (increased in area)	Mean	–	−0.32	−0.32
	Median	–	−0.32	−0.32
	SD	–	0.003	0.003
	Minimum	–	−0.317	−0.317
	Maximum	–	−0.313	−0.313
	n	0	2	2

increase in SBF for the Dermagraft patients was 25 BPU (SD 43) and the mean increase in SBF for the control group was 6.1 BPU (SD 33).

Capillary count in histological sections did not reveal significant changes in either group. The mean change in the average number of capillaries (final − initial value) for the Dermagraft patients was an increase of 2.9 (SD 5.5) and for the control patients was an increase of 2.1 (SD 3.2)—this was not statistically significant ($P = 0.55$).

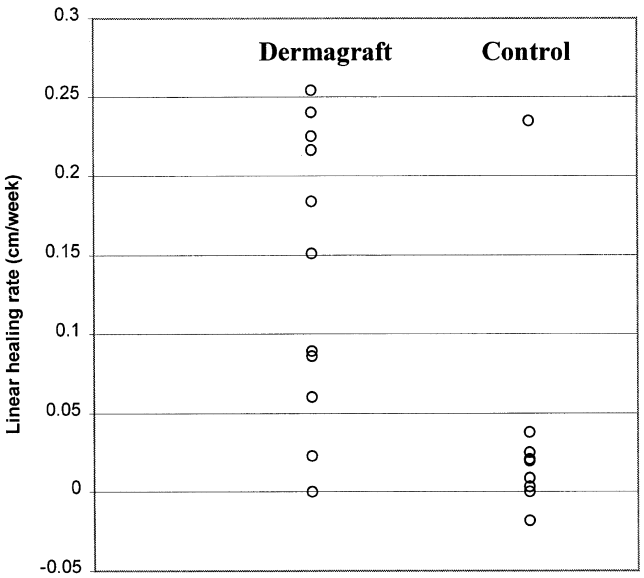
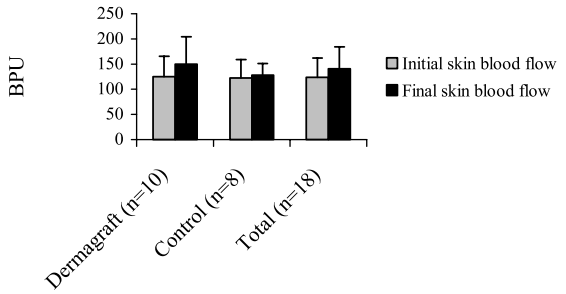


Fig. 3. Linear rate of healing by Gilmans's equation ($D = \Delta A/P$), where D, linear healing; A, change in area; P, mean perimeter of the initial and final ulcers.

Discussion

The healing of venous leg ulcers is a complex process, and different treatment regimens are available to facilitate this process. Tissue engineering offers the possibility of replacing the damaged or destroyed dermis of patients suffering from a deep ulcer with a living human fibroblast-derived dermal implant. In this study, Dermagraft was used as an adjunct to compression therapy. The precise mechanism by which Dermagraft stimulates chronic wounds to heal has not yet been established. Possible mechanisms include providing the wound bed with a metabolically active dermal matrix and/or promoting the expression of important mediators (VEGF, TGF-β, HGF/SF) and thus stimulating angiogenesis and subsequent wound healing.[16,38]

In this study, measurements of both the total ulcer healing rate (which could be influenced by the initial size and shape of the ulcer) and the linear healing of the ulcer edge (incorporating the initial ulcer area and perimeter) were used in order to reflect the *true healing rates* of the ulcers. Despite the limitation of a small sample size, the percentage reduction in ulcer area was significantly higher in the Dermagraft treated patients ($P = 0.002$) during the 12-week study period. The median percentage reduction was 95 and 17%, respectively. The difference between these healing rates could be explained by the initial ulcer size (and shape) in the Dermagraft and control groups, 9.5 and 12 cm^2, respectively. The larger initial ulcer size in the control group may explain this groups' poor results when compared with other published studies,



* Skin blood flow measured by blood perfusion units (BPU)

Fig. 4. Mean peri-ulcer skin blood flow (skin blood flow measured by blood perfusion units (BPU)) at initial and final assessments.

although a Cochrane review reported a wide variability in healing rates (40–84%) with compression bandaging in the treatment of venous leg ulcers.[39] The initial ulcer size and shape can have a great effect on calculating the total area healed over time, where small ulcers may heal more quickly than large ulcers, producing misleading results when comparing rates of healing. However, the linear healing of the wound edge is not affected by the initial ulcer geometry. Linear healing incorporates the ulcer size and perimeter in the calculation, and gives a more accurate representation of the rate of healing of an ulcer. In this study, there was a statistically significant difference between the Dermagraft and control groups ($P = 0.006$) in the linear rate of healing.

Actions of Dermagraft in wound repair include colonisation by cells and provision of growth factors and cytokines, both activities dependent on living cells. Low fibroblast proliferative potential has been reported in venous stasis ulcers.[40] Studies have shown that proliferation can be stimulated by fibroblast growth factor (FGF) and epidermal growth factor to a significant extent in these ulcers.[40] In a study of Dermagraft in the treatment of diabetic foot ulcers, Mansbridge *et al.*[41] found that cells in the cryopreserved culture showed 60% viability by dye exclusion and, when isolated, were able to proliferate in monolayer culture. Protein synthesis by Dermagraft was inhibited 70–98% by cryopreservation, but, if within the therapeutic range, recovered to 45–85% of the prefreeze value over 48 h. More recently, Newton and co-workers[42] found that blood flow at the base of diabetic foot ulcers increased in patients treated with Dermagraft. These data demonstrate the critical dependence of the therapeutic properties of this living dermal implant on recovery of protein synthesis, growth factor expression, and angiogenesis, determined by metabolic activity.[41] Growth factor secretion is a function of live cells, so the ability of live fibroblasts in implants to colonise wound beds is of great importance. Cells derived from a single implantation of Dermagraft were identified by Mansbridge and colleagues[43] in female patients by detection of *SRY* using nested polymerase chain reaction (PCR) were detected up to 6 months (the longest time-point tested), indicating they were able to colonise the wound bed and survive the host environment.

Angiogenesis is an essential part of the usual process of healing.[44] This process requires the migration and proliferation of endothelial cells in order for new functioning blood vessels to form. Growth factors also play a major role in regulating the overall process of angiogenesis.[45] Our results showed a considerable mean increase in peri-ulcer skin perfusion in the Dermagraft group (20%) compared with the control group (4.9%). Dermagraft treated patients also had a greater increase in SBF from the initial to the final assessment, although this difference was not statistically significant ($P = 0.36$). The median increase in SBF was also higher in the Dermagraft group, 22 BPU versus 8.5 BPU in the control arm of the study. This increase in peri-ulcer skin perfusion may reflect an early stimulation of angiogenesis and subsequent blood vessel formation. However, further studies are required to confirm these findings.

The data from this pilot study suggests that Dermagraft may expedite the healing of chronic venous leg ulcers. These initial results may form a basis for future clinical investigations and justify the conduct of a larger study with prolonged follow up to confirm their validity, such a study is currently being planned.

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References

1 Bergan JJ. Overview of potential benefits of direct venous reconstruction. *Int Angiol* 1985; **4**(4):463–466.
2 Hafner J, Bounameaux H, Burg G, Brunner U. Management of venous leg ulcers. *VASA* 1996; **25**(2):161–167.
3 O'Brien JF, Grace PA, Perry IJ *et al.* Randomized clinical trial and economic analysis of four-layer compression bandaging for venous ulcers. *Br J Surg* 2003; **90**(7):794–798.
4 Moody M. Comparison of Rosidal K and SurePress in the treatment of venous leg ulcers. *Br J Nurs* 1999; **8**(6):345–355.
5 Margolis DJ, Berlin JA, Strom BL. Which venous leg ulcers will heal with limb compression bandages? *Am J Med* 2000; **109**(1):15–19.
6 Colgan MP, Dormandy JA, Jones PW *et al.* Oxpentifylline

treatment of venous ulcers of the leg. *BMJ* 1990; **300**(6730): 972–975.
7 Rudofsky G. Intravenous prostaglandin E1 in the treatment of venous ulcers—a double-blind, placebo-controlled trial. *VASA Suppl* 1989; **28**:39–43.
8 Moneta GL, Porter JM. Non-operative treatment of chronic venous insufficiency. In: Rutherford RB, ed. *Vascular Surgery.* Philadelphia: WB Saunders, 2002: 1999–2007. Chapter 146.
9 Shami SK, Sarin S, Cheatle TR, Scurr JH, Smith PD. Venous ulcers and the superficial venous system. *J Vasc Surg* 1993; **17**(3): 487–490.
10 Zimmet SE. Venous leg ulcers: modern evaluation and management. *Dermatol Surg* 1999; **25**(3):236–241.
11 Douglas WS, Simpson NB. Guidelines for the management of chronic venous leg ulceration. Report of a multidisciplinary workshop. British Association of Dermatologists and the Research Unit of the Royal College of Physicians. *Br J Dermatol* 1995; **132**(3):446–452.
12 Simka M, Majewski E. The social and economic burden of venous leg ulcers: focus on the role of micronized purified flavonoid fraction adjuvant therapy. *Am J Clin Dermatol* 2003; **4**(8):573–581.
13 Olin JW, Beusterien KM, Childs MB *et al.* Medical costs of treating venous stasis ulcers: evidence from a retrospective cohort study. *Vasc Med* 1999; **4**(1):1–7.
14 Tanaka M, Nakakita N, Kuroyanagi Y. Allogeneic cultured dermal substitute composed of spongy collagen containing fibroblasts: evaluation in animal test. *J Biomater Sci Polym Ed* 1999; **10**(4):433–453.
15 Sacks MS, Chuong CJ, Petroll WM, Kwan M, Halberstadt C. Collagen fiber architecture of a cultured dermal tissue. *J Biomech Engng* 1997; **119**(1):124–127.
16 Naughton G, Mansbridge J, Gentzkow G. A metabolically active human dermal replacement for the treatment of diabetic foot ulcers. *Artif Organs* 1997; **21**(11):1203–1210.
17 Naughton GK, Applegate D. Cryopreserved dermal implants. In: Atala A, Lanza RP, eds. *Methods of tissue engineering*. New York: Academic Press, 2002: 1157–1176. Chapter 104.
18 Falanga VJ. Tissue engineering in wound repair. *Adv Skin Wound Care* 2000; **13**(2 Suppl):15–19.
19 Kern A, Liu K, Mansbridge J. Modification of fibroblast gamma-interferon responses by extracellular matrix. *J Invest Dermatol* 2001; **117**(1):112–118.
20 Gentzkow GD, Iwasaki SD, Hershon KS *et al.* Use of dermagraft, a cultured human dermis, to treat diabetic foot ulcers. *Diabetes Care* 1996; **19**(4):350–354.
21 Purdue GF. Dermagraft-TC pivotal efficacy and safety study. *J Burn Care Rehabil* 1997; **18**(1 Pt 2):S13–S14.
22 Purdue GF, Hunt JL, Still Jr JM *et al.* A multicenter clinical trial of a biosynthetic skin replacement, Dermagraft-TC, compared with cryopreserved human cadaver skin for temporary coverage of excised burn wounds. *J Burn Care Rehabil* 1997; **18**(1 Pt 1): 52–57.
23 Hansbrough JF, Dore C, Hansbrough WB. Clinical trials of a living dermal tissue replacement placed beneath meshed, split-thickness skin grafts on excised burn wounds. *J Burn Care Rehabil* 1992; **13**(5):519–529.
24 Kantor J, Margolis DJ. A multicentre study of percentage change in venous leg ulcer area as a prognostic index of healing at 24 weeks. *Br J Dermatol* 2000; **142**(5):960–964.
25 Meyer FJ, Burnand KG, Lagattolla NR, Eastham D. Randomized clinical trial comparing the efficacy of two bandaging regimens in the treatment of venous leg ulcers. *Br J Surg* 2002; **89**(1):40–44.
26 Moffatt CJ, Simon DA, Franks PJ *et al.* Randomised trial comparing two four-layer bandage systems in the management of chronic leg ulceration. *Phlebology* 1999; **14**(4):139–142.
27 Hansbrough JF, Mozingo DW, Kealey GP *et al.* Clinical trials of a biosynthetic temporary skin replacement, Dermagraft-Transitional Covering, compared with cryopreserved human cadaver skin for temporary coverage of excised burn wounds. *J Burn Care Rehabil* 1997; **18**(1 Pt 1):43–51.
28 Economou TP, Rosenquist MD, Lewis II RW, Kealey GP. An experimental study to determine the effects of Dermagraft on skin graft viability in the presence of bacterial wound contamination. *J Burn Care Rehabil* 1995; **16**(1):27–30.
29 Hanft JR, Surprenant MS. Healing of chronic foot ulcers in diabetic patients treated with a human fibroblast-derived dermis. *J Foot Ankle Surg* 2002; **41**(5):291–299.
30 Marston WA, Hanft J, Norwood P, Pollak R. The efficacy and safety of Dermagraft in improving the healing of chronic diabetic foot ulcers: results of a prospective randomized trial. *Diabetes Care* 2003; **26**(6):1701–1705.
31 Stacey MC, Burnand KG, Layer GT, Pattison M, Browse NL. Measurement of the healing of venous ulcers. *Aust N Z J Surg* 1991; **61**(11):844–848.
32 Patete PV, Bulgrin JP, Shabani MM, Smith DJ. A non-invasive, three-dimensional, diagnostic laser imaging system for accurate wound analysis. *Physiol Meas* 1996; **17**(2):71–79.
33 Laudanska H, Gustavson B. In-patient treatment of chronic varicose venous ulcers. A randomized trial of cadexomer iodine versus standard dressings. *J Int Med Res* 1988; **16**(6):428–435.
34 Gorin DR, Cordts PR, LaMorte WW, Manzoian JO. The influence of wound geometry on the measurement of wound healing rates in clinical trials. *J Vasc Surg* 1996; **23**(3):524–528.
35 Griffin JW, Tolley EA, Tooms RE, Reyes RA, Clifft JK. A comparison of photographic and transparency-based methods for measuring wound surface area. *Phys Ther* 1993; **73**(2):117–122.
36 Plassmann P. Measuring wounds. *J Wound Care* 1995; **4**(6): 269–272.
37 Gilman TH. Parameter for measurement of wound closure. *Wound* 1990; **2**(3):95–101.
38 Jiang WG, Harding KG. Enhancement of wound tissue expansion and angiogenesis by matrix-embedded fibroblast (dermagraft), a role of hepatocyte growth factor/scatter factor. *Int J Mol Med* 1998; **2**(2):203–210.
39 Cullum N, Nelson EA, Fletcher AW, Sheldon TA. Compression for venous leg ulcers. *Cochrane Database Syst Rev* 2001; (2):CD000265.
40 Stanley AC, Park HY, Phillips TJ, Russakovsky V, Menzoian JO. Reduced growth of dermal fibroblasts from chronic venous ulcers can be stimulated with growth factors. *J Vasc Surg* 1997; **26**(6):994–999. discussion 999–1001.
41 Mansbridge J, Liu K, Patch R, Symons K, Pinney E. Three-dimensional fibroblast culture implant for the treatment of diabetic foot ulcers: metabolic activity and therapeutic range. *Tissue Engng* 1998; **4**(4):403–414.
42 Newton DJ, Khan F, Belch JJ, Mitchell MR, Leese GP. Blood flow changes in diabetic foot ulcers treated with dermal replacement therapy. *J Foot Ankle Surg* 2002; **41**(4):233–237.
43 Mansbridge JN, Liu K, Pinney RE *et al.* Growth factors secreted by fibroblasts: role in healing diabetic foot ulcers. *Diabetes Obes Metab* 1999; **1**(5):265–279.
44 Pettet G, Chaplain MA, McElwain DL, Byrne HM. On the role of angiogenesis in wound healing. *Proc R Soc Lond B Biol Sci* 1996; **263**(1376):1487–1493.
45 Bennett NT, Schultz GS. Growth factors and wound healing: Part II. Role in normal and chronic wound healing. *Am J Surg* 1993; **166**(1):74–81.

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