Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 10, Issue, 05, pp.69854-69858, May, 2018

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

QUANTIFICATION OF NEOVASCULARISATION IN CHRONIC NON HEALING ULCERS AFTER AMNIOTIC MEMBRANE DRESSING VS COLLAGEN SPONGE APPLICATION

[1,*]Datta Prasad, M. and [2]Ananda Rama Rao, B.

[1]Junior Resident (Surgery), SVS Medical College, Mahbubnagar, Telangana
[2]Dean & Professor of Surgery SVS Medical College, Mahbubnagar, Telangana

ARTICLE INFO

Article History:
Received 14th February, 2018
Received in revised form
10th March, 2018
Accepted 09th April, 2018
Published online 31st May, 2018

Key words:

Amniotic membrane,
Collagen sheet,
Chronic non healing ulcers.

***Corresponding author*

ABSTRACT

Collagen sheath and amniotic membrane has unique properties like enhanced wound healing through neovascularisation, anti adhesive effects, bacteriostatic, without inducing immunological reactions. There are no studies to quantify neovascularisation with any agent applied to chronic non healing ulcers. This is a comparative study between collagen sheath and amniotic membrane application. This study is done to evaluate effects of healing in chronic ulcers as evidenced by quantum of neovascularisation by collagen sheath and amniotic membrane application for a period of eight weeks in 30 patients, 15 patients with collagen sheath and 15 patients with amniotic membrane. Collagen sheath is commercially available. Amniotic membrane is to be harvested from placenta taken from caesarean section and applied over ulcers. Patients are evaluated at first, second, fourth, sixth and eighth week. In the first two weeks there is faster rate of neovascularisation as seen by counting the number of capillaries in an average of five high power fields (p-value 0.000), later on there is congestion of vessels with formation of granulation tissue which peaks at fourth week (p- <0.0001), leading to reduction in ulcer size. When compared to collagen sheath amniotic membrane showed significant increase in number of capillary formation per high power field (p- <0.0001). There is significant reduction in ulcer size (p- 0.000) with both collagen sheath and amniotic membrane, inter alia there is no significant difference in decrease in ulcer size between collagen sheath group and amniotic membrane group (p->0.05). The cost effectiveness, readily availability, ease of application makes collagen sheath a better choice for treating chronic ulcers.

Citation: Datta Prasad, M. and Ananda Rama Rao, B. 2018. "Chasing a mirage in Afghanistan", *International Journal of Current Research*, 10, (06), 69854-69858.

INTRODUCTION

Healing of an ulcer involves complex biological process, which needs interactions among different cell types along with growth of new blood vessels into the wound (neovascularisation/angiogenesis), to promote adequate delivery of nutrients and regulatory factors required for tissue remodeling and regeneration. In chronic wounds, there is disruption of normal healing process due to either poor general health or local factors like inadequate blood supply to the wound. Healing of chronic leg ulcers constitute major clinical challenge not only because of high prevalence, refractory nature, impact on quality of life of patients, but also their economic consequences on health care system. Many modalities of wound care have come up to assist a surgeon, to treat chronic wounds. One such development is topical application of biological material i.e. amniotic membrane allograft over the wound and collagen sheath. Amniotic membrane is the innermost layer of placenta, with highly attractive characteristics needed for wound healing. It is a semitransparent membrane which is found to be immunologically inert as it do not express HLA-A, B, C and DR or β2 micro globulin, hence graft rejections are negligible (Dua, 2004; Chen, 2010 and Sakuragawa, 1995). Material used is collagen in sheet form produced from Bovine sources composed mostly Type 1 and Type 3 collagen. Collagen is packed in neutral glass vials containing sterile preservative liquid medium sterilized by Ethylene oxide and available in various sizes (Krishnan, ?). R Krishnan studied role of collagen sheet as a temporary cover for raw area on dogs and concluded that it prevents exogenous infections, promotes rapid epithelization, frequent dressing is not required, lesser time to heal as compared to other conventional dressing (Krishnan, ?). Jeschke G concluded that collagen matrix accelerates epidermal regeneration and locally increases growth factor concentration (Marc, 2005). Snap L et al studied chronic ulcer with collagen sheet to conclude that it encourages early healing with uniformity in granulation (Snap, 2004). Chengcan Yao et al, found that wound healing was enhanced in traumatic ulcers with application of absorbable collagen sponge (Yao, 2006). P Halankar found that there was hematoma beneath the collagen dressing but there was complete re-epithelization beneath the hematoma (Halankar, 2005). Vivekananda B R et al (2014)

have done a research on effectiveness of amniotic membrane dressing and concluded that it was superior to conventional dressing in terms of epithelialization, graft uptake and prevention of infection at ulcer sites (Vivekananda, 2014). Almost all the studies take reduction of wound size as the end point by applying amniotic membrane and collagen sheath. Till date there is no study available in literature to quantify the wound healing in terms of neovascularisation, hence this study comparing amniotic membrane and collagen sheath.

METERIAL AND METHODS

Study design: This comparartiv, controlled study was done in the Department of General Surgery, SVS Medical College and Hospital, Mahbubnagar; on 15 patients with chronic non-healing ulcers in lower limbs with amniotic membrane 15 patients with collagen sheath.

Inclusion criteria

- Patients between 30 to 60yrs of both sexes.
- Chronic non healing ulcers on lower limb due to diabetes , varicose veins , and of non- malignant aetiology
- Ulcers of size 4*4cms or more (i.e. equal to or more than 16cm^2 surface area of the ulcer) with no tendency of healing in past two months despite conventional treatment.

Exclusion criteria

- Age of patient <30yrs and >60yrs
- Patients with deep vein thrombosis
- Significant arterial insufficiency
- Severe neuropathy
- Severe systemic disease
- Renal insufficiency
- Malignant ulcers
- Parasitic ulcers

METHODS

- Informed consent is taken from the patients.
- Detailed history of the patient with chronic non healing ulcer of the leg is taken.
- Debridement of ulcer is done to remove necrotic slough.
- The initial ulcer size is measured at its maximum diameters by using 0.5*0.5cm graph printed over transparent sheath up to one decimal in centimetres.
- Initial biopsy of 0.5cm is taken from floor of ulcer and sent for histopathology in 2% formalin solution. Routine histopathology of the specimen is carried out and under 40x magnification, the number of blood vessels present per field is counted at four corners of the field and centre; and average of five fields is taken.
- Amniotic membrane is obtained from patients undergoing elective Caesarean section. The patient should not be positive for HIV and HBsAg and other STD. The amniotic fluid should be clear. If meconium staining is found such amniotic membrane is not taken for study. Collagen is packed in neutral glass vials containing sterile preservative liquid medium sterilized by Ethylene oxide and available in various sizes. Expose denuded area thoroughly cleaned. Selected right dimension collagen sheet is washed thoroughly in sterile normal saline. Apply local collagen sheet firmly on raw area with no air bubble in between.
- **Extraction of amniotic membrane:** After delivering the baby from uterus, placenta with its covering is taken into a sterile tray. The membranous coverings are cut with scissors. The amniotic membrane is separated from chorion and placed in another sterile tray. The separated amniotic membrane is thoroughly washed with normal saline and such freshly harvested membranes are used for clinical application.

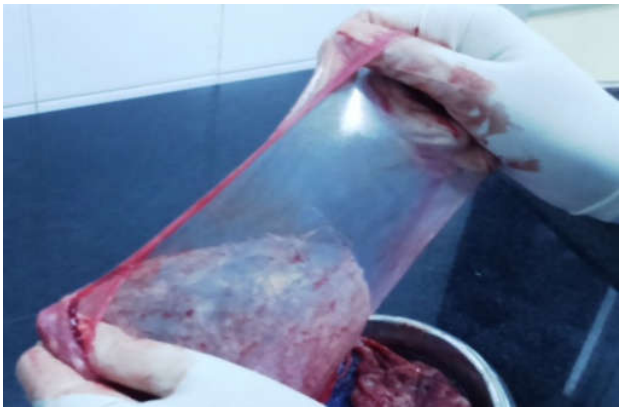


Fig. 1. extraction of amniotic membrane

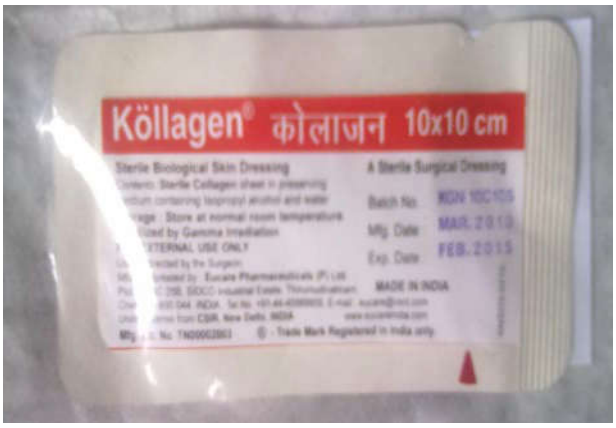
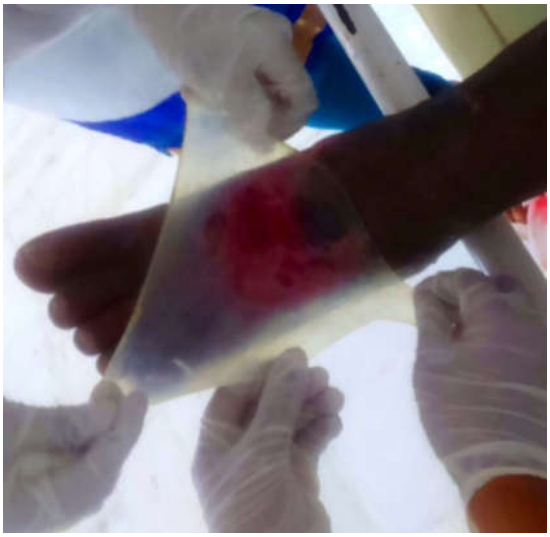


Fig. 2&3. Collagen sheath

- The amniotic membrane and collagen sheath thus obtained is applied on chronic ulcer bed. Wound is inspected at 1st , 2nd, 4th, 6th, and 8th weeks. Healing is measured by taking digital photo with Sony coolpix

camera using 4x magnification from a distance of 20cms.



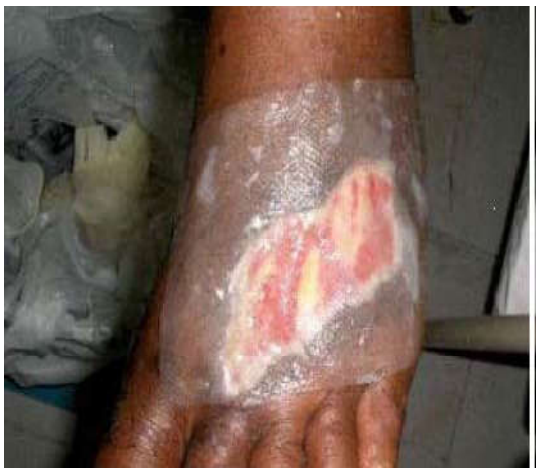


Fig. 4&5. Collagen sheath application on wound

- Biopsy is taken from the wound and subjected to histopathogy examination as done initially during the above mentioned weeks. The number of new blood vessels formed are counted in five fields and average is taken. The vascular congestion and stroma formation are also noted.
- The wound healing status at the end of eight weeks is taken as the end point for this study.
- The results are plotted in the proforma.

RESULTS

Table 1. Comparison of no.Of capillaries per high power field with collagen vs. amniotic membrane

Treatment	Collagen (mean+/- SD)	Amniotic membrane (mean+/- SD)	P value
Day 0	5.40+/-1.35	5.800+/-1.146	0.390
1week	8.53+/-1.73	10.67+/-2.127	0.005
2nd week	11.53+/-1.73	14.20+/-2.242	0.001
4th week	14.00+/-1.65	18.40+/-2.501	<0.0001
6th week	15.87+/-1.51	19.67+/-1.291	<0.0001
8th week	18.27+/-1.49	19.87+/-0.5164	0.001

Comparison of no. Of capillary count with collagen and amniotic membrane showed there is significant increase in capillary count with amniotic membrane with p value <0.05.

Table 2. Comparison of ulcer size progression with amniotic membrane and collagen sheath

Treatment	Collagen (mean+/- SD)	Amniotic membrane (mean+/- SD)	P value
Day 0	32.40+/-17.45	38.58+/-17.20	0.337
1week	30.80+/-17.01	35.92+/-16.00	0.403
2nd week	25.70+/-14.67	31.35+/-15.46	0.3133
4th week	20.27+/-12.48	24.73+/-14.20	0.3681
6th week	16.00+/-11.09	19.00+/-11.72	0.4778
8th week	11.73+/-10.69	14.57+/-10.28	0.466

The comparison of ulcer size progression in two groups from day 0 to 8th week showed that there is no significant difference in decrease in size from day 0 to 8th week. The p value from day 0to 8th week is>0.05 shows that there is no significant difference between these two treatments in terms of ulcer size compared.

DISCUSSION

Wound healing is a complex method to achieve anatomical and functional integrity of disrupted tissue by various components, in an organized staged pathway. It is divided into 3 phases namely inflammatory, proliferative and maturational. The process of formation of new blood vessels called angiogenesis is very essential for wound healing. It begins with mechanical and chemical stimulation (Dua, 2004). Increased muscle contraction leads to release of nitric oxide causing vasodilatation. Chemical stimulators being angiogenic proteins (angiopoietin), angiogenic growth factors like FGF 1, FGF 2, and VEGF etc activate receptors on endothelial cells in pre existing blood vessel, which release proteases that degrade the basement membrane thereby the endothelial cells escape from parent vessel. These cells proliferate in the matrix forming sprouts then loops and become full fledged vessel lumen and get connected to neighbouring vessel. Angiogenesis inhibitor can be endogenous like delta like ligand 4(Dll4) or come from outside. There are different methods of dressing which include, conventional dressing with saline, foams and sprays, films, vacuum assisted dressing, biological dressing with collagen, amniotic membrane etc. Both collagen sheet and amniotic membrane helps in wound healing by increase in capillary count in granulation tissue. Comparison of both the methods in our study showed amniotic membrane is better choice against collagen in terms of increase in capillary count.The amniotic membrane is the innermost layer of placenta and develops from extra embryonic tissue. It consists of fetal and maternal components which are kept together by chorionic villi. The amniotic membrane at ultrastuctural level is a thin, tough, transparent, avascular composite membrane (Hassan, 2008; Chopra and Thomas, 2013 and Bruce Werber, 2013). The amniotic epithelium contains intracellular cytoskeletal filaments like actinin, desmoplakin, cytokeratin, vimentinetc which has role in structural integrity and modulation of shape of cell of healing tissue (Vivekananda, 2014). Amniotic membrane decreases the secretion of pro inflammatory cytokines like TNF-alpha, interferon etc and increase anti inflammatory cytokines IL-10, 4 etc. Various immune cells like T cells, dendritic cells, B cells are actively suppressed[7]. There is induction of vascular endothelial growth factor (VEGF) from the cells of amniotic membrane which results in formation of new blood vessels, thereby increasing granulation tissue and promotes wound healing (Bruce Werber, 2013; Burgos, 1983 and Cheung, 2004).

Material used is collagen in sheet form produced from Bovine sources and is composed of mostly Type 1 and Type 3 collagen. Collagen is packed in Neutral glass vials containing sterile preservative liquid medium sterilized by Ethylene oxide and available in various sizes. Collagen plays a relevant role in cutaneous tissue repair and represents a valid therapeutic option when used as a bioactive advanced dressing in chronic wound management. It improves fibroblast deposition in the dermal matrix and stimulates angiogenesis, granulation tissue formation, and reepithelization (Stern, 1913). In this study we compared both collagen sheet and amniotic membrane angiogenic properties by quantifying the capillary count on biopsy. Here are the results discussed.

Ulcer size

The mean of ulcer size for 15 patients at the start of the study i.e. at day 0 was 37.6cm. Amniotic membrane was applied over the ulcer bed. All the patients were followed for eight weeks. The mean of ulcer size at the end of the study period was 14.6cm. This shows there was 61% reduction in ulcer size by the end of eight weeks. In two patients there was complete healing of the ulcer by the end of study period. The decrease in ulcer size with application of collagen sheath in ulcer showed day 0 the mean ulcer size is32.40+/-17.45 cm. At the end of 8th week the meanof ulcer size is 11.73+/-10.69cm. There is significant decrease in ulcer size at a given point of time when compared to previous week with p value <0.0001. According to study done by Isabelle Mermet et al (2007) on15 chronic venous ulcers of size 5-25cm^2, there was a reduction of 80% of ulcer size by the end of 12 weeks. Complete healing of the ulcer was observed in three patients by the end of the study.[16]
A study done by Charles M Zalen et al on 13 diabetic foot ulcers of size 5-25cm^2, complete healing was seen in 12 patients by 6 weeks period. There was 92% reduction in the size of the ulcer by the end of study period (Zelen, 2013). Omkar Singh study reveals that regarding Collagen Dressing Versus Conventional Dressings in 120 patients with chronic wounds of varied aetiologies and with mean age 43.7yrs, with two weeks of treatment, 60% of the 'collagen group' wounds and only 42% of the 'conventional group' wounds were sterile (P=0.03). Healthy granulation tissue appeared earlier over collagen-dressed wounds than over conventionally treated wounds (P=0.03). After eight weeks, 52 (87%) of 'collagen group' wounds and 48 (80%) of 'conventional group' wounds were >75% healed (P=0.21).

Eight patients in the 'collagen group' and 12 in the 'conventional group' needed partial split-skin grafting (P=0.04). Collagen-treated patients enjoyed early and more subjective mobility. No significant better results in terms of completeness of healing of burn and chronic wounds between collagen dressing and conventional dressing were found (Onkar Singh, 2011). In study done by Harish Rao regarding collagen dressings versus conventional dressings in wound healing of 100 patients with diabetic foot ulcer. In 75 patients collagen dressing was applied, whereas conventional dressing in 25 patients. On enrollment, the median wound size was 33.5 cm2 in collagen dressing group and 48 cm2 in conventional dressing group. Healing time (4.02 ± 0.59 Vs 7.6 ±1.38), duration of antibiotic therapy (15.12±4.55 Vs 24.08± 6.5) and mean follow up period (2.40 ± 0.61 Vs 2.96 ± 1.2) were significantly less in collagen dressing group as compared to conventional dressing group (P<0.001). Collagen dressing is safe and effective in the treatment of foot ulcer and significantly reduces healing time, duration of antibiotic therapy and follow up time (Dr. Harish Rao, 2012).

Rate of neovascularisation

Comparison of neovascularisation with collagen and amniotic membrane showed, amniotic membrane showed significant increase in capillary count. With collagen sheath application mean+/- SD at day0 is 5.40+/-1.35 and is significantly increased to 18.27+/-1.49. With amniotic membrane at day 0 mean+/- SD capillary count is 5.800+/-1.146 and significantly increased to the 19.87+/-0.5164 at the end of 8th week. When compared with collagen sheath amniotic membrane group showed significant increase in capillary count with p value <0.05. The fact of neovascularisation after amniotic membrane graft over ulcer is a known and well studied fact[13, 20]. But there is no study till date which has quantified this fact. This makes the present study unique. In the current study, there was increase in number of capillaries with decrease in size of ulcer from day o to eight week of the study. These two parameters have been chi squared and the p-value is found to be 0.011 which was statistically significant. The correlation of these parameters have been done with r-value being -0.913, which means there was negative relation between these two parameters and the strength being 91.3%.

Formation of granulation tissue

Apart from the blood vessels getting congested, at second week patients started developing granulation tissue or stroma. From fourth week onwards, granulation tissue was found to increase rapidly and by sixth week it reached moderate to high level. By the end of the study of eight weeks, very high amount of granulation tissue was found in all the patients. The development of granulation tissue with time has been chi squared and the result was found to be statistically significant (p-value=0.000). To summarise, the rate of increase in number of capillaries was high in first two weeks which later slowed down and congestion and stroma formation has increased; reached to its peak around fourth week. The formation of granulation tissue along with epithelialisation resulted in significant reduction in size of ulcer from fourth week onwards.

Conclusion

The amniotic membrane and collagen sheath application enhanced wound healing by increasing the number of capillaries in the wound. Rapid neovascularisation is seen in first two weeks of application, followed by slow increase in number of capillaries with congestion around newly formed blood vessels. In comparison with collagen sheath amniotic membrane showed increase in capillary count and stroma formation that in turn helps in wound healing. In terms of neovascularisation amniotic membrane is superior to collagen sheath. This increase in blood vessels enhances formation of stroma and granulation tissue there by leading to reduction of wound size. Other advantages of this membrane include immunologically inert, cost effectiveness, readily available, ease of application and better scar formation.

REFERENCES

Bruce Werber, Erin Martin. 2013. A Prospective Study of 20 Foot and Ankle Wounds Treated with Cryopreserved

Amniotic Membrane and Fluid Allograft. *The Journal of Foot & Ankle Surgery* xxx 1–7.

Burgos H. 1983. Angiogenic and growth factors human amnio-chorion and placenta. *Europ J Clin Invest*., 13:289.

Chen EH, Tofe AJ. 2010. A literature review of the safety and biocompatibility of amnion tissue. *J ImplAdvClin Dent*. 2(3):67–75.

Cheung CY. 2004. Vascular endothelial growth factor activation of intramembranous absorption: a critical pathway for amniotic fluid volume regulation. *J SocGynecolInvestig*., 11:63–74.

Chopra and Thomas, J Biomim. 2013. Amniotic Membrane: A Novel Material for Regeneration and Repair. *Biomater Tissue Eng*., 18:1.

Dr. Harish Rao, Dr. Ashwin Pai, Dr.Irshad Hussein, Dr. Hs Shankar Ram, Dr. Sheila R Pai, and Dr. Shobha Pai. 2012. A Comparative Study between Collagen Dressings and Conventional Dressings in Wound Healing. *Int j collab res on int med and pub health,* 4(5):

Dua HS, Gomes JA., King AJ, Maharajan VS. 2004. The amniotic membrane in ophthalmology. *Survey of Ophthalmology*. (Jan);49(1):51–77.

Halankar, P., D Cunha-Gomes, C. Chaudhari. 2005. Collagen Dressing in the management of donor site of split thickness skin grafts. *Bombar Hospital J.,* 47(2).

Hassan Niknejad, Habibollah Peirovi. 2008. Properties of the Amniotic Membrane for potential use in tissue engineering. European cells and materials;15:88-99.

Isabelle Mermet, Nathalia Pottier. 2007. Use of amniotic membrane transplantation in the treatment of venous leg ulcers.Wound Repair and Regeneration, 15 459-464.

Krishnan R. Role of collagen sheet as temporary cover for raw area an experimental study. Published by Eucarepharma, Private Limited in Kollagen Monograph. Ind J Plast Surg. 14(1).

Marc G. Jeschke, Sandmann G, Schubert T, Klein D. 2005. Effect of oxidized regenerated cellulose/collagen matrix on dermal and epidermal healing and growth factors in an acute wound. *Wound Repair and Regeneration.* 13(3): 324-331.

Onkar Singh, Shilpi Singh Gupta, and Raj Kumar Mathur. 2011. Collagen Dressing Versus Conventional Dressings in Burn and Chronic Wounds: A Retrospective Study. *J CutanAesthet Surg.* Jan-Apr; 4(1): 12–16.

Sakuragawa, N., Tohyama, J., Yamamoto, H. 1995. Immunostaining of human amniotic epithelial cells: possible use as a transgene carrier in gene therapy for inborn errors of metabolism. Cell Transplant, 4: 343-346.

Sheikh, E.S., Sheikh, E.S., Fetterolf, D.E. 2013. Use of dehydrated human amniotic membrane allografts to promote healing in patients with refractory non healing wounds. *Int Wound J*., doi: 10.1111/iwj.12035

Snap L, Donahue K, Falanga V. 2004. Clinical classification of bioengineered skin use and its correlation with healing of diabetic and venous ulcers. *Dermatol Surg.,* 30:1524-32.

Stern M. 1913. The grafting of preserved amniotic membrane to burned and ulcerated surfaces, substituting skin grafts. *JAMA*. 60(13):973-974.

Vivekananda B R, Sachin S Shetty and Gautham J Shetty. 2014. Effectiveness of amniotic membrane dressing versus conventional dressing in non-healing lower limb ulcers. IJBR 05 (05).

Yao CYP, Wu H, Zha Z. 2006. Acceleration of wound healing in traumatic ulcers by absorbable collagen sponge containing recombinant basic fibroblast growth factor. Biomed Materials. 1(1):33

Zelen CM, Serena TE, Denoziere G, Fetterolf DE. 2013. A prospective randomized comparative parallel study of amniotic membrane wound graft in the management of diabetic foot ulcers. *Int Wound J,* 10:502–507.

****