REVIEW ARTICLE

A systematic review on clinical outcomes of human amniotic membrane preparations in the management of venous leg ulcers

K. Lakmal[1], D. Hettiarachchi[1], M. R. N. Cassim[2], A. P. Malalasekera[1]
[1] Department of Anatomy, Faculty of Medicine, University of Colombo, Sri Lanka
[2] Department of Surgery, Faculty of Medicine, University of Colombo, Sri Lanka

Keywords: Human amniotic membrane; venous leg ulcers; chronic ulcers

Abstract

Introduction

Venous leg ulcers are the commonest type of chronic lower leg ulcers worldwide. It impacts the patient's quality of life significantly. Our objective was to assess current evidence on using human amniotic membranes (HAM) in venous leg ulcer management.

Methods

Google Scholar, PubMed, and the Cochrane library were utilized to search the following search terms (MeSH terms in PubMed) in the abstract field or in the title, "Amnion" OR "Placenta" AND "Varicose ulcer "OR "Stasis ulcer" OR "Chronic venous ulceration" in studies published until the 1st of March 2022. We used standard methods to assess the quality of the published articles. The articles thus included were cohort studies (both retrospective and prospective) and randomized control trials.

Results

When the above criteria were used in the search, 12, 8, 15,6, and 4 citations were found in MEDLINE, Cochrane Library, Google Scholar, Embase, and Web of Science respectively. The 15 nonduplicate studies were screened with the inclusion and exclusion criteria, we selected 7 studies for this review. However, the amniotic membrane preparations used in these studies were not uniform. All randomized controlled trials (n=3) have concluded that there is an improvement in healed ulcer percentage at the end of the study in the interventional group when compared to the control group, which was statistically significant ($p<0.05\%$). The percentage of ulcers that had healed at the end study was 60% in interventional groups of the above trials. One prospective study showed that the recurrence rate was less than 30% at a 3-year follow-up examination. We couldn't perform a meta-analysis due to study heterogeneity.

Conclusion

Current scientific evidence indicates that amniotic membrane preparations can be utilized in promoting chronic forms of venous leg ulcers adjunctive to traditional treatment or as second-line therapy.

Correspondence: Dineshani Hettiarachchi
E-mail: dineshani@anat.cmb.ac.lk
https://orcid.org/0000-0002-1732-7339
Received: 25-01-2022 Accepted: 15-07-2022
DOI: http://doi.org/10.4038/sljs.v40i2.8934

Introduction

The entity of venous leg ulcers is becoming a major health challenge over the past years [1]. They have emerged as the commonest type of lower extremity ulcers as it has been shown that 80% of lower extremity ulcers have a venous component [2]. Venous leg ulcers have higher rates of recurrence making them a chronic health problem and refractory to conventional treatments of wound debridement, wound dressing, and compression therapy. It is shown that less than 50% of ulcers achieve healing in 12 weeks with traditional treatment [3]. Even after the correction of aetiology for venous hypertension like saphenofemoral, sapheno-popliteal, and superficial venous insufficiency and other contributing factors like anaemia, cellulitis, and oedema, still, a significant proportion of patients are left with non-healing venous leg ulcers [4,5]. Owing to poor healing, which is usually slow and painful, venous leg ulcers are associated with significant morbidity and poor quality of life [6].

The mainstay of venous leg ulcer management is graduated compression bandaging. Various types of surgical dressings such as collagen products, polymer sponges, hydrogel, hydrocolloids, and membranes, have been used for treating refractory or non-healing ulcers [7]. The Human Amniotic Membrane (HAM) is considered a dressing that is natural or biological and had been used as an allograft in several studies. In past studies in patients with diabetic foot ulcers human amniotic membrane (AM) has shown properties of healing and proven its potential as an allograft [8]. In addition, it has been shown that the human amniotic membrane produces certain well-known wound healing compounds such as epidermal growth factors (FGFs), platelet-derived growth factors (PDGFs), and epidermal growth factor (EGF), and transforming growth factor-beta (TGF-β) [9]. Another mechanism by which the amniotic membrane facilitates wound healing is by accelerating the regenerative process of

damaged tissue by delivering hyaluronan polymers [10].

Biological dressings based on amniotic membranes have been used over the past few decades. Due to their inherent biological properties, they can facilitate the healing of ulcers of different etiologies such as burns, diabetes, neuropathic, and bedsores [11-13]. Amniotic membranes have been processed and manufactured in different ways. Such as cryopreserved, dehydrated, or stem cell extractions to utilize in wound treatment [14]. There are several studies conducted on patients with venous leg ulcers to investigate the efficacy of HAM preparations with or without comparing with standard management.

A previous systematic review has assessed the cost-effectiveness of using HAM preparations. However, this study has not evaluated the clinical outcome of this method [15]. We aimed at assessing the suitability of amniotic membrane preparations as potential grafts for venous leg ulcers to facilitate the healing process in this systematic review through published studies. To the best of our knowledge, this is the first systematic review aimed at assessing the clinical outcomes of amniotic membrane preparations.

Methods

We searched the following databases Google Scholar, PubMed, and the Cochrane library using Mesh terms, "Amnion" OR "Placenta" AND "Varicose ulcer" OR "Stasis ulcer" OR "Chronic venous ulceration". (MeSH terms) in the abstract field or in the title of studies published before 1st March 2022. Additionally, a non-English database named APAMED was also utilized in the search to minimise publication bias. To identify any additional publications that we would have missed we screen the reference lists of the full papers.

Using the Downs and Black checklist, the quality of the studies was assessed. The articles thus included were cohort studies (both retrospective and prospective) and randomized control trials. However, case reports were excluded from the present study. We exclusively selected studies conducted on human subjects and other studies such as those done on animal models or in-vitro studies were excluded. The main aim of the study was to assess the success of HAM preparations in the treatment of venomous leg ulcers as measured by wound healing. The secondary objectives were to assess its safety, and future recurrence rates.

We performed the initial screening for eligibility based on the abstracts and their titles from the electronic databases. Full texts were screened using the inclusion and exclusion criteria. We sought the opinion of the senior investigator in doubtful situations. Two independent reviewers evaluated the study's eligibility to be included. The studies which were included used different HAM preparations namely stem cell extractions, cryopreserved, and dehydrated preparations.

We included the studies which have used different preparations of the amniotic membrane allografts (cryopreserved, dehydrated, and stem cell extractions). Randomized control trials, that compared the amniotic membrane treatment with standard care (multilayer compression therapy) were selected. Those of animal models or in vitro were excluded. Studies that were performed aiming at the analysis of the molecular or chemical factors without measuring the clinical outcome of the HAM allograft treatment were excluded (figure 1).

From the studies included in the review following data were extracted: Study setting, year, trial designs (study designs), characteristics of the participants, details of the amniotic membrane preparations, outcome measures, and statistical significance of the results. Outcome measures were the healing percentage, healing time, adverse outcomes, and recurrence during the follow-up.

Results

From the respective databases, we identified the following number of articles when searched with the search terms: Google scholar (n=15), PubMed (n=12), Cochrane (n=8),

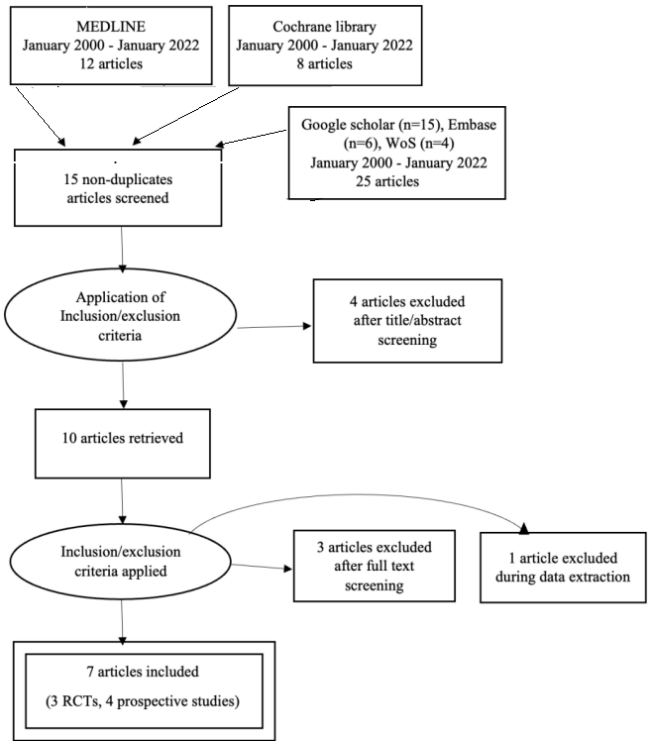

Figure 1. Prisma flow chart

Embase(n=6), and Web of Science(n=4). After removing the duplicates and the applications of the inclusion and exclusion criteria, we included a total of seven articles in the present study. A summary of the search strategy is depicted in figure 1. The studies included here were all conducted after the year 2000. We found 3 randomized control trials [16,17,18] and all were multicenter trials conducted in the United States. Out of 4 prospective studies, 2 were done in India [19,20] and one each in France [21] and Spain [22].

The three randomized controlled trials (RCTs) had a total of 169 and 152 participants in the interventional and control groups respectively. A total of 155 patients were treated with HAM preparations in the prospective studies. The mean ages of the participants in both groups were 60.9 years and 60.6 years in the interventional group and the control group respectively. Whereas in the prospective studies the mean age was 48.9 years. Ulcer location was the gaiter area in many participants, as it is a typical location of venous leg ulcers. Mean ulcer duration was more than 10 months in the RCTs and more than 12 weeks in prospective studies. In RCTs patients with ulcer size, more than 5cm2 were in both interventional and control groups. Notably, participants in one prospective study [20] had a larger baseline ulcer area (more than 16cm2). Only in one study surgery has been done for varicose veins before the treatment with amniotic membrane preparations. (Table-1).

In the above studies, different preparations of HAM had been used such as EpiFix (commercially available preparation, manufactured by MIMEDX) [16,17], dehydrated amnion/chorion membrane (dHAM) [18,22], and cryopreserved amniotic membrane (prepared at the institutes) [21]. The frequency of assessment of the patients was weekly in majority of the studies. Duration of treatment and follow-up periods were also different in the studies. In all RCTs, investigators have spent initial 2 weeks for screening. All RCTs have concluded with statistically significant ($p<0.05\%$) improvement in healed ulcer percentage at the end of the study in the interventional group compared to the control group. In the RCT interventional groups, the healed percentage of ulcers was 60%. In one RCT [17] a notable reduction in the baseline surface area of the ulcers in patients treated with HAM was observed. A prospective study conducted by Francis et al [19] demonstrated less than a 30% of recurrence rate of venous leg ulcers during a 3-year follow-up period. Furthermore, Hanumanthappa et al [20] in their prospective comparative study have shown that 80% of ulcers achieved epithelialization at 3 weeks with HAM dressing and it was statistically significant. ($p<0.005$). The results of each study are summarized in table 2.

Discussion

This review was focused on evaluating the current literature and scientific evidence on the effectiveness of the use of HAM in the management of venous leg ulcers. All studies that used HAM preparations on venous ulcers that had not seen significant improvement on conventional therapy or had recurred after conventional therapy were assessed. HAM was not a popular first-line mode of treatment. It remains an experimental therapy for venous ulcers that had failed to achieve re-epithelialization with conventional therapy such as compressive bandage and wound debridement. We have included altogether 6 studies in this review. There was a notably higher rate of wound closure compared to conventional treatment observed in all randomized controlled trials. Adverse effects attributable to HAM products were not observed in the 3 studies which included adverse outcomes. This indicates amniotic membrane treatment has a good safety profile.

All the RCTs were conducted in the United States. Different preparations had been used for treatment. Data was limited to assess the efficacy of the different preparations. Except study done by Francis et al, in other studies, an adjunct compressive bandage has been used. Long-term recurrence after therapy had been assessed in only one study (less than 30% in 3 years) [19]. Previous epidemiological studies imply the recurrence rate is 26-70% [23]. With these previous studies, amniotic membrane therapy has a comparatively lower recurrence rate. More studies are required to support the evidence of HAM as a potential allograft in venous leg ulcer treatment. Despite these drawbacks, we were able to do qualitative analysis. We couldn't perform a metanalysis owing to study heterogeneity. Some factors that influenced the heterogeneity of the studies included clinical diversity, duration, variability of the study design, and different amniotic membrane preparations. Due to the variability of the methods employed to assess the outcomes of the intervention and publication bias was not assessed due to the availability of a small number of studies for comparison.

The use of HAM as a biological dressing or allograft is more expensive than the conventional treatment methods. It has been applied weekly in most of the studies. But when compared with the biocompatible skin graft which has been used for refractory venous ulcers, HAM is relatively cost-effective [15]. A study done by Hanumanthappa et al [20] has described a cost-effective method of harvesting amniotic membrane from the placenta during cesarean section, preservation, and application methods. In comparison with the results of other biological allografts amniotic membrane preparation has been shown higher healing rate in the study done by Bianchi et al [17], (Epifix 60% compared with the

Table 1. Study group characteristics

Author	Year	Location (setting)	Study type	Size of the study	Mean age in (years)/SD	Ulcer Location/s	Mean ulcer duration (weeks)/SD	Median Ulcer duration (weeks)/ (range)	Mean baseline ulcer size(cm^2)/SD
Bianchi et al[16]	2019	Multicenter USA	RCT	128 (I=64, C=64)	I=62.2(14.3) C=60.3(11.4)	Medial, anterior, lateral around malleolus, low gaiter	I= 40.0(55.6) C=61.5(71.6)	I=20(4-312) C=39(4-384)	I=7.4(5.8) C=8.6(6.8)
Marinello et al[22]	2018	Single-center Spain	Prospective study	10	76.1 (15.4)	Leg, ankle	52.8	105.2	21.75(18.17)
Bianchi et al[17]	2017	Multicenter USA	RCT	109 (I=52, C=57)	I=61.5(14.9) C=60.0(10.6)	Medial, anterior, lateral around malleolus, low gaiter	I=41.9(60) C=58.9(72.6)	I=17.5(4-312) C=35(4-384)	I=7.6(6.1) C=8.3(6.7)
Serena et al[18]	2014	Multicenter USA	RCT	84 (I=53, C=31)	I=59(17.75) C=62.6(13.53)	Gaiter area	I=55.2(83.2) C=52(65.6)	I=16 C=22	I=6.0(4.33) C=6.3(5.27)
Francis et at[19]	2013	India	Prospective study	40	45	Leg, ankle, and foot	Mean ulcer duration not mentioned 12-24 weeks- 8 >24 weeks -32	NA	Mean ulcer size not mentioned 3-5cm^2 -14 >5cm^2-26
Hanumanthappa et al[20]	2012	Mangalore India	Prospective comparative study	200 (I=100, C=100)	I=46.5 C=45.5	Leg, ankle	I=18 C=20	Range I=12-24 C=12-28	>16
Merment et al[21]	2007	Besancon, France	Prospective study	15	79	Ankle, gaiter area	NA	>12 weeks	4.59(2.49)

SD-Standard deviation; RCT-Randomized control trial; I-intervention group; C- control group; USA-United States of America NA- Not Available

Table 2. Outcomes and Interventions of the studies

Author	Year	Type of study	Intervention and size of the group	Frequency of evaluation	Study duration	Ulcer area reduction	Healed percentage	Recurrences	Adverse outcomes. (Amniotic membrane product related)
Bianchi et al[16]	2019	RCT	EpiFix-64 SOC-64	Weekly	16 weeks (2-week screening,12-week treatment, 2 week follow-up)	NA	Healed at 12 weeks – I=50%, C=31% (p=0.0473) Healed at 16 weeks – I=59%, C=39%(p=0.0335)	NA	NA
Marinello et al[22]	2018	Prospective study	dHACM-10	Weekly	8 weeks	80% mean reduction	Complete healing percentage -66.7%	NA	Increased exudate in 4 patients
Bianchi et al[17]	2017	RCT	EpiFix-52 SOC-57	Weekly	16 weeks (2-week screening,12-week treatment, 2-week follow-up)	At 12 weeks – p=0.0435 At 16 weeks- p=0.0098	Healed at 12 weeks – I=60%, C=35% (p=0.0128) Healed at 16 weeks – I=71%, C=44%(p=0.00625)	NA	Not observed
Serena et al[18]	2014	RCT	dHACM-53 SOC-31	Weekly	6weeks (2-week screening, 4-week treatment phase,)	NA	At 4 weeks- I=62% and C=32% showed greater than 40% closure. (p=0.005)	NA	Not observed
Francis et al[19]	2013	Prospective study	Amnion transfer-40	Follow up at 10,30 1st 90 days. Long-term follow-up at 1 and 3 years	Up to 3 years of follow up	At 30 days a)>75% area reduction in 47.5% b)50-75% area reduction in 32.5%	NA	Less than 30% recurrences 3 year follow up	NA
Hanumanthappa et al[20]	2012	Prospective comparative study	Amniotic membrane dressing-100 SOC-100	Weekly	3 weeks	NA	I=81% and C=40% showed epithelialization (p<0.005)	NA	NA
Merment et al[21]	2007	Prospective study	Cryopreserved AM graft-15	Weekly	3 months follow up	Granulation tissue 17% on day 0 to 69% on day14 Decrease of the fibrinous slough from 36% on day o to 16% on day 14 Mean ulcer area from 4.59cm^2 to 2.91cm^2 on day 30	At 3 months - 80% healed	NA	Not observed

RCT- Randomized Control Trials; SOC-Standard of Care; NA-Not Available; I-intervention group; C- Control group; dHACM – dehydrated Human Amnion/Chorionic Membrane; AM- Amniotic membrane.

Apligraf-31% [24] and Dermograf-38% [25]). With this background, it is suitable as second-line treatment or as an adjunct in the management of venous ulcers.

A study done in 2018 showed that healing of leg ulcers was faster if early endovenous ablation of superficial venous reflux was achieved [26]. Current literature indicates similar ulcer healing rates between superficial venous surgery and the use of compression. However, with a lesser recurrence rate [27]. Therefore, the effect of superficial venous surgery on ulcer healing cannot be disregarded in the current study. Most of the studies included in this review have been done before 2018 and only one study mentioned that the patients in their study had undergone surgery for varicose veins before the treatment with amniotic membrane allografts [19]. Two studies [20,21] mentioned that the patients in the study had not received surgical treatment for varicose veins.

Limitations

One of the main drawbacks was that the study methods weren't uniform and there was heterogeneity among them. As a result, the differences in outcomes could be due to the study methods and cannot be solely attributed to the use of HAM. Another drawback was the smaller number of RCTs.

Conclusion

Data available in the literature is limited at the moment. Available scientific evidence indicates that amniotic membrane preparations can be utilized to promote the healing of chronic venous leg ulcers as an adjunct to traditional treatment or as second-line therapy. Further studies which compare standard therapy with HAM are needed. These studies need to be conducted on a larger cohort.

All authors disclose no conflict of interest. The study was conducted in accordance with the ethical standards of the relevant institutional or national ethics committee and the Helsinki Declaration of 1975, as revised in 2000.

References

1. Zenilman J, Valle MF, Malas MB, Maruthur N, Qazi U, Suh Y, et al. Chronic venous ulcers: a comparative effectiveness review of treatment modalities. [Internet]. Rockville, MD:Agency for Healthcare Research and Quality (US); 2013 Dec;2013.
2. O'Meara S, Al-Kurdi D, Ovington LG. Antibiotics and antiseptics for venous leg ulcers. Cochrane Database Syst Rev 2008; (1): CD003557.
3. McLafferty RB. Venous leg ulcers. In: Mowatt-Larssen E, Desai SS, Dua A, Shortell CEK, eds. Phlebology, Vein Surgery and Ultrasonography: Diagnosis and Management of Venous Disease. Cham, Switzerland: Springer;2014:341-355.
4. Cornwall JV, Doré CJ, Lewis JD (1986) Leg ulcers: epidemiology and aetiology. Br J Surg 73:693–696
5. Baker SR, Stacey MC, Singh G, Hoskin SE, Thompson PJ (1992) Aetiology of chronic leg ulcers. Eur J Vasc Surg 6:245–251
6. Persoon A, Heinen MM, van der Vleuten CJ, de Rooij MJ, van de Kerkhof PC, van Achterberg T. Leg ulcers: a review of their impact on daily life. J Clin Nurs 2004; 13: 341–54.
7. Patel NP, Labropoulos N, Pappas PJ (2006) Current management of venous ulceration. Plast Reconstr Surg 117:254S–260S
8. Thompson, Patricia, et al. "Comparing human amniotic allograft and standard wound care when using total contact casting in the treatment of patients with diabetic foot ulcers." Advances in skin & wound care 32.6 (2019): 272-277
9. Parolini O, Solomon A, Evangelista M, Soncini M. Human term placenta as a therapeutic agent: from the first clinical applications to future perspectives. In: Berven E, editor. Human placenta: structure and development. Hauppauge, NewYork: Nova Science Publishers, 2010: 1–48.
10. Jiang D, Liang J and Noble PW: Hyaluronan in tissue injury and repair. Ann Rev Cell Dev Biol 23: 435-461, 2007.
11. Torrati FG, Rossi GA, Ferreira E et al (2000) Analysis of cost of dressings in the care of burns patients. Burns 26:289–293
12. Shun A, Ramsey-Stewart G (1983) Human amnion in the treatment of chronic ulceration of the leg. Med J Aust 2:279–283
13. Quinby WC, Hoover HC, Scheflan M, Walters PT, Slavin SA, Bondoc CC (1982) Clinical trials of amniotic membranes in burn wound care. Plast Reconstr Surg 70:711–716
14. Su, Ya-Na, et al. "Human amniotic membrane allograft, a novel treatment for chronic diabetic foot ulcers: A systematic review and meta-analysis of randomized controlled trials." International Wound Journal 17.3 (2020): 753-764.
15. Gutiérrez-Moreno S, Alsina-Gibert M, Sampietro-Colom L, Pedregosa-Fauste S, Ayala-Blanco P. Estudio Cost-benefit analysis of amniotic membrane transplantation for venous ulcers of the legs that are refractory to conventional treatment. Actas Dermosifiliogr. 2011 May;102(4):284-8. Spanish. doi: 10.1016/j.ad.2011.01.003. Epub 2011 Mar 25. PMID: 21440240
16. Bianchi C, Tettelbach W, Istwan N, Hubbs B, Kot K, Harris S, Fetterolf D. Variations in study outcomes relative to intention-to-treat and per-protocol data analysis techniques in the evaluation of efficacy for treatment of venous leg ulcers with dehydrated human amnion/chorion membrane allograft. Int Wound J. 2019 Jun;16(3):761-767. doi: 10.1111/iwj.13094. Epub 2019 Mar 12. PubMed PMID: 30864259; PubMed Central PMCID: PMC6850648.
17. Bianchi C, Cazzell S, Vayser D, Reyzelman AM, Dosluoglu H, Tovmassian G; EpiFix VLU Study Group. A multicentre randomised controlled trial evaluating the efficacy of dehydrated human amnion/chorion membrane (EpiFix(®)) allograft for the treatment of venous leg ulcers. Int Wound J. 2018 Feb;15(1):114-122. doi: 10.1111/iwj.12843. Epub 2017 Oct 11.PubMed PMID: 29024419.
18. Serena TE, Carter MJ, Le LT, Sabo MJ, DiMarco DT; EpiFix VLU Study Group. A multicenter, randomized, controlled clinical trial evaluating the use of dehydrated human amnion/chorion membrane allografts and multilayer compression therapy vs. multilayer compression therapy alone in the treatment of venous leg ulcers. Wound Repair Regen. 2014

Nov-Dec;22(6):688-93.
doi: 10.1111/wrr.12227. Epub 2015 Jan 8. PubMed PMID: 25224019.

19. Francis J, Shajimon CR, Bhat AK, Kanakambaran B. Use of Amnion Transfer in Resistant Nonhealing Venous Leg Ulcers. Indian J Surg. 2015;77(Suppl 2):457-462.
doi:10.1007/s12262-013-0875-7

20. Hanumanthappa M.B, Gopinathan S, Guruprasad Rai D. Amniotic membrane dressing versus conventional dressing in the lower limb varicose ulcer. International Journal of biological and medical research.2012;3(2):1616-1620

21. Mermet I, Pottier N, Sainthillier JM, Malugani C, Cairey-Remonnay S, Maddens S, Riethmuller D, Tiberghien P, Humbert P, Aubin F. Use of amniotic membrane transplantation in the treatment of venous leg ulcers. Wound Repair Regen. 2007 Jul-Aug;15(4):459-64.
doi: 10.1111/j.1524-475X.2007.00252.x. PMID: 17650088

22. Marinello J, S.-P. M., Albuxeich C, Carreño P, Lopez-Palencia J,Palomera E,Alos J (2018).Safety and Efficacy of Amniotic Membrane Implant in Venous Leg Ulcers. Journal of Vascular Medicine & Surgery. 2018 6(5).
doi: 10.4172/2329-6925.1000373

23. Mościcka P, Szewczyk MT, Cwajda-Białasik J, Jawień A. The role of compression therapy in the treatment of venous leg ulcers. Adv Clin Exp Med. 2019;28(6):847-852

24. Marston WA, Sabolinski ML, Parsons NB, Kirsner RS. Comparative effectiveness of a bilayered living cellular construct and a porcine collagen wound dressing in the treatment of venous leg ulcers. Wound Repair Regen 2014;22:334–40. https://doi.org/10.1111/wrr.12156.

25. Krishnamoorthy L,Harding K, Griffiths D, MooreK, Leaper D, Poskitt K, Sibbald RG, Brassard A, Dolynchuk K, Adams J, Whyman M. The clinical and histological effects of Dermagraft® in the healing of chronic venous leg ulcers. Phlebology 2003;18(1):12–22.
https://doi.org/10.1258/026835503321236858.

26. Gohel MS, Heatley F, Liu X, et al. A Randomized Trial of Early Endovenous Ablation in Venous Ulceration. N Engl J Med. 2018;378(22):2105-2114. doi:10.1056/NEJMoa1801214

27. Ren, Shi-Yan et al. "Strategies and challenges in the treatment of chronic venous leg ulcers." World journal of clinical cases vol. 8,21 (2020): 5070-5085.
doi:10.12998/wjcc.v8.i21.5070