Title: A RCT Evaluating Efficacy of Type-I Collagen Skin Substitute vs. Human Amnion Membrane in Treatment of Venous Leg Ulcers

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Introduction:

Venous leg ulcers (VLUs) are the most common type of chronic lower extremity ulcerations, accounting for 60-80% of all leg ulcers [1]. They affect approximately 0.18% to 1.35% of the general population and impose a substantial economic burden on healthcare systems worldwide [2]. Chronic venous insufficiency leads to sustained hypertension in the venous system, resulting in capillary damage and impaired healing [3]. These ulcers present a significant public health challenge, leading to impaired quality of life, substantial healthcare expenditures, and recurrence rates exceeding 70% [4]. Standard treatments include compression therapy and conventional wound dressings; however, healing rates remain suboptimal, often resulting in prolonged healing times and high recurrence rates [5]

The pathophysiology of venous leg ulcers involves complex interactions between venous hypertension, inflammatory processes, and impaired cellular function within the wound environment. [6] Chronic venous insufficiency leads to increased hydrostatic pressure, capillary leakage, and tissue hypoxia, creating a hostile microenvironment that impedes normal wound healing processes. [7, 8]. The presence of elevated matrix metalloproteinases, chronic inflammation, and bacterial colonization further contributes to delayed healing and wound chronicity. [9]

Wound healing is a complex biological process involving phases of inflammation, proliferation, and remodelling. Traditional wound care often fails to adequately stimulate this cascade in chronic wounds, necessitating the use of advanced biologic dressings. [10]

Recent advances in wound care have led to the development of bioengineered skin substitutes designed to address the underlying molecular and cellular deficits in chronic wounds. [11] Advanced wound care options such as collagen-based dressings and allografts have been developed to enhance the wound healing microenvironment. Among these, Type-I collagen-based skin substitutes (HPTC) (such as Helicoll®) and dehydrated human amnion/chorion membrane (dHACM) have gained prominence [12-15].

HPTC is a high purity Type-I collagen matrix derived from bovine tendon and processed to preserve native triple-helix structure, providing a biocompatible scaffold for cellular ingrowth and promote angiogenesis [16]. dHACM comprises preserved amniotic tissue layers, rich in cytokines and growth factors, and extracellular matrix components that theoretically promote wound healing through regenerative mechanisms. Clinical studies have demonstrated the efficacy of dHCAM in various chronic wound types [17].

Recent systematic reviews indicate that bioengineered skin substitutes may improve healing outcomes in venous leg ulcers, with healing probabilities ranging from 0.11 to 0.65 over variable time periods. Clinical trials with dHACM have demonstrated healing rates of 75% at 12 weeks in venous leg ulcers [18-20].

Prior studies in diabetic foot ulcers have reported promising results using HPTC compared to dHACM [21, 22]. While numerous studies have evaluated treatments individually, direct comparative studies evaluating their relative efficacy in venous leg ulcer management is null. The present study aims to bridge this gap by conducting a randomized, controlled clinical trial comparing the efficacy of HPTC and dHACM in terms of healing efficacy and patient-reported outcomes.

Materials and Methods:

This single-centre, prospective, randomized, open label, single-blinded, controlled, parallel-group clinical trial was conducted at a tertiary care centre over a 6-month period. The study protocol was reviewed and approved by the Adichunchanagiri Institute of Medical Sciences Institutional Ethics Committee with approval number AIMS/IEC/004/2025 and conducted in accordance with the Declaration of Helsinki. All participants provided written informed consent before enrolment. Additionally, all study products used in this study were manufactured, handled, and stored in accordance with applicable Good Manufacturing Practices. The study was registered in Clinical Trials.gov with identity number NCT06831760. Confidentiality was maintained with all patient records.

Sixty patients aged 18–80 years with non-infected chronic venous ulcers of more than 4 weeks' duration, measuring between 2 cm^2 and 25 cm^2, with adequate arterial inflow (Ankle Brachial Index > 0.8) and willingness to comply with follow-up were included. Exclusion criteria included active infection requiring systemic antibiotics, uncontrolled diabetes, peripheral arterial disease, autoimmune disorders, malignancy, immunosuppression, pregnancy and known allergies to bovine or human-derived products. Patients were randomly allocated into two equal groups using a computer-generated sequence:

- Group A: Treated with High Purity Type-1 collagen-based skin substitute (HPTC) (n=30)
- Group B: Treated with dHACM (n=30)

Intervention: Application protocols followed manufacturer instructions. Wounds were debrided before initial grafting. All patients received standard wound cleaning and compression therapy. In addition:

- Group A received application of HPTC, a high-purity Type-I collagen dressing. The product was sized to cover the entire wound surface with a 1-cm margin and secured with appropriate secondary dressings
- Group B received dHACM graft. The membrane was rehydrated and applied to cover the wound bed completely
- Standard Wound Care for Both Groups - First layer of a non-adherent and porous paraffin gauze followed by Second layer of absorbent gauze pads and then third layer of a soft roll and crepe bandage.

Infection control measures were taken and patient education regarding wound care and leg elevation were given. Repeat application was done if deemed necessary.

Outcome Measures:

Primary Outcome Measure:

i. Percentage wound area reduction - percentage wound area reduction from week 1 through week 6 plus 1 week follow up measured manually with digital photography

Secondary Outcome Measure:

i. **Infiltration of vascularity in the ulcer bed -** Infiltration of vascularity in the ulcer bed on day 5 of application. Vascularity assessment will be done using biopsy on Day 0 of application to be compared with Day 5 after application of Type-I Collagen-based Skin Substitute or Human Amnion/Chorion Membrane. [Time Frame: 6 days]. Histopathological Assessment – Before application and on the 5th day post-application of either HPTC or dHCAM, a 2mm punch biopsy was obtained from the wound edge extending into the wound bed under local anaesthesia (2% lidocaine without epinephrine). Biopsy samples were immediately fixed in 10% neutral buffered formalin for 24 hours, processed through graded alcohols, and embedded in paraffin blocks. Serial sections of 4µm thickness were prepared and stained with:
    i. Hematoxylin and Eosin (H&E) for general morphology
    ii. Masson's Trichrome for collagen assessment
    iii. CD31 immunohistochemistry for capillary density evaluation
    iv. α-SMA immunohistochemistry for fibroblast activity

Histological Parameters Evaluated

1. Vascular Infiltration: Assessed by counting new blood vessels per High Power Field (hpf) **(0-3 scale)**
    - 0: Minimal vascular ingrowth (<5 vessels/hpf)
    - 1: Mild infiltration (5-10 vessels/hpf)
    - 2: Moderate infiltration (11-20 vessels/hpf)
    - 3: Abundant infiltration (>20 vessels/hpf)
2. Neo-epithelialization: Measured as epithelial migration distance from wound edge **(0-3 scale)**
    - 0: No epithelial migration
    - 1: Minimal migration (<25% wound coverage)
    - 2: Moderate migration (25-75% coverage)
    - 3: Extensive migration (>75% coverage)

3. Fibroblast Activity: Quantified by counting α-SMA positive fibroblasts per HPF and assessment of fibroblast morphology **(0-3 scale)**
    - 0: Sparse, inactive fibroblasts
    - 1: Moderate cellularity, minimal matrix production
    - 2: High cellularity, active-matrix synthesis
    - 3: Very high activity with extensive matrix deposition
4. Capillary Density: Evaluated using CD31 staining, counted as vessels per mm^2 of tissue
5. Inflammatory Response: Graded semi-quantitatively **(0-3 scale)**
    - 0: Minimal inflammatory infiltrate
    - 1: Mild chronic inflammation
    - 2: Moderate mixed inflammation
    - 3: Severe acute inflammation
6. Collagen Deposition: Assessed using Masson's Trichrome staining **(0-3 scale)**
    - 0: Minimal collagen matrix
    - 1: Loose, immature collagen
    - 2: Moderate organized collagen
    - 3: Dense, mature collagen architecture

All histological assessments were performed by two independent pathologists blinded to treatment allocation. Inter-observer agreement was assessed using Cohen's kappa coefficient.

ii. Time to achieve complete wound closure - the time to achieve complete wound closure (defined as 100% epithelialization with no drainage) of the target ulcer by the end of 6 weeks [Time Frame: 6 weeks]

iii. Proportion of subjects to obtain complete closure - the proportion of subjects that obtain complete closure over the 6-week treatment period [Time Frame: 6 Weeks]

iv. Mean number of repeated application - mean number of repeated applications of the Advanced Skin Substitute & Human Amnion/Chorion Membrane used to obtain wound closure [Time Frame: 6 Weeks]

v. Incidence of adverse events - incidence of adverse events related to the intervention (e.g., infection, allergic reactions) [Time Frame: 6 Weeks]

Other Pre-specified Outcome Measures:

i. Change in Pain - change in pain measured by a Visual Analog Scale with score range from 0 to 10, wherein 0="no pain" to 10="severe pain" [Time Frame: 7 weeks including 1-week follow-up]. Pain scores were assessed weekly.

ii. Change in quality of life - change in quality of life assessed using the Wound-QoL questionnaire measured as 'not at all', 'a little', 'moderately', 'quite a lot' and 'very much' for 17 questions [Time Frame: 7 weeks including 1-week follow-up]

iii. Healed Wound appearance assessment using Manchester Scar Scale - the resultant new skin is assessed and documented at each visit using the Manchester Scar Scale assessing colour, finish, contour, distortion & texture with values given from 1 to 4 wherein 1 denotes excellent and 4 means poor [Time Frame: 7 weeks including 1-week follow-up]

Data Collection and Monitoring

a. Baseline data: Demographics, medical history, ulcer characteristics.
b. Weekly follow-ups: Wound measurements (photographic documentation), assess healing progress, pain scores, record adverse events and patient-reported outcomes.
c. Histopathological specimens were obtained at baseline and day 5 for vascularity assessment.
d. End-of-Study Visit (Week 6): final wound assessment, collect patient feedback on treatment experience, quality of life and scar assessment.
e. Data entry and monitoring was managed using an electronic data capture system.