Title: Multicentric RCT Comparing High Purity Type I Collagen-Skin Substitute vs dHACM in Treatment of Diabetic Foot Ulcers

NCT No: NCT07046403

Date: November 09, 2025

INTRODUCTION

Diabetic foot ulcers (DFUs) represent one of the most serious complications of diabetes mellitus, affecting approximately 15-25% of diabetic patients during their lifetime [1]. It remains a major global health problem associated with infection, limb loss and increased mortality. The global burden of DFUs continues to escalate, with direct healthcare costs exceeding $30 billion annually in the United States alone [2]. In India, the prevalence of DFUs among diabetic patients is estimated at 6.2%, with regional variations ranging from 5.6% to 9.5% [3]. The consequences of DFUs extend beyond immediate healthcare costs, as these wounds are associated with significant morbidity, prolonged hospitalizations, and increased mortality rates [4]. Recurrence is common and healing is often protracted despite best practice wound care.

The pathophysiology of DFUs involves multiple interconnected factors including peripheral neuropathy, impaired circulation, biomechanical stress, and compromised immune function [5]. Traditional wound care approaches often prove insufficient, resulting in delayed healing, increased infection rates, and ultimately, amputation in 20% of cases [6]. The complex nature of diabetic wounds necessitates advanced therapeutic interventions that can address the underlying cellular and molecular deficits.

Recent advances in regenerative medicine have introduced novel treatment modalities, including bioengineered skin substitutes and biological matrices, which have shown promise in accelerating wound healing and improving clinical outcomes [7]. Among these, high-purity type-I collagen- based skin substitutes (HPTC) and dehydrated human amnion/chorion membrane (dHACM) have emerged as promising therapeutic options [8, 9]. Type-I collagen, comprising 97% structural similarity across species, provides an optimal scaffold for cellular attachment and tissue regeneration [10]. This bioengineered collagen matrix mimics the native extracellular architecture and creates a conducive microenvironment for cell migration, proliferation, and differentiation. The inherent non-immunogenic properties of highly purified type-I collagen, which are attributed to the absence of sulphur-containing amino acids that typically trigger immune responses, make it particularly suitable and well-tolerated for wound healing applications [11].

Conversely, dHACM utilizes the natural regenerative properties of placental membranes, which have been employed clinically for over a century [12]. The amnion and chorion membranes contain growth factors, cytokines, and extracellular matrix components that facilitate wound healing through anti-inflammatory and pro-regenerative mechanisms [13].

However, processing methods may affect the bioactivity of these products, potentially limiting their therapeutic efficacy [14].

Previous single-centre studies have suggested potential advantages of HPTC over dHACM in treating chronic wounds [15, 16]. However, the evidence base remains limited, with small sample sizes and single-centre designs limiting the generalizability of findings. Furthermore, most comparative studies lack comprehensive histological analysis to elucidate the underlying mechanisms of action.

The objective of this multicentre randomized controlled trial was to compare the safety and efficacy of HPTC versus dHACM in treating DFUs, with particular emphasis on wound healing kinetics, histological parameters, and patient-centred outcomes. We desire to reiterate our previous single centre study result [17], that HPTC would demonstrate superior wound healing outcomes through enhanced cellular activity and improved tissue regeneration.

MATERIALS AND METHODS

Study Design and Setting

This prospective, multicentre, randomized, controlled, two parallel group, open-label trial based on pooled design was conducted across four tertiary care centres with study protocol approved by the institutional ethics committees of all participating centres: Adichunchanagiri Institute of Medical Sciences (AIMS), B. G. Nagara, JSS Medical College and Hospital (JSS), Mysore and Mysore Medical College and Research Institute (MMC&RI), Mysore and Rajarajeswari Medical College and Hospital (RRMCH), Bangalore, all centres in the state of Karnataka, India. The study was done under the supervision of primary investigator Dr Naveen N (AIMS) with the other principal investigators included Dr Ravi Shivaiah, Dr Vijay Kumar and Dr Kamal Kumar M, respectively. The study protocol was approved by the institutional ethics committees of all participating centres and registered with ClinicalTrials.gov (Identifier: NCT07046403; Protocol Record Id: AIMS/IEC/206/2025). The investigators adhered to the applicable regulatory requirements and the trial was conducted in accordance with Good Clinical Practice guidelines and the Declaration of Helsinki. Written informed consent was obtained from all participants and patient confidentiality was rigorously
maintained.

Patient screening and eligibility

The study population consisted of patients seeking treatment for DFUs. Eligible patients were those willing to participate and comply with scheduled visits on days 7, 14, 21, 28 (intervention period) and 35 (follow-up period). The study included two phases: screening and treatment. The screening phase aimed to determine patient eligibility for the treatment phase. During the screening, a series of assessments were conducted, including demographics, medical history, concomitant medications, vital, signs, physical examination, foot ulcer history, clinical infection signs at the ulcer site, and ankle-brachial index measurement.

Inclusion criteria: Patients aged 18-75 years with type 1 or type 2 diabetes mellitus presenting with chronic foot ulcers were screened for eligibility. Target ulcer size between 5.0-20.0 cm^2 measured post-debridement; ulcer duration of 4-20 weeks; adequate circulation documented by ankle-brachial index (ABI) 0.7-1.3; and glycated haemoglobin (HbA1c) <12%.

Exclusion criteria included: Patients were excluded from the study if they met any of the following criteria: presence of active infection requiring systemic antibiotics, confirmed

osteomyelitis or exposed bone in the wound bed, current use of immunosuppressive therapy, diagnosis of active malignancy, pregnancy or lactation, end-stage renal disease requiring dialysis, acute Charcot foot deformity, previous amputation that would affect target ulcer offloading capacity, and participation in other investigational studies within the preceding 30 days.

Randomization and Blinding

Eligible patients were randomized 1:1 using computer-generated random sequence with variable block sizes (4, 6, 8) stratified by site. Allocation concealment was maintained using sequentially numbered opaque sealed envelopes. Due to the nature of the interventions, participants and investigators could not be blinded to treatment allocation. However, outcome assessors for wound parameters (photograph-based planimetry was performed by a central reader), histological analysis and quality of life measurements were blinded to treatment assignment.

Interventions

All participants received standardized wound care including debridement, infection control, glycaemic optimization, and pressure offloading using total contact casting or removable cast walkers. Subjects who met the study's inclusion criteria after the screening period were randomized into one of two groups:

a. The HPTC arm (n=60) received high-purity type-I collagen-based skin substitute (Helicoll®, Encoll Corp, USA) that was carefully trimmed to precisely cover the wound bed, followed by application of standard of care (SOC) wound dressing. The reapplication schedule for HPTC was determined by the treating investigator's clinical judgment based on wound assessment and healing progress.
b. The dHACM group (n=60) received dehydrated human amnion/chorion membrane applied according to manufacturer instructions with concurrent application of standard of care (SOC), with repeat applications performed as clinically indicated based on wound healing response.

During the four-week treatment phase, patients were re-evaluated on days 7, 14, 21, and 28. The SOC bandage in both groups included an identical three-layer dressing systems: non-adherent paraffin gauze (primary layer), absorbent gauze pads (secondary layer), and soft roll with crepe bandage (tertiary layer) - standardized 3-layer dressing system.

If the study ulcer was found to be 100% re-epithelialized during the visit, no further study procedures were conducted at that time. The patient was then scheduled for a follow-up visit after one week to confirm the healing. If complete healing was not observed, an assessment was performed to check for signs of clinical infection. If an infection was diagnosed, treatment with topical antimicrobials (betadine, chlorhexidine) or oral antibiotics was allowed, but the use of topical antibiotics (erythromycin, polymyxin, mupirocin) was prohibited.

Following the infection assessment, the ulcer was cleaned, photographed, and debrided at the investigator's discretion to ensure a clean, granulating ulcer base with minimal adherent slough. The SOC was then reapplied, and the patient was instructed to keep the bandaging dry. The patient was also advised to contact or visit the study site if the bandage became soiled or was removed. Topical antibiotics specifically on the wound were prohibited. Systemic antibiotics were permitted when clinically needed.

Study completion

Patients completed the study four weeks after their first treatment visit. However, if a patient's study ulcer closed before the four-week mark, they were considered to have completed the study at that time. Complete healing of the study ulcer was defined as 100% re-epithelialization with no drainage. Throughout the treatment period, patients had the right to refuse participation or withdraw from the study at any time without prejudice. If a patient chose to withdraw from the study, their last recorded wound measurement was carried forward and used to calculate the change in wound size and their final outcome.

Outcome Measures

Primary Outcome:

1. Proportion of participants achieving wound size reduction at 4 weeks, measured using digital planimetry. Wound area was monitored at visits on days 7, 14, 21, 28 (intervention period) and 35 (follow-up period)

Secondary Outcomes:

1. Histological assessment of vascular incursion and wound biology was performed using punch biopsies (2mm diameter) obtained from the wound edge on day 5 post-treatment initiation. Histological parameters were evaluated using standardized scoring scales, including:

a. vascular infiltration assessed on a 0-3 scale based on vessel count per high-power field,
b. neo-epithelialization measured on a 0-3 scale based on epithelial migration distance from the wound edge,
c. fibroblast activity quantified on a 0-3 scale based on α-SMA positive fibroblast count and cellular morphology,
d. capillary density measured as vessels per mm² using CD31 immunohistochemical staining,
e. inflammatory response graded on a 0-3 semi-quantitative scale, and
f. collagen deposition assessed on a 0-3 scale using Masson's Trichrome staining.

Additional secondary outcomes included

2. Time to complete wound closure defined as 100% re-epithelialization with no drainage,
3. Percentage wound area reduction measured weekly using standardized digital photography,
4. Mean number of treatment applications representing the total applications required per participant during the 4-week treatment period,
5. Adverse events including infection and other complications monitored throughout the study period,
6. Quality of life assessed using patient-reported visual analogue scale (VAS) measuring change from baseline to follow-up week, and
7. Scar quality evaluated using the Vancouver Scar Scale at 5 weeks post-treatment

Vascularity assessment was done using biopsy on day zero of application to be compared with day five after the application of HPTC or dHCAM. For histopathological assessment, before application and on the fifth day after the application of either HPTC or dHCAM, a 2 mm 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 alcohol, and embedded in paraffin blocks. Serial sections of 4 μm thickness were prepared and stained with hematoxylin and eosin (H&E) for general morphology, Masson’s trichrome for collagen assessment, CD31 immunohistochemistry for capillary density evaluation, and α-SMA immunohistochemistry for fibroblast activity. Histological parameters that were evaluated included vascular infiltration, neo-epithelialization, fibroblast activity, capillary density,

inflammatory response, and collagen deposition (Table 1). All histological assessments were performed by two independent pathologists blinded to treatment allocation. Inter-observer agreement was assessed using Cohen's kappa coefficient.

Table 1: Histological parameters evaluated in the ulcer bed at baseline and on day five of application

Parameter	Measurement tool	Criteria	Score
Vascular infiltration	Assessed by counting new blood vessels (0–3 scale)	Minimal vascular ingrowth (<5 vessels/HPF)	0
		Mild infiltration (5–10 vessels/HPF)	1
		Moderate infiltration (11–20 vessels/HPF)	2
		Abundant infiltration (>20 vessels/HPF)	3
Neo-epithelialization	Measured as epithelial migration distance from wound edge (0–3 scale)	No epithelial migration	0
		Minimal migration (<25% wound coverage)	1
		Moderate migration (25–75% coverage)	2
		Extensive migration (>75% coverage)	3
Fibroblast activity	Quantified by counting α-SMA positive fibroblasts per HPF and assessment of fibroblast morphology (0–3 scale)	Sparse, inactive fibroblasts	0
		Moderate cellularity, minimal matrix production	1
		High cellularity, active-matrix synthesis	2
		Very high activity with extensive matrix deposition	3
Capillary density	Evaluated using CD31 staining, counted as vessels per mm² of tissue		
Inflammatory response	Graded semi-quantitatively (0–3 scale)	Minimal inflammatory infiltrate	0
		Mild chronic inflammation	1
		Moderate mixed inflammation	2
		Severe acute inflammation	3
Collagen deposition	Assessed using Masson's trichrome staining (0–3 scale)	Minimal collagen matrix	0
		Loose, immature collagen	1
		Moderate organized collagen	2
		Dense, mature collagen architecture	3

*Data presented as scoring criteria; all parameters assessed using standardized histological grading scales

Sample Size Calculation

Based on previous studies, we anticipated 85% of HPTC patients and 55% of dHACM patients would achieve wound reduction. With 80% power to detect a 25% absolute difference in complete wound closure at 5 weeks, 5% significance level, and assuming 10% dropout rate, a total of n=120 with 60 participants per group were required, pooled in from across 4 centres.

Statistical Analysis

Statistical analyses were performed using SPSS version 28.0 (IBM Corp, Armonk, NY, USA) and R version 4.3.0. Continuous variables were expressed as mean ± standard deviation or median (interquartile range) based on normality testing using Shapiro-Wilk test. Categorical variables were presented as frequencies and percentages.

Between-group comparisons for continuous variables used independent t-tests or Mann-Whitney U tests. Chi-square or Fisher's exact tests were employed for categorical variables. Time-to-event analysis used Kaplan-Meier curves with log-rank tests. Mixed-effects models analysed repeated measures data. All analyses followed the intention-to-treat principle, with sensitivity analyses using per-protocol populations. A two-sided p-value of less than 0.05 was considered statistically significant for all analyses, with p-values less than 0.001 indicating highly significant differences between groups.

Data collection and follow-up

Demographic data, medical history, comorbidities, and baseline wound characteristics were recorded. Laboratory parameters, including haemoglobin, albumin, and glycated haemoglobin (HbA1c), were obtained at baseline and follow-up visits.

Patients were evaluated at baseline, day 5, day 7, day 14, day 21, day 28, and day 35. At each visit, wound measurements were performed using standardized digital planimetry, with wound area calculated in cm^2. Histopathology examination results were recorded on baseline day 0 and day 5 after application. Digital photographs were taken for documentation and independent assessment. Complete wound closure was defined as 100% epithelialization without drainage or dressing requirements. Any adverse events, if they occurred, were duly noted and notified. Scar quality and durability were assessed at each visit, with patient satisfaction assessed at the fifth week, during the one-week follow-up.

Ethical Considerations

The study was conducted in accordance with ethical principles outlined in the Declaration of Helsinki. Written informed consent was obtained from all participants before enrolment. Participant confidentiality was maintained throughout the study period. Data safety monitoring was performed by an independent committee.