Statistical Analysis Plan (SAP): ENC-HEL-DFU-02

A Randomized Controlled Clinical Trial Evaluating The Efficacy Of A Unique Advanced Bioengineered Skin Substitute with Standard of Care Versus An Active Comparator with Standard of Care In The Treatment Of Non-Healing Diabetic Foot Ulcers

Sponsor: EnColl Corporation

CONFIDENTIAL

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Table 1. Version history.

Version Number	Release Date	Change(s)	Reason for Change
1.0	November 5, 2024	N/A	Initial Release

Brief Trial Description

This study is a multi-center, single-blinded (healing outcomes), parallel group, randomized prospective trial designed to evaluate the use of a human keratin graft (Helicoll®) that is 510K approved for application in the management of multiple wound types including the diabetic foot ulcer (DFU). Helicoll® is a translucent, off-white, semi-occlusive, self-adhering and pre-sterilized Type-I Collagen Sheet for use as a bioactive membrane. It is a reconstituted collagen sheet free of contaminants like lipids, elastin, and other immunogenic proteins, and is flexible with moderate tackiness.

The purpose of this clinical evaluation is to compare patient outcome data on Helicoll® versus another commercially available 510K FDA–cleared advanced skin substitute, Epifix.

In this trial following a short screening period of up to 2 days, subjects with Wagner 1 diabetic foot ulcers (DFUs), will receive standard of care (SOC) treatment for their condition and randomized to either Epifix® or Grafix®, or Helicoll®. Subjects were seen weekly until 5 weeks after randomization unless the DFU is healed or the subject withdrawn or lost to follow-up.

SOC in this study is offloading of the DFU, appropriate sharp or surgical debridement, and infection management as deemed appropriate by the investigator. Patients will be provided with a cast walker to offload their wounds or total contact casting if walkers cannot be made to fit the participant's index foot. Primary dressings for the SOC group are plain foam (Allevyn gentle, Mepilex) or Mepital / Adaptic Touch) and a final three-layer dressing (4x4 gauze, soft cast roll, ace bandage / Coban).

General

Descriptive statistical methods will be used to summarize the data from this study. These include number of subjects (n), mean and standard deviation (SD) for continuous variable data (medians and IQR [interquartile range] values will be given when the distribution of variable values is non-normal), and frequencies and percentages for categorical variable data. There will be no hypothesis testing performed in regard to trial endpoints. All data collected during the study will be reported and analyzed.

Unless specified otherwise, all statistical testing will be two-sided and performed using a significance (alpha) level of 0.05.

All statistical analyses will be conducted using IBM SPSS statistics (v27).

Sample Size Calculations

No sample size calculations were performed as a convenience sample of 24 subjects will be enrolled at up to 5 sites.

Subject Replacement

Not applicable to this study.

Interim Analysis

No formal interim analysis is planned or conducted.

Trial Stopping Rules

Subjects will be permitted to withdraw at any time and investigators can withdraw a patient based on the occurrence of an AE/SAE interfering with treatment or placing the subject's health in jeopardy.

There were no overall trial stopping rules.

Patient Populations

The populations defined for the analysis include intent-to-treat (ITT), per protocol (PP), and safety populations.

Intent-to-treat population (ITT)

The ITT population will consist of all randomized subjects, regardless of actual treatment received, with analysis conducted according to original treatment assignment group. Subjects who were randomized but later found to be ineligible for the study because they failed to meet all inclusion and exclusion criteria will be excluded.

Per protocol (PP)

The PP population will consist of randomized subjects with analysis conducted according to treatment received. The following subjects will be excluded: (a) subjects randomized but later found to be ineligible for the study because they failed to meet all inclusion and exclusion criteria; (b) subjects who did not complete the study; and (c) subjects with major protocol violations.

Safety Population

The safety population will be all subjects who were randomized and received at least one treatment.

Definitions

Baseline

For all parameters, baseline will be defined as the last available data point before the first treatment, which occurs at TV1.

Visit window data including study endpoints

For each weekly visit a window of ± 2 days will be adopted for data. If a specified time endpoint is outside of the window the last available visit data prior to the specified endpoint will be used.

Healing of Index Ulcer

Healing is defined as complete epithelialization of the ulcer without drainage observed in the index ulcer at two separate visits 2 weeks apart. If a subject fails this healing confirmation visit then they can continue in the trial with their assigned treatment provided that the index wound did not heal at the EOS visit.

Date of healing

The date of healing is defined as the date of the first assessment of index ulcer healing provided the index ulcer is still healed 2 weeks later.

Percentage area reduction from baseline

The percent change in the surface area of the Index Ulcer (PAR) will be calculated using the following formula:

((A1–A2)/A1)*100

Where A1 is the baseline area (at randomization), and A2 is the area at the specified time point.

Statistical Methodology

Missing data

Missing data may be the result of missed visits, subjects lost to follow-up or who have died, and may be endpoint data or covariate data.

The outcome of complete wound healing will be scored as **not** healed for the following events:

- The subject dies

- The subject has an amputation that affects the index ulcer
- The subjects is lost to follow-up
- The subjects is withdrawn from the trial
- The subject withdraws consent.

Missing PAR endpoint data within 5 weeks from TV1 will be not imputed.

Data QC

1. Inspect for accuracy of input:
    1.1. Plausible values
    1.2. Outliers or out-of-range values
2. Evaluate degree and distribution of missing data; for missing non-endpoint, out-of-range or non-plausible data, query PERI.
3. Identify non-normal continuous variables; data for non-normal continuous variables will include median/IQR metrics.

Statistical Analysis

Analysis will be started once database lock for the trial is initiated and the final version of the SAP is released into EnColl's system.

Research hypotheses

Because this trial is not powered to statistically test endpoints, no primary research hypotheses (hypotheses tested using statistical tests) will be tested.

General

The following analyses will be conducted:

1. Flow chart (subjects) with disposition according to CONSORT criteria (see shell Figure 1).
2. Overall trial statistics (enrollment dates, site numbers, numbers of subjects, group assignments)
3. Screen failure rate and screen failure reasons according to exclusion criteria.
4. Deaths, withdrawals, and loss to follow-up (total and by group assignment) with week of discontinuation and reason.

Demographics (subject-related and wound-related)

Patient demographics between groups at baseline: patient age, smoking status, BMI, HbA1c and creatinine levels; diabetes duration; number of concurrent DFUs and prior DFUs; amputations (broken down into major and minor amputation counts); history of DFU recurrence; history of and significant foot deformities.

Wound demographics between groups at baseline: DFU positions and location; ulcer duration and area at randomization; offloading type and offloading history; debridement count.

Baseline variables between groups will be tested using t tests, Mann-Whitney, chi square, and Fisher exact tests (as appropriate) without adjustment for multiplicity testing (see shell Tables 1 and 2).

Selected comorbidities will be presented along with total comorbidity count (extracted from medical history; will be tested between treatment groups) (see shell Table 3)

Endpoints

Endpoint analysis will conducted using the ITT population.

Primary

1. Primary endpoint analysis: PAR of index wounds at 5 weeks (mean and SD) by treatment group presented in a table; a separate figure will be presented for both treatment groups for mean PAR at weeks 1-5.

Secondary

The following endpoints will be calculated for each treatment group:

2. Proportions of index wounds healed by 5 weeks.
3. Time to heal within 5 weeks: mean time to heal (days) with 95% confidence intervals. A Kaplan-Meier plot for both treatment groups will be presented.
4. Number of applications of the intervention product for healed wounds only by treatment group.

Analysis will be done for the ITT population.

Safety Analysis

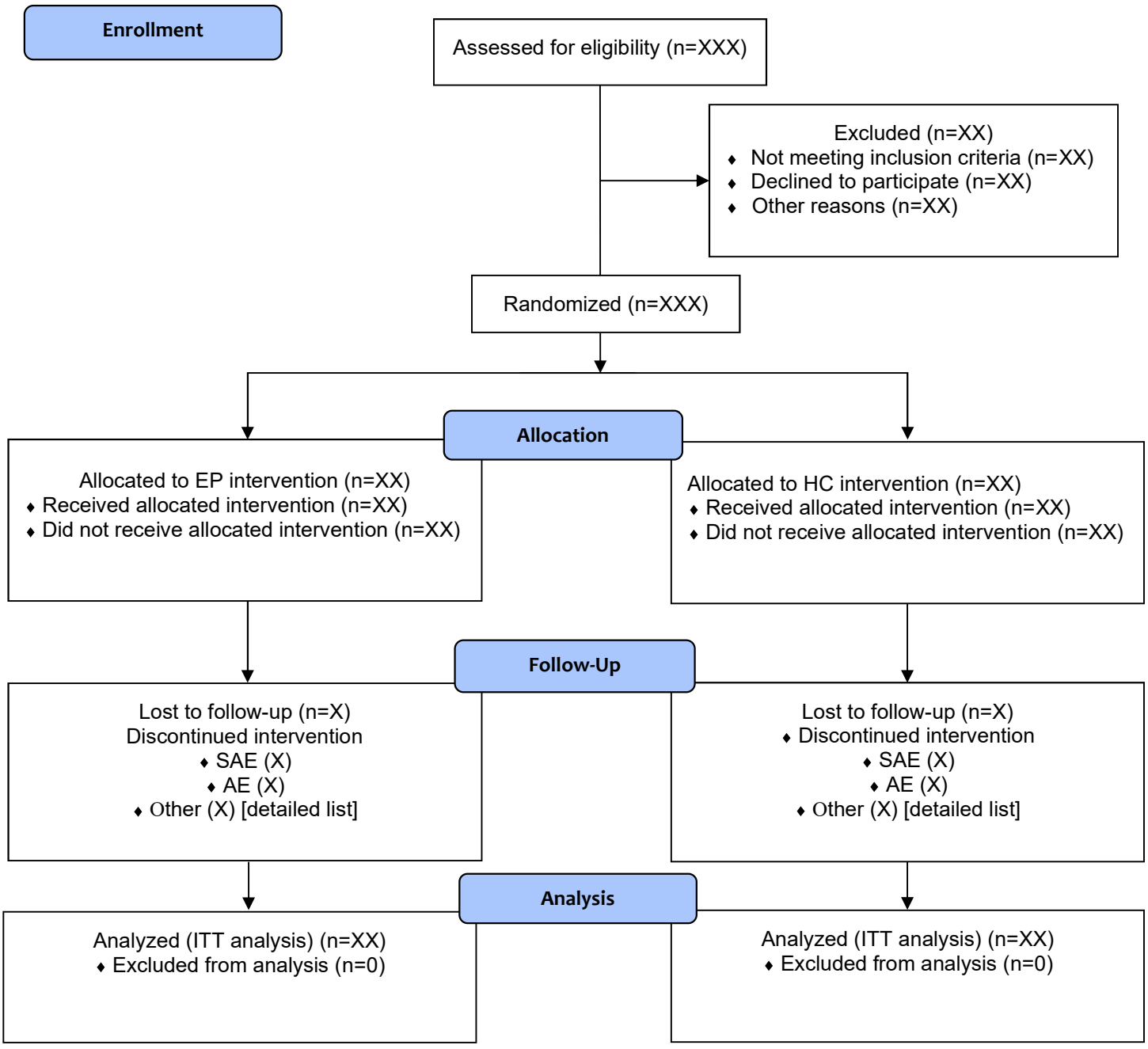
The following will be conducted:

Summary statistics for each treatment group:

- Count of AEs per treatment group/per patient
- Summary rates per group
- Notes of unexpected occurrences during the study; relevant decisions made) (text in report)
- Counts of SAEs/AEs by treatment group (listing of subjects); see shell Table 4.

- Complete listing of all AEs by subject, system organ class and preferred term(s). SAEs will be **bolded** in the listing (see shell Table 5).
- Counts of AEs by severity and relatedness to intervention product (see shell Table 6).

Shell Figure 1. CONSORT subject flow chart. EP: Epifix; HC: Helicoll.



Shell Table 1. Patient-related variables, except for comorbidities and concomitant drugs. Figures in parentheses will be percentage for categorical variables and standard deviation (SD) of the mean for continuous variables; for non-normal variables, such as initial wound area, medians and interquartile ranges (IQR) will be added.

Variable	Epifix	Helicoll	p
Age (years)	XX.X (XX.XX)	XX.X (XX.XX)	XX
Race			
Caucasian	X (X%)	X (X%)	
African American	X (X%)	X (X%)	XX
Other	X (X%)	X (X%)	
Ethnicity			
Hispanic	X (X%)	X (X%)	XX
Not Hispanic	X (X%)	X (X%)	
Gender			
Male	X (X%)	X (X%)	XX
Female	X (X%)	X (X%)	
BMI	XX.X (XX.XX)	XX.X (XX.XX)	XX
Smoker			
Current	X (X%)	X (X%)	
Former	X (X%)	X (X%)	XX
Never smoked	X (X%)	X (X%)	
HbA1c (%)			
At randomization	XX.X	XX.X	XX
Creatinine (mg/dL)	XX.X (XX.XX)	XX.X (XX.XX)	XX
Diabetes duration (years)	XX.X (XX.XX)	XX.X (XX.XX)	XX
Subject age when first DFU appeared (years)	XX.X (XX.XX)	XX.X (XX.XX)	XX
Prior DFU count	XX.X (XX.XX)	XX.X (XX.XX)	XX
Other concurrent DFUs (at screening	X (X%)	X (X%)	XX
History DFU recurrence	X (X%)	X (X%)	XX
Amputations, minor			
0	X (X%)	X (X%)	
1	X (X%)	X (X%)	
2	X (X%)	X (X%)	XX
3	X (X%)	X (X%)	
≥4	X (X%)	X (X%)	
Major amputations	X (X%)	X (X%)	XX

Variable	Epifix	Helicoll	p
Foot deformities Deformity 1 {Continue for all subjects with identified different deformities}	X (X%)	X (X%)	XX

Shell Table 2. Wound-related variables. Figures in parentheses will be percentage for categorical variables and standard deviation (SD) of the mean for continuous variables; for variables that have a non-normal distribution of values, such as initial wound area, medians and interquartile ranges (IQR) will be added.

Variable	Epifix	Helicoll	p
Wound area (cm^2)	XX.X (XX.XX) XX (XX)	XX.X XX (XX) XX (XX)	XX
Wound age (weeks)	XX.X (XX.XX) XX (XX)	XX.X XX (XX) XX (XX)	XX
Vertical location Plantar Dorsal	 X (X%) X (X%)	 X (X%) X (X%)	XX
Position Medial Lateral	 X (X%) X (X%)	 X (X%) X (X%)	XX
Anatomical location Toe Forefoot Midfoot Heel Ankle	 X (X%) X (X%) X (X%) X (X%) X (X%)	 X (X%) X (X%) X (X%) X (X%) X (X%)	 XX
Offloading type: Type 1 {Continue with summary statistics for all subjects with identified different types of offloading}	 X (X%)	 X (X%)	 XX
Number of debridements	XX.X (XX.XX)	XX.X (XX.XX)	XX

Shell Table 3. Selected comorbidities noted at first physical examination by treatment group.

Comorbidity	Epifix	Helicoll	p
CKD	X (X%)	X (X%)	X (X%)
Hypertension	X (X%)	X (X%)	X (X%)
PAD	X (X%)	X (X%)	X (X%)
CHF	X (X%)	X (X%)	X (X%)
Leg edema	X (X%)	X (X%)	X (X%)
Restricted mobility*	X (X%)	X (X%)	X (X%)
Venous disease	X (X%)	X (X%)	X (X%)
Peripheral neuropathy	X (X%)	X (X%)	X (X%)
Any psychiatric condition	X (X%)	X (X%)	X (X%)
Comorbidity count**	XX.X	XX.X	XX

*Restricted mobility: any subject who uses a walker, wheelchair, crutches or canes, and/or has an inability to move freely because of a physical or mental disability, handicap or restriction.

**Based on all identified comorbidities from medical history.

Shell Table 4. Count of SAEs (bolded) and AEs by subject, and totals by treatment group (figures in parentheses represent percentage of total AEs).

AE Category	Epifix	Helicoll
Subject ID SAE count {Continue for all subjects with SAEs}	XX-XXX x	XX-XXX x
TOTAL (SAEs)	xx (xx)	xx (xx)
Subject ID AE count {Continue for all subjects with AEs}	XX-XXX x	XX-XXX x
TOTAL (AEs)	xx (xx)	xx (xx)

Shell Table 5. Categorization of AEs by medDRA terms for each subject. For AEs categorized as SAEs, **listing**s **will be bolded**.

TEAE Category	Epifix	Helicoll
Subject ID System organ class Preferred term(s)	XX-XXX Text Text	XX-XXX Text Text
{Continue for all system organ classes for each subject. Start a new page for each subject}		

Shell Table 6. Categorization of AEs by severity and relatedness.
Figures in parentheses represent percentages for each treatment group.

AE Category	Epifix	Helicoll
Severity		
Mild	xx (xx)	xx (xx)
Moderate	xx (xx)	xx (xx)
Severe	xx (xx)	xx (xx)
Life-threatening	xx (xx)	xx (xx)
Fatal	xx (xx)	xx (xx)
Relatedness		
Not related	xx (xx)	xx (xx)
Unlikely to be related	xx (xx)	xx (xx)
Possibly related	xx (xx)	xx (xx)
Probably related	xx (xx)	xx (xx)
Definitely related	xx (xx)	xx (xx)