Intraoperative Cellular Reprogramming–Enhanced Skin Transplantation for Diabetic Foot Ulcers: A Randomized Clinical Trial

Trial Registration: ClinicalTrials.gov Identifier: NCT02070835

Study Protocol (version 2_0720_2017)

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Introduction

Diabetic foot ulcers (DFUs) represent a debilitating complication of diabetes, affecting 15% to 25% of patients and accounting for the majority of non-traumatic lower-limb amputations worldwide.[1-4]. Despite multidisciplinary care, outcomes remain suboptimal; substantial rates of non-healing and recurrence persist, imposing a heavy burden on patients and healthcare systems. [5, 6, 7, 8]

The failure of DFUs to heal stems from a hostile wound microenvironment characterized by persistent inflammation, ischemia, and defective remodeling.[9] Clinically, split-thickness skin grafting (STSG) is a standard reconstructive strategy to provide immediate closure. However, in the diabetic context, STSG is intrinsically limited because it provides structural coverage without addressing the inflammatory and angiogenic deficits of the wound bed, resulting in poor graft integration and a high risk of recurrence. [10, 11]

Autologous cell-based therapies have been proposed to restore this compromised microenvironment. [12-15] While promising, most strategies rely on cell delivery alone, lacking the immediate barrier function of a graft. Previously, our group demonstrated that combining autologous skin cell suspension with STSG could accelerate re-epithelialization.[16] Building on this, we focused on Epidermal Stem Cells (EpiSCs). Emerging evidence suggests that EpiSCs are not merely regenerative building blocks but potent paracrine regulators capable of modulating immune responses and promoting angiogenesis. [17 18. 19-25.26 27 28]

Based on this rationale, we developed Intraoperative Cellular Reprogramming–Enhanced Skin Transplantation (CREST). This technique repurposes residual skin tissue—normally discarded during grafting—to create an EpiSC-enriched suspension that is applied intraoperatively to biologically optimize the wound bed. We hypothesized that this "point-of-care" modification of standard STSG would enhance healing rates and durability. This prospective, randomized clinical trial was designed to compare the efficacy and safety of CREST versus conventional STSG in patients with chronic diabetic foot ulcers.

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1 STUDY DESIGN

1.1 STUDY CONTENT

1) Efficacy: The primary objective of this study is to evaluate the clinical efficacy of Cellular Reprogramming-Enhanced Skin Transplantation (CREST)—specifically the combination of an autologous epidermal stem cell (EpiSC)-enriched suspension with split-thickness skin grafting (STSG)—in the repair of chronic diabetic foot ulcers (DFUs). This evaluation is conducted through a prospective, randomized, controlled, evaluator-blinded clinical trial. The study aims to determine whether the addition of the autologous EpiSC-enriched suspension provides a superior rate of complete wound healing and accelerated wound healing compared to standard split-thickness skin grafting (STSG) alone. Secondary efficacy endpoints include time to complete healing, the long-term recurrence rate, and safety outcomes.

2) Safety: The study aims to evaluate the safety and postoperative complication rate of CREST for the treatment of DFUs, including local infection, graft necrosis, donor site morbidity, and the incidence of treatment-emergent adverse events.

1.2 STUDY CONFIGURATION

This study is a prospective, randomized, controlled, evaluator-blinded clinical trial. The clinical phase was conducted from August 2017 and December 2023 .

Sample Size: The intended sample size is 108 patients, with participants randomized in a 1:1 ratio to either the CREST experimental group or the standard treatment (STSG alone) control group. This sample size ensures sufficient statistical power (80% power, two-sided α of 0.05) to detect a difference in the primary endpoint (complete healing rate at 2 weeks), based on on previous pilot studies and clinical data, the complete healing rate was assumed to be 60% in the control group, We hypothesized that the CREST therapy would achieve a superior healing rate of 85% (an absolute increase of 25 percentage points).

1.3 PARTICIPATING CENTERS

This research is a prospective, randomized, controlled, evaluator-blinded clinical trial conducted at a single clinical site. The Department of Burn and Wound Repair of the First Affiliated Hospital of Sun Yat-sen University serves as the sole research unit responsible for subject enrollment, surgical interventions, and clinical follow-up. All study procedures and data

collection are centralized within this department to ensure consistency and quality control throughout the trial period.

2 ENROLLMENT OF SUBJECTS

2.1 INCLUSION CRITERIA

Patients were eligible for the study if they met all of the following criteria: Aged 18 years or older; Diagnosed with type 1 or type 2 diabetes mellitus according to World Health Organization criteria; Presence of a chronic diabetic foot ulcer (Wagner grade 2–3) on the foot (below the ankle), with an area measuring between 5 cm^2 and 80 cm^2 after debridement; The target ulcer had been present for at least 4 weeks and had not healed despite receiving standard wound care (in accordance with the International Working Group on the Diabetic Foot guidelines) for a minimum of 4 weeks prior to randomization; Had a glycated hemoglobin (HbA1c) level of <12% measured during screening or within 3 months before randomization; If a revascularization procedure had been performed on the affected limb, it must have been completed at least 4 weeks prior to randomization; Willing and able to provide written informed consent, understand the study procedures, and comply with all planned study visits and follow-up assessments.

2.2 EXCLUSION CRITERIA

Patients were excluded from the study if they met any of the following criteria: Active infection of the target ulcer requiring systemic antibiotic therapy, or the presence of necrosis, purulence, or sinus tracts that could not be removed by debridement during the screening visit; Significant peripheral arterial disease, defined as an abnormal ankle-brachial index (ABI; ABI <0.7 or >1.3); End-stage renal disease requiring renal dialysis, or an estimated glomerular filtration rate (eGFR) of <20 mL/min/1.73 m^2; Active malignancy, uncontrolled autoimmune disease, or current treatment with systemic corticosteroids, cytotoxic agents, or other immunosuppressive therapies; Known hematological disorders that may impair wound healing; Known human immunodeficiency virus (HIV) infection or active hepatitis; A revascularization procedure on the affected limb was planned or had been performed within the 4 weeks preceding randomization; Participation in another interventional clinical trial within 3 months prior to randomization; Pregnancy, lactation, or intention to become pregnant during the study period; Any condition that, in the judgment of the investigator, would compromise the patient's ability to

understand the study, provide informed consent, or complete the required follow-up; Any other medical or psychosocial condition deemed by the investigator to potentially jeopardize the patient's safety or the reliability of the study results.

2.3 ELIMINATION CRITERIA

Participants who have been selected for this clinical study and falling into any of the following criteria will be considered as eliminated cases in the statistical analysis.

1) Participants who fail to meet the inclusion criteria are identified post-enrollment;

2) Participants with fewer than 2 follow-up visits post-enrollment will be excluded from the objective efficacy evaluation but can still be evaluated for adverse reactions;

3) Participants who fail to comply with the trial protocol.

2.4 WITHDRAWAL CRITERIA

Participants have the right to withdraw from the clinical trial at any point and for any reason. The investigator has the authority to request participants to cease their involvement in the study in order to protect their welfare. Before withdrawing from the trial, it is essential to conduct the most recent follow-up visit and assess all relevant efficacy and adverse reaction indicators. Additionally, it is essential to maintain a thorough documentation of the reason and date of withdrawal.

If any of the specified scenarios arise for a participant during the trial, it is the responsibility of the investigator to discontinue the treatment and remove the participant from the trial:

1) Inadequate compliance with medical advice

2) Occurrence of adverse reactions judged by investigator that inappropriate to continue this clinical study

3) Participant's voluntary withdrawal from the clinical study due to unwillingness to continue.

3 STUDY SCHEDULE

Participants are assigned randomly to either the CREST experimental group or the standard treatment control group through randomized grouping. The enrollment and grouping of participants should be reassessed as part of the monitoring of the clinical trial.

3.1 VISIT 1 SCREENING (DAY -60 TO DAY 0)

Prior to enrolling in the trial, each participant is required to fulfill the specified requirements,

with screening outcomes remaining valid for a period of 2 months prior to the surgical intervention.

1) A signed informed consent form

2) Review of the medical history

3) Fundamental personal information

4) Current treatment and medication regimen

5) Routine physical examinations encompass measurements of height, weight, and vital signs

6) Specialized physical examinations include wound assessment, arterial palpation, and neurological examination, etc.

7) Measurements of glycated hemoglobin and serum/urine pregnancy test

3.2 VISIT 2 ENROLLMENT (DAY 1)

3.2.1 REVIEW Of Adverse Events

The monitoring and documentation of adverse events will continue to be conducted and recorded in the case record form.

3.2.2 RANDOMISATION GROUPING

Randomly assign participants to either the CREST group or the standard treatment group. Randomization is performed using a computer-generated randomization sequence with block sizes of four. Allocation concealment is maintained using sealed opaque envelopes.

3.2.3 SURGICAL PROTOCOL

The control group will receive standard split-thickness skin grafting (STSG) alone, whereas the experimental group will undergo the Cellular Reprogramming-Enhanced Skin Transplantation (CREST) procedure, which involves spraying an autologous epidermal stem cell (EpiSC)-enriched basal cell suspension onto the wound bed prior to STSG application.

1) For the CREST experimental group:

a) Wound bed preparation and debridement.

b) Measure the longest diameter and maximum width of the wound. Design a rectangular donor area to ensure that, after trimming, residual STSG fragments will cover $\geqslant$2.5% (1/40) of the original wound area. Harvest a split-thickness skin graft (STSG) of uniform thickness (0.25 mm) from the donor site (scalp or thigh) using an electric dermatome.

c) Process the residual STSG fragments using the Myseed® kit to obtain an epidermal stem

cell (EpiSC)-enriched basal cell suspension.

d) Spray the EpiSC-enriched suspension onto the prepared wound bed.

e) Overlay and secure the remaining split-thickness skin graft (STSG) using a tie-over bolster dressing.

f) Postoperatively, change the outer dressing in accordance with standard treatment protocols. Additionally, it is important to closely monitor and record the wound's healing progress as outlined in the trial protocol. Complete the surgical record in the Case Report Form (CRF) table.

2) For standard treatment control group:

a) Wound bed preparation and debridement.

b) Harvest a split-thickness skin graft (STSG) of uniform thickness (0.25 mm) from the donor site (scalp or thigh) using an electric dermatome.

c) Immediately cover the wound with the STSG and secure it using a tie-over bolster dressing.

d) Following the surgery, it is essential to regularly change the outer dressing, as well as systematically monitor and document the advancement of the wound as per the trial protocol. Complete the surgical record in the Case Report Form (CRF) table.

3.3 VISIT 3 (POSTOPERATIVE DAY 6 ± 1)

1) Physical examination and vital signs.

2) Monitor Adverse Events (AE) and concomitant medications.

3) Initial Wound Assessment: Evaluation of early graft adherence and dressing change.

3.4 VISIT 4 (POSTOPERATIVE DAY 14 ± 2)

1) Primary Efficacy Evaluation: Incidence of complete wound healing within 2 weeks, defined as epithelialization without drainage confirmed by blinded assessors.

2) Measurement of wound reduction rate.

3) Record Adverse Events and physical signs.

3.5 VISIT 5 (HEALING TRACKING - WEEKLY)

1) Healing Time Recording: Participants are followed weekly (or daily via remote photo monitoring) to record the exact date of complete epithelialization for Kaplan-Meier analysis.

2) Record total days to healing for both groups.

3.6 VISIT 6 (FOLLOW-UP AT 1, 3, AND 6 MONTHS)

1) To minimize attrition, assessments are conducted via telemedicine (telephone and digital image transmission, e.g., WeChat) to allow for visual screening of ulcer recurrence.

2)Recurrence Verification: Any patient-reported suspicion or ambiguous visual evidence triggers an immediate in-person clinical assessment for confirmation.

3.7 VISIT 7 (LONG-TERM FOLLOW-UP - 12 MONTHS)

1) Final Efficacy Evaluation: Calculation of the 1-year recurrence rate via remote visual survey (with in-person verification for suspected cases).

2) Safety Assessment: Final recording of any late-occurring Adverse Events.

4 EVALUATION OF EFFICACY

4.1 PRIMARY EFFICACY ENDPOINT

The primary endpoint is the incidence of complete wound healing within 2 weeks postoperatively, defined as 100% epithelialization without drainage, as confirmed by blinded assessors.

4.2 SECONDARY EFFICACY ENDPOINTS

1) Time to complete healing: The number of days from surgery to complete epithelialization.

2) Ulcer recurrence rate during the 12-month follow-up period: Defined as the re-appearance of an ulcer at the original site after complete healing.

3) Incidence of adverse events and complications.

4.3 TIME OF EFFICACY EVALUATION

In this study, an evaluation of efficacy is carried out post-surgical intervention and throughout the follow-up period. All participants must undergo an efficacy evaluation upon completion or withdrawal of the trial. The primary endpoint is assessed at 2 weeks postoperatively. Secondary endpoints are assessed during weekly healing tracking and at the 12-month follow-up visit.

4.4 METHODS OF EFFICACY EVALUATION

In order to ensure the reliability of the assessment, the evaluation of wound healing is conducted by blinded assessors who are not involved in the surgical procedure and are unaware of the participants' group assignment. The findings should be accurately documented in the original medical record as objective evidence.

5 SAFETY EVALUATION

All adverse events that occur during the trial must be documented. The causal relationship between the adverse event and the study intervention should be judged by the investigator. Any abnormal laboratory results that manifest during the trial and persist until the conclusion of the trial should be followed up by the investigator until they normalize or the clinical presentation stabilizes.

5.1 SAFETY INDICATORS

5.1.1 VITAL SIGNS AND PHYSICAL EXAMINATION

The vital signs and physical examinations, including wound examination, heart rate, blood pressure, body temperature, and respiratory rate, need to be documented before the commencement of the study and at every follow-up visit.

5.1.2 INDICATORS OF LABORATORY TESTS

Prior to commencing the study, the clinical research associate (CRA) will record the range of normal values for each laboratory test indicator. The laboratory items listed below will be necessary for Visit 1 during the study period.

a) Glycated hemoglobin

b) Serum/urine pregnancy test (required for women of childbearing age)

5.1.3 ADVERSE REACTIONS

After the surgical procedure, investigators must monitor the presence of adverse reactions, including tissue responses at the recipient site, local inflammatory reactions like congestion, edema, ulceration, and oozing, systemic reactions like fever, rashes, itching, and other allergic manifestations, as well as infections, among other potential outcomes. The adverse reactions will be documented as part of adverse events.

5.2 ADVERSE EVENT EVALUATION

Adverse events will be evaluated and documented in accordance with the Adverse Event Evaluation Form.

6 ADVERSE EVENT RECORDING AND REPORTING

6.1 DEFINITIONS

Any medical incident, regardless of its connection to the intervention, that occurs subsequent to a surgical procedure in the context of a clinical trial is considered an adverse event. It can be any unfavorable and undesired abnormal outcomes, including signs, symptoms, or laboratory test results, irrespective of their correlation with the surgical procedure. All adverse events should be recorded in the Case Report Form (CRF).

Clinical research associates (CRA) at study centers are required to systematically collect and verify information on adverse events while reviewing participants' clinical history records (original data verification). Adverse events that remain unresolved at the time of assessment have to be monitored until their resolution. For adverse events that cannot be resolved during the clinical observation period (e.g., amputation, death, etc.), the investigator will classify the event as permanently unresolved. The time of resolution of the event will be left blank on the Case Report Form (CRF).

6.2 ADVERSE EVENT CORRELATION

The investigator is required to assess the potential correlation between the adverse event and either the surgical procedure or the concurrent administration of the medication, referring to the following 5-level classification criteria:

1) Definitely related: the reaction appears in a reasonable postoperative chronological sequence, and the reaction is consistent with the adverse reaction known to associate with the suspected skin grafting.

2) Possibly related: the reaction appears in a reasonable postoperative chronological sequence, and the reaction is consistent with the adverse reaction known to associate with the suspected skin grafting; the patient's clinical status or simultaneous treatment modalities may also play a role in the reaction.

3) Possibly unrelated: the appearance of the reaction does not align with a reasonable postoperative chronological order, and the reaction is not quite consistent with the adverse reaction known for the suspected skin grafting; the patient's clinical status or simultaneous treatment modalities may contribute to the reaction.

4) Unrelated: the reaction does not align with a reasonable postoperative chronological order, the reaction is in line with the reaction known to occur with concomitant use of medications; the patient's clinical status or other treatment modalities may contribute to the reaction. Enhancements

in the health state or discontinuation of simultaneous treatments may lead to the disappearance of reactions, while the reintroduction of other treatment modalities could potentially initiate the onset of reactions.

5) Unable to assess: the reaction appears without a clear relationship to the chronology of the post-surgical period, but aligns with known reactions associated with skin grafting, and other medications used concurrently may be attributed to the comparable reaction.

The study delineates the aforementioned items (1), (2), and (5) as adverse reactions.

The formula for calculating the adverse reaction incidence rate: number of cases of adverse reactions / total number of cases × 100%.

6.3 CRITERIA FOR GRADING ADVERSE EVENTS

In the capacity of a practicing physician, the investigator will evaluate all adverse events in the following manner:

1) Mild: an awareness of signs or symptoms that are easily tolerated (for pediatric clinical studies, defined as being aware of symptoms but easily tolerated）

2) Moderate: discomfort sufficient to interfere with daily activities (for pediatric clinical studies, defined as a definite problem with the child's movements)

3) Severe: disability that unable to engage in work or perform daily activities (for pediatric clinical studies, defined as the presence of extreme pain or inability to perform daily activities)

6.4 SERIOUS ADVERSE EVENTS

A serious adverse event is defined as an undesirable occurrence following a surgical procedure that fulfills one or more of the following criteria:

1) results in death

2) Poses a life-threatening risk due to the treatment

3) results in hospitalization or an extended hospital stay

4) results in permanent or significant loss of function or disability

5) results in teratogenesis or carcinogenesis.

Some events requiring hospitalization or prolonged stays in a hospital may not be classified as serious adverse events, including:

1) hospitalization for social reasons other than an adverse event

2) hospitalization for elective surgery, examinations, or other treatments for which an

appointment had been scheduled before enrollment in the study

3) hospitalization for a pre-existing condition that do not exacerbate during the study period

6.5 REPORTING PROCESS

6.5.1 REPORT FORM

The Serious Adverse Events (SEA) Report Form is necessary for documenting and reporting serious adverse events.

6.5.2 REPORTING PROCEDURES

In case of a serious adverse event, regardless of its connection to the skin grafting, the investigator is required to submit the initial report to the Ethics Committee of the First Affiliated Hospital of Sun Yat-sen University (Tel: 020-87755766-8185) within 24 hours. Additionally, the investigator should provide a follow-up report to the same department promptly upon completion of the final report, which should include details of the serious adverse event. If the serious adverse event results in death, there is no need for retrospective submission of the final report.

7 RULES FOR WITHDRAWAL AND DISCONTINUATION FROM THE STUDY

7.1 PATIENT WITHDRAWAL

A participant has the right to withdraw from a clinical trial at any point, regardless of the reason, without impacting the Investigator's ability to provide treatment for the participant's condition. In the pursuit of safeguarding the well-being of research participants, the investigator is entitled to request the withdrawal of a participant for various reasons, including but not limited to concurrent illness, adverse events, or treatment inefficacy. The Clinical Research Core Team retains the authority to request a participant to discontinue their involvement in the study due to protocol violations, administrative considerations, or other legitimate and ethical reasons.

While minimizing participant withdrawal is preferred in clinical research, it is crucial to acknowledge that participant withdrawal does indeed occur. Whenever a participant terminates involvement in a study for any reason, a final study evaluation must be conducted, and the reason for withdrawal should be documented. All documentation pertaining to the participant should be comprehensive. As long as the consent form has been not revoked, the individual who has withdrawn must continue to be followed up and has the disease status documented as if there is no withdrawn.

The investigator is required to conduct further inquiries with individuals who withdraw from the study due to poor compliance to ascertain the reasons for their absence from follow-up appointments. Withdrawals resulting from concurrent illness or adverse events should be recorded in detail on the observation form, along with all relevant and valuable details.

7.2 PREMATURE TERMINATION OF STUDIES

Potential reasons for premature termination of a clinical trial may include external factors, recurrence of serious adverse events, elevated treatment-related mortality, or inadequate participant enrollment. All investigators will receive written notification in the event of premature termination of a clinical study. Every investigator who wishes to terminate the involvement in this clinical study must promptly notify the Principal Investigator of the decision.

8 FOLLOW-UP OF THE STUDY

8.1 DURATION OF FOLLOW-UP

Participants will be followed up at postoperative day 6 ± 1, day 14 ± 2, weekly until healing, and at 1, 3, 6, and 12 months postoperatively.

8.2 CONTENT OF FOLLOW-UP VISITS

Follow-up visits involve recording the participants' general vital signs, examining their wounds, and assessing the recipient site of the skin grafting. Record the above follow-up in the original medical record. The evaluation content should be filled in the Case Report Form (CRF).

9 STATISTICAL METHODS

9.1 SAMPLE SIZE CALCULATION

Based on previous pilot studies and clinical data, the complete healing rate was assumed to be 60% in the control group. We hypothesized that the CREST therapy would achieve a superior healing rate of 85% (an absolute increase of 25 percentage points). Using a two-sided chi-square test with a significance level (α) of 0.05 and a power (1- β) of 80%, the calculated sample size required was approximately 49 patients per group. Accounting for an estimated dropout rate of 10% during the 1-year follow-up period, the target enrollment was increased to 54 patients per group, resulting in a total planned sample size of 108 patients.

9.2 PRIMARY ENDPOINT INDICATORS

The primary endpoint indicator in this trial is the incidence of complete wound healing within 2 weeks postoperatively. Complete wound healing is defined as epithelialization without drainage, as confirmed by blinded assessors.

9.3 SECONDARY ENDPOINTS INDICATORS

1) Time to complete healing;

2) Ulcer recurrence rate during the 12-month follow-up period;

3) Incidence of adverse events and complications.

9.4 ANALYSIS

The baseline data will be subject to a balanced analysis through descriptive frequency lists. A chi-square test will be used to compare the baseline data between the two groups. The resemblance of each relevant characteristic between the two groups will be analyzed through tables. It is necessary to present the figures and proportions of participants who undergo screening, and randomized grouping, and the primary cause of screening failure or treatment discontinuation. Demographic variables (e.g., age, gender, ethnicity), primary and secondary diagnoses, prior therapy, and combination therapy are summarized across different treatment groups through descriptive statistical values or categorical tables.

9.4.1 EFFICACY ANALYSIS

Data will be analyzed using SPSS version 26.0 (IBM Corp., USA). Continuous variables will be expressed as mean ± standard deviation or median (interquartile range) and compared using Student's t-test or Mann–Whitney U test as appropriate. Categorical variables will be presented as frequencies and percentages and analyzed using chi-square or Fisher's exact test. Time-to-event data (time to complete healing) will be analyzed using Kaplan–Meier survival curves and log-rank tests. Multivariable logistic regression will be used to identify independent predictors of healing. A two-tailed P value <0.05 will be considered statistically significant.

9.4.2 EFFECTIVENESS ANALYSIS

Categorical data, such as adverse events and serious adverse events, will be depicted through frequency lists and summary charts. A comparative analysis between the two groups will be conducted utilizing chi-square tests, Fisher's exact probability tests, or Cochran-Mantel-Haenszel (CMH) methods.

Quantitative data, such as laboratory test results, will be analyzed using measures like the

arithmetic mean or median to characterize the trends in their concentrations. Additionally, measures such as standard deviation or interquartile range will be utilized to describe the distribution patterns of the data. The t-test or non-parametric test method will be used to compare the two groups.

For the analysis of relevant prognostic factors, the Logistic regression model will be employed for short-term follow-up data, whereas the multivariate Cox regression model will be utilized for long-term follow-up data. Furthermore, the analysis will incorporate the end-observation carry-over method to address any missing data.

9.5 DEFINITION OF THE ANALYSIS SET

1) The analysis will be performed on the intention-to-treat (ITT) population, which includes all randomized participants. This population will serve as the primary group for conducting efficacy analysis.

2) A per-protocol (PP) analysis may be performed as a sensitivity analysis, comprising participants who adhered closely to the study protocol. If the PP population exceeds 95% of the ITT population, conducting a separate PP analysis may be deemed unnecessary.

3) The safety analysis population will comprise all individuals who underwent the assigned surgical intervention.

10 RESEARCH ITEMS

The following items will be provided to research units and investigators. The investigators must sign and date the receipt form upon receiving the items.

1) Research Protocol

2) Informed Consent Form

3) Case Report Form

4) Manual for the Investigator

5) Files from the trial center

11 ETHICS

11.1 RESPONSIBILITY OF THE INVESTIGATOR

It is the responsibility of the investigator to ensure that the clinical trial is conducted in strict

compliance with the trial protocol, Chinese Good Clinical Practice (GCP) guidelines, and relevant regulations.

11.2 INFORMED CONSENT FORM

Before participants are enrolled in the trial, the investigator is required to provide a comprehensive explanation regarding the trial's objectives, methodology, potential benefits, risks, and any discomfort that may arise. Participants will be informed that their participation in the trial is voluntary, and they will have the option to withdraw at any time. It will also be conveyed that their decision to participate or not will not impact the treatment of their disease. The privacy rights of the participant will be protected.

Participants and their legal guardians should be given sufficient time to review the informed consent form and ask any questions. Before enrollment, the participants and their guardian are required to sign the informed consent form, with a copy kept by the participants.

11.3 GOOD CLINICAL PRACTICE (GCP)

This clinical study will be conducted in accordance with the Declaration of Helsinki and the Chinese Good Clinical Practice (GCP) guidelines. Prior to implementation, the study protocol needs to obtain approval from the ethics committee of the unit overseeing the clinical study. The investigator will ensure that this clinical study is conducted in compliance with the laws, regulations, scientific principles, and ethical standards of the People's Republic of China regarding medical research. If revisions to the study protocol are deemed necessary during the study's progression, the modified protocol must be resubmitted to the Ethics Committee of the overseeing unit for documentation and approval prior to execution. If important new information concerning the investigational drug arises, the informed consent document will be updated in writing and submitted to the Ethics Committee of the responsible unit for review. Subsequently, the participants will be required to provide their consent again.

11.4 CONFIDENTIALITY OF SUBJECTS' PERSONAL DATA

The data collection in this trial will be restricted to the necessary information for investigating the efficacy and safety of skin grafting. The collection and utilization of this data will be in compliance with the relevant laws and regulations governing privacy protection.

12 MANAGEMENT REQUIREMENTS AND QUALITY CONTROL

12.1 MODIFICATION OF THE PROTOCOL

The investigator does not authorized to make arbitrary modifications to the trial protocol. If modifications are deemed necessary throughout the trial, the investigator will propose all protocol modifications to Sun Yat-sen University. Subsequently, the revised protocol will be released following deliberation and approval.

12.2 COMPLETION OF THE CASE REPORT FORM

The investigator initially identifies the patients to be included based on the information provided in the Screening Form. Once enrolled, participants will be examined and treated as claimed by the requirements of the program, and the relevant contents will be documented in the original medical records. The original medical records and examination results will be strictly filled in the Observation Form of the Case Report Form (CRF), whereas the Combined Medication Form will be finalized at the conclusion of each trial cycle. At the end of the study, the healthcare professional in charge of completing the case report form (CRF) should collaborate with the superior doctor to carefully review the CRF for consistency with the original records. Significant deviations or data falling outside the clinically acceptable range have to be verified and the responsible physicians are required to provide the necessary explanations.

12.3 DATA QUALITY ASSURANCE

In order to ensure the comprehensiveness, precision, and reliability of the data, the study will implement the following measures (an additional Standard Operating Procedure (SOP) is available to provide further detailed regulations for this clinical trial).

1) The selection of qualified and experienced research units and researchers should be conducted.

2) Prior to the initiation of the study, comprehensive details regarding the study protocol are imparted to the investigators through lectures, written materials, and other means. Additionally, collaborative efforts will be made to devise solutions for potential issues that may arise.

3) All participants who have signed the informed consent form and meet the eligibility criteria for participation in the trial must have their information accurately and comprehensively documented in the case record form. It is essential to ensure that there are no missing data or omissions, and any vacant spaces should be clearly marked with an underline.

4) Ensuring the accuracy of data in the case record form necessitates cross-referencing it with

the participant's original medical record to identify and rectify any discrepancies.

5) As raw data, any corrections made to the original medical record chart should be underlined only, with the revised data noted nearby. The investigators are required to affix their initials and dates.

6) The Clinical Research Associate (CRA) will regularly verify the completeness and accuracy of the data.

7) If data are deemed questionable, it is advisable to communicate this problem to the investigator promptly for confirmation or correction. Additionally, blind review should be conducted during the statistical phase. If bias exists, it should either confirm or corrected by the investigator.

12.4 SITE MONITORING VISIT (SMV)

12.4.1 PURPOSE OF SMV

To ensure the adherence of the clinical trial to the protocol and relevant regulations, as well as to ensure the completeness, reliability, and consistency of the multicenter data, simultaneously to coordinate the uniformity of the trial progress.

12.4.2 CONTENT OF SMV

Clinical Research Associates (CRAs) routinely visit clinical research units to supervise and document the trial's progress. The Clinical Research Associate (CRA) is authorized to verify the original data of all participants involved in the research. Monitoring primarily encompasses several key aspects: verifying if the participants meet the enrollment criteria; ensuring the timely, accurate, complete, and credible completion of the case report form (CRF); confirming that the participants adhere to the prescribed method and dosage of medication as per the protocol requirements; documenting all adverse events in the CRF form; and guaranteeing that any errors or omissions are rectified, signed, and dated by the investigator.

In case of serious adverse events or fatalities (including those associated with chemotherapy) occurring either in connection with or independently of the study throughout the clinical trial, the responsible physicians or hospital will promptly implement necessary actions.

13 STUDY COMPLETION/DISCONTINUATION

13.1 END OF STUDY

Upon the conclusion of the follow-up period for the final subject in the trial, the investigator is required to inform the sponsor. This notification marks the point at which the study can be deemed as concluded. The project must be finalized within two years as specified by Sun Yat-sen University.

13.2 STUDY DISCONTINUATION

The Principal Investigator retains the authority to terminate the trial or cease operations at a study site at any time. Reasons include, but are not limited to:

1) Inability of the investigator to comply with the trial protocol;

2) Inability of the investigator to recruit sufficient participants;

3) Safety concerns;

4) Sufficient evidence demonstrating the absence of effectiveness.

13.3 AUDIT

The Clinical Research Associate (CRA) or Principal Investigator conducts regular audits of all study documentation for the trial, which includes the original records, and cross-references them with the Case Report Form (CRF). The study site will be notified of the audit and is expected to make appropriate preparations. Comparable inspections could be conducted by the relevant Drug Administration. In such instances, the investigator should promptly inform the research unit.

14 PROGRESS OF THE STUDY

A total of 108 participants were enrolled in this prospective, randomized, controlled, and evaluator-blinded clinical investigation spanning over a period of 2 years.

Commencement of the trial: January 2022 Conclusion of the trial: December 2023

In this clinical study, participants were randomized in a 1:1 ratio to either the CREST experimental group or the standard treatment control group. Accounting for participant screening against inclusion criteria and potential dropouts, the successful collection and follow-up of 108 cases required a continuous period of 2 years.

1. Armstrong DG, Boulton AJM, Bus SA. Diabetic Foot Ulcers and Their Recurrence. *N Engl J Med.* Jun 15 2017;376(24):2367-2375.
2. Armstrong DG, Tan TW, Boulton AJM, Bus SA. Diabetic Foot Ulcers: A Review. *Jama.* Jul 3 2023;330(1):62-75.
3. Pecoraro RE, Reiber GE, Burgess EM. Pathways to diabetic limb amputation. Basis for prevention. *Diabetes Care.* May 1990;13(5):513-521.
4. Singh N, Armstrong DG, Lipsky BA. Preventing foot ulcers in patients with diabetes. *Jama.* Jan 12 2005;293(2):217-228.
5. Rice JB, Desai U, Cummings AK, Birnbaum HG, Skornicki M, Parsons NB. Burden of diabetic foot ulcers for medicare and private insurers. *Diabetes Care.* 2014;37(3):651-658.
6. Zhang P, Lu J, Jing Y, Tang S, Zhu D, Bi Y. Global epidemiology of diabetic foot ulceration: a systematic review and meta-analysis (†). *Ann Med.* Mar 2017;49(2):106-116.
7. Margolis DJ, Kantor J, Berlin JA. Healing of diabetic neuropathic foot ulcers receiving standard treatment. A meta-analysis. *Diabetes Care.* May 1999;22(5):692-695.
8. Sheehan P, Jones P, Caselli A, Giurini JM, Veves A. Percent change in wound area of diabetic foot ulcers over a 4-week period is a robust predictor of complete healing in a 12-week prospective trial. *Diabetes Care.* Jun 2003;26(6):1879-1882.
9. Falanga V. Wound healing and its impairment in the diabetic foot. *Lancet.* Nov 12 2005;366(9498):1736-1743.
10. Rose JF, Giovinco N, Mills JL, Najafi B, Pappalardo J, Armstrong DG. Split-thickness skin grafting the high-risk diabetic foot. *J Vasc Surg.* Jun 2014;59(6):1657-1663.
11. Ramanujam CL, Stapleton JJ, Kilpadi KL, Rodriguez RH, Jeffries LC, Zgonis T. Split-thickness skin grafts for closure of diabetic foot and ankle wounds: a retrospective review of 83 patients. *Foot Ankle Spec.* Oct 2010;3(5):231-240.
12. Sood R, Roggy DE, Zieger MJ, Nazim M, Hartman BC, Gibbs JT. A comparative study of spray keratinocytes and autologous meshed split-thickness skin graft in the treatment of acute burn injuries. *Wounds.* Feb 2015;27(2):31-40.
13. Esteban-Vives R, Choi MS, Young MT, et al. Second-degree burns with six etiologies treated with autologous noncultured cell-spray grafting. *Burns.* Nov 2016;42(7):e99-e106.
14. Laschke MW, Menger MD. Vascularization in tissue engineering: angiogenesis versus inosculation. *Eur Surg Res.* 2012;48(2):85-92.
15. Zhao H, Chen Y, Zhang C, Fu X. Autologous epidermal cell suspension: A promising treatment for chronic wounds. *J Tissue Viability.* Feb 2016;25(1):50-56.
16. Hu ZC, Chen D, Guo D, et al. Randomized clinical trial of autologous skin cell suspension combined with skin grafting for chronic wounds. *Br J Surg.* Jan 2015;102(2):e117-123.
17. Blanpain C, Fuchs E. Epidermal homeostasis: a balancing act of stem cells in the skin. *Nat Rev Mol Cell Biol.* Mar 2009;10(3):207-217.
18. Yang R, Liu F, Wang J, Chen X, Xie J, Xiong K. Epidermal stem cells in wound healing and their clinical applications. *Stem Cell Res Ther.* Jul 29 2019;10(1):229.
19. Hu Z, Guo D, Liu P, et al. Randomized clinical trial of autologous skin cell suspension for accelerating re-epithelialization of split-thickness donor sites. *Br J Surg.* Jun 2017;104(7):836-842.
20. Huang S, Hu Z, Wang P, et al. Rat epidermal stem cells promote the angiogenesis of full-thickness wounds. *Stem Cell Res Ther.* Aug 8 2020;11(1):344.

21. Li X, Wu H, Yang H, et al. Efficacy and safety of epidermal cell regeneration for postoperative non-healing wounds: a randomized, partially blinded, parallel-controlled trial. *Stem Cell Res Ther.* Jun 3 2025;16(1):284.

22. Wang P, Hu Z, Cao X, et al. Fibronectin precoating wound bed enhances the therapeutic effects of autologous epidermal basal cell suspension for full-thickness wounds by improving epidermal stem cells' utilization. *Stem Cell Res Ther.* Sep 11 2019;10(1):154.

23. Wang P, Theocharidis G, Vlachos IS, et al. Exosomes Derived from Epidermal Stem Cells Improve Diabetic Wound Healing. *J Invest Dermatol.* Sep 2022;142(9):2508-2517.e2513.

24. Wang Z, Xu H, Yang H, et al. Single-stage transplantation combined with epidermal stem cells promotes the survival of tissue-engineered skin by inducing early angiogenesis. *Stem Cell Res Ther.* Mar 23 2023;14(1):51.

25. Xu H, Yang H, Wang Z, et al. Epidermal Stem Cell Derived Exosomes Alleviate Excessive Autophagy Induced Endothelial Cell Apoptosis by Delivering miR200b-3p to Diabetic Wounds. *J Invest Dermatol.* May 2024;144(5):1134-1147.e1132.

26. Yang H, Xu H, Wang Z, et al. Analysis of miR-203a-3p/SOCS3-mediated induction of M2 macrophage polarization to promote diabetic wound healing based on epidermal stem cell-derived exosomes. *Diabetes Res Clin Pract.* Mar 2023;197:110573.

27. Guo J, Dardik A, Fang K, Huang R, Gu Y. Meta-analysis on the treatment of diabetic foot ulcers with autologous stem cells. *Stem Cell Res Ther.* Oct 16 2017;8(1):228.

28. Gao W, Chen D, Liu G, Ran X. Autologous stem cell therapy for peripheral arterial disease: a systematic review and meta-analysis of randomized controlled trials. *Stem Cell Res Ther.* May 21 2019;10(1):140.