

## 1. Cover Page

**Official Study Title:**

Air-Dried Human Amniotic Membrane Terminally Sterilized by Gamma Irradiation for Multidisciplinary Clinical Application

**NCT Number:**

Not applicable

**Document Date:**

22 February, 2026

**Document Type:**

Study Protocol and Statistical Analysis Plan

## 2. Study Protocol

### 1. Background

Human amniotic membrane (hAM) is extensively applied in regenerative medicine due to its biocompatibility, extracellular matrix structure, and cytokine content. Terminal sterilization by gamma irradiation is required to ensure safety, but may affect structural and biochemical properties. Optimizing sterilization dose is critical for clinical application in ocular surface reconstruction and diabetic foot ulcer treatment.

### 2. Objectives

- **Primary Objective:** Evaluate the effect of gamma irradiation (15, 20, 25 kGy) on the structural integrity, sterility, and cytokine preservation of air-dried human amniotic membrane (AD-hAM).
- **Secondary Objective:** Assess preliminary clinical outcomes in ocular surface reconstruction and Wagner grade 3 Diabetic Foot Ulcer healing.

## Study Status

**Recruitment Status:** Not yet recruiting

**Study Start Date:** February 2026

**Primary Completion Date:** March 2026

**Study Completion Date:** December 2026 (Final data collection for all outcome measures)

**Study Phase:** Not Applicable

**Enrollment:** 25 placental donors; 4 preliminary clinical cases

**Enrollment Type:** Actual

**Last Known Status Update:** March 3, 2026

### 3. Study Design

- **Study Type:** Interventional (Clinical Trial)
- **Allocation:** Non-randomized, single-arm
- **Intervention Model:** Single group assignment
- **Primary Purpose:** Treatment
- **Estimated Enrollment:** 4 patient case series (clinical assessment)
- **Start Date:** February 2026
- **Primary Completion Date:** : March 2026
- **Study Completion Date:** March 2026

### 4. Eligibility Criteria

**Ages Eligible for Study:**

- Adults 18 years and older

**Sexes Eligible for Study:**

- All

**Accepts Healthy Volunteers:**

- No

**Inclusion Criteria**

1. Patients requiring ocular surface reconstruction due to chemical/thermal burns, persistent epithelial defects, or conjunctival defects.
2. Patients with chronic non-healing diabetic foot ulcers (DFU) of at least 4 weeks duration.
3. Ability to understand the study procedures and provide written informed consent.
4. For tissue donors (placenta): healthy mothers undergoing elective cesarean delivery with negative infectious disease screening (HIV, HBV, HCV, syphilis).

## **Exclusion Criteria**

1. Active systemic infection or sepsis.
2. Immunocompromised patients (HIV positive, ongoing immunosuppressive therapy).
3. Pregnant or breastfeeding women.
4. History of malignancy in the past 5 years.
5. Known allergy or hypersensitivity to human amniotic tissue or materials used in graft preparation.
6. Participation in another investigational clinical trial within 30 days prior to enrollment

## **5. Interventions**

- **Intervention Name:** Gamma-irradiated AD-hAM
- **Description:** Air-dried human amniotic membrane sterilized with cobalt-60 at 15, 20, or 25 kGy.
- **Mode of Administration:** Surgical graft placement for ocular or skin wound healing.

## **6. Outcome Measures**

### **Primary Outcome Measures:**

1. **Epithelialization and wound healing rate in ocular surface reconstruction**
  - **Measure:** Time to complete epithelialization and re-epithelialization of the conjunctival surface after AD-hAM transplantation
  - **Time Frame:** Up to 2 months post-surgery
  - **Description:** Assessed by slit-lamp examination and photographic documentation to confirm full epithelial coverage and graft integration
2. **Wound contraction and granulation tissue formation in diabetic foot ulcer (DFU)**
  - **Measure:** Percentage reduction in ulcer area and improvement in granulation tissue
  - **Time Frame:** Up to 120 days post-AD-hAM application
  - **Description:** Measured by clinical photographs and planimetric analysis of ulcer dimensions

### **Secondary Outcome Measures:**

1. **Preservation of structural integrity of AD-hAM**

- **Measure:** Histological assessment of epithelial and stromal layers
- **Time Frame:** Before and after gamma irradiation, stored for 1–2 years
- **Description:** Hematoxylin & eosin staining to evaluate tissue morphology, basement membrane continuity, and stromal organization
- 2. **Cytokine levels in AD-hAM after processing and sterilization**
  - **Measure:** Quantitative analysis of bFGF, TGF- $\beta$ 1, IL-6, and IL-8
  - **Time Frame:** After sterilization and storage for up to 2 years
  - **Description:** Measured by ELISA to assess preservation of bioactive molecules
- 3. **Microbial safety of AD-hAM**
  - **Measure:** Sterility and microbial impermeability
  - **Time Frame:** Up to 2 years post-sterilization
  - **Description:** Bioburden testing according to ISO 11737-1, microbial impermeability tested against representative Gram-positive and Gram-negative bacteria

## 7. Study Procedures

- Placenta collection and processing under aseptic conditions.
- Air-drying and gamma sterilization at designated doses.
- Biochemical and structural analysis (FTIR, total protein, ELISA for cytokines).
- Clinical application in ocular surface or diabetic foot ulcer patients.
- Follow-up and documentation of healing outcomes.

## 8. Ethical Considerations

- Approved by Bioethics Committee, Libyan Biotechnology Research Center, Tripoli (Ref No. BEC-BTRC6-2021).
- Conducted per Declaration of Helsinki and IAEA tissue banking protocol.

## 3. Statistical Analysis Plan (SAP) Summary

### 1. Objectives of Analysis

- Determine dose-dependent effects of gamma irradiation on protein content, cytokines, and structural integrity.
- Evaluate preliminary clinical efficacy of AD-hAM grafts.

### 2. Analysis Populations

- **Laboratory Assessment:** All collected AD-hAM samples (n=25)
- **Clinical Assessment:** Patients receiving AD-hAM grafts (n=4 cases)

### 3. Statistical Methods

- **Continuous Variables (protein, cytokine levels):**
  - Descriptive statistics (mean  $\pm$  SD)

- Comparisons using paired or unpaired Student's t-test
  - Significance threshold:  $p < 0.05$
- **Categorical Variables (clinical outcomes, sterility):**
  - Counts and percentages
  - Qualitative assessment of epithelialization and wound healing

#### **4. Missing Data Handling**

- Laboratory duplicates/triplicates used; missing values addressed using mean imputation.
- Clinical follow-up data reported as observed; no imputation due to small sample size.

#### **5. Software**

- GraphPad Prism v9.0

#### **6. Reporting**

- Results summarized in tables and figures (H&E, FTIR spectra, cytokine levels, wound photos).

#### **7. Overall Contact:**

- **Name:** Dr. Fawzi Ebrahim
- **Role:** Principal Investigator
- **Phone:** +218 916423664
- **Email:** fowzi.omar@yahoo.com