# Characterizing Partially Healed Wounds Treated With 3D Printed Adipose Grafts: Material Analysis

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## Introduction

Adipose has served as a successful scaffold for wound healing (1, 2) however the impacts of extracellular matrix (ECM) formation remain unstudied.

In dermal tissue, the interplay between cellular constituents and the extracellular matrix (ECM) serves as a cornerstone for physiological equilibrium and the wound healing process (3). Epithelial cells, located within the confines of the ECM, orchestrate vital functions encompassing the degradation of aged ECM components and new ECM synthesis, pivotal processes underpinning re-epithelialization and subsequent wound repair (3). Throughout the course of re-epithelialization, alterations in surface morphology and biomechanical properties manifest at the micro and nanoscale levels, signifying profound transformations within the wound bed (4).

The quantification of surface topography and characterization of tissue samples furnish indispensable insights into the dynamics of tissue regeneration, presenting a key avenue for understanding and assessing the efficacy of regenerative interventions.

In this study, we undertake a comprehensive characterization of 3D printed grafts crafted from adipose tissue, employing scanning electron microscopy (SEM). A full thickness wound model in a rat, described elsewhere (5) was used evaluating the wound healing of wounds treated with 3D printed adipose versus untreated wounds, thereby contributing to the refinement of future therapeutic strategies.

## Methods and Materials

**Bioink preparation -** Lipoaspirate human adipose fat was purchased from Zenbio (Durham, NC). The samples are first prepared using a tissue homogenizer (BioSpec, Bartlesvile, OK, USA). Samples are then micronized as described below using a series of micronizer blades (ROKIT Healthcare, South Korea).

Samples were prepared using a series of sharp blade systems with pore sizes from 4000 µm, 2400 µm ,600 µm and 200 µm in a push/pull manner using a 10 ml syringe. Samples were then transferred to 50 ml syringes and a saline solution was added with ratio of 2:5 v/v. Then, the bioink was left to rest for 20 min until two immiscible phases formed, and the saline was discarded.

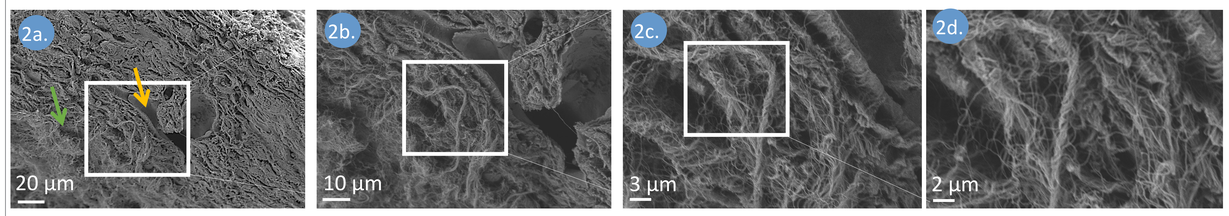
**3D printing grafts:** an FDA approved 3D printer (ROKIT Healthcare, South Korea) was used to create grafts. Briefly, the wound image area was scanned using AI assisted AiD region software (ROKIT, South Korea). Poly caprolactone (PCL) filament was 3D printed around perimeter of the wound area, and the adipose bioink was printed inside of the PCL region. A Peltier temperature control system reduced the temperature of the matrix plate to -20°C to solidify the 3D printed graft.

**Animal wound model:** 2 cm X 2 cm animal wound structure was created at the back of Sparge Dawley rat and frozen grafts were transferred into the wound structure and the grafts were covered immediately. The grafted were then explanted after 7 days and fixed using 10% (v/v) Paraformaldehyde solution.

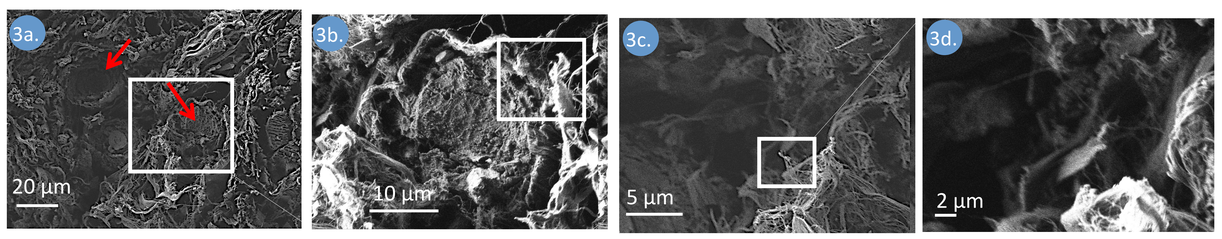
**SEM sample preparation:** Tissue samples were further fixed using 2% glutaraldehyde, 3% paraformaldehyde and 5% sucrose in phosphate buffer saline. Samples were then dehydrated using a series of ethanol solutions (v/v) (1:50, 1:75, 1:80, 1:90, 1:95,100%). Samples mounted on sample holders, and they were critically point dried with CO2 using a Tousimis (Rockville, MD, USA) followed by 20 nm sputter coating with palladium. A Zeiss Gemini 360 FE-SEM SEC system was used with a electron high tension (EHT) of 3.00 KV and with an SE2 detector.

## Bio-fabrication of Human Adipose Graft


### Control – 7D Explanted Wound with No Graft




### Treatment Group – 7D Explanted Wounds Treated with Human 3D Printed Adipose Grafts




Scanning electron microscopy of explanted wound with no graft after 7 days (Figure 2 - top row) and explanted human adipose after 7 days (Figure 3 - bottom row). (a-d) collagen fiber structure is observed in the wound matrix at the control with relatively less density. (Figures 2 and 3) Adipose grafts poses debris and various cell structure in the grafts. (2a) SEM of the fibrillated collagen in the wound matrix (green), along with microvasculature formation (yellow). The scale bar set for 20 µm. (2b) Enlarged view of segment of the image in (A) with scale bar of 10 µm. Enlarged view of (2c) fibrillated collagen & (2d) collagen fibers. (3a) SEM image of adipose graft showing various cells and dense tissue structure. The scale bar represent 20 µm. (3b) Enlarged view of (3c) showing the cell geometry and cell membrane morphology. Scale bar is 10 µm. (3d) Enlarged view of image F showing the membrane structure of the cell geometry. The scale bar represent 5 µm. (H) Enlarged view of the cell membrane. Scale bar = 2 µm.

## Discussion

The scanning electron microscopy (SEM) analysis of cross-sectional images of explanted wound samples offers valuable insights into the morphological characteristics of the tissue within the wound bed. Specifically, examination of the morphological structure of control wounds without grafts at the 7-day mark reveals a fibrillated collagen structure with distinctive large porosity evident in the extracellular matrix. This observation underscores the dynamic remodeling processes occurring within the wound microenvironment.

Furthermore, the presence of tubular structures within the tissue confirms the existence of microvasculature, indicative of the initiation of angiogenesis within the wound bed. This early angiogenic response suggests the onset of re-epithelialization and cell infiltration, highlighting the intricate interplay between cellular processes and the surrounding ECM during wound healing initiation.

In contrast, analysis of adipose graft explanted tissue reveals a notably different morphology. The presence of a high cell density within the matrix structure suggests that tissue re-epithelialization has progressed to a more advanced stage compared to the non-grafted wounds. The observed cellular structures, varying in size and geometric arrangement, likely encompass various types of fibroblasts and macrophages. This diversity in cell types indicates a complex cellular milieu enriched with growth factors, immune mediators, and regenerative cytokines, which collectively promote tissue regeneration and facilitate wound healing.

The distinct morphological features observed in the adipose graft explanted tissue underscore the potential efficacy of adipose grafts in enhancing tissue regeneration and wound healing processes. Further elucidating the cellular and molecular mechanisms underlying these observations may unveil novel therapeutic strategies aimed at optimizing wound healing outcomes.

## Conclusions

- SEM analysis highlights distinct morphological differences between wounds with and without adipose grafts, showcasing the potential of adipose grafts to enhance tissue regeneration.
- Presence of fibrillated collagen and tubular microvasculature in adipose graft explanted tissue indicates advanced re-epithelialization and angiogenesis, fostering an optimal environment for tissue repair.
- High cell density and diverse cellular structures in adipose graft explanted tissue suggest enrichment of growth factors, immune mediators, and regenerative cytokines, further supporting the efficacy of adipose grafts in promoting wound healing.

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