Personalized Extrusion-Based 3D Printing of Human Lipoaspirate Scaffolds for Accelerated Healing of Full Thickness Wounds in Rats

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Introduction

Chronic wounds present a formidable challenge in healthcare due to their intricate and diverse healing environments, varying significantly among patients. Addressing this complexity, we explore the use of personalized scaffolds generated through extrusion-based 3D printing to revolutionize the treatment of complex chronic wounds. This innovative approach allows tailoring of the scaffolds to individual patient needs, which is particularly crucial for healing to take place in chronic wound beds (1).

In this investigation, an FDA-approved extrusion 3D printer was employed to fabricate scaffolds from human lipoaspirate, subsequently evaluated for their healing efficacy. A full-thickness defect model in Sprague Dawley rats, featuring large-sized defects (2 cm x 2 cm), served as a challenge model for complete healing, enabling us to discern differences compared to conventional advanced wound dressings, such as amniotic membranes, at a partially healed stage at 7 days. An additional time point of 21 days was introduced to assess complete wound healing and compare outcomes against controls.

Histological analysis was employed to ascertain cell infiltration levels, granulation tissue formation, and re-epithelialization at each time point. This study presents a promising avenue for advancing personalized wound care strategies, demonstrating the potential of extrusion-based 3D printing in tailoring scaffolds for enhanced and accelerated healing of chronic wounds.

Methods

1. Survival of Micronized Adipose Cells
To test the survival of the adipose tissue cells and morphology after the processing through the adinizer blades, live & dead cell assays (Invitrogen, L3224) and morphology characterization by H&E staining were performed on the freshly harvested human lipoaspirate and a micronized adipose tissue sample. Micronized adipose tissue was prepared by passing a volume of lipoaspirates serially through the four adinizer blades (4000, 2600, 600, 2000 um).

For the live & dead cell assay, the lipoaspirate and micronized adipose tissue samples were both washed twice with the 1X Dulbecco's Phosphate Buffered Saline (D-PBS, Welgene, LB 001-02), followed by incubation in 2 µM calcein AM and 4 µM EthD-1 solutions at 30℃ . Observations made with the fluorescent microscope (Nikon, Ts2-FL).

2. Full Thickness Wound Model in Rats
Frozen 3D printed grafts were prepared by Tides Medical using an FDA-approved 3D printer. Grafts were kept frozen until time of implantation in the wound. Two 2x2 cm full thickness wounds were created on the dorsal side of each rat, along either side of the spine. Each 2x2cm implant was placed into a full thickness wound, and implant location was randomized in location among the rats. Healing was assessed at two time points, 7 days and 21 days.
Histological analysis of each explanted wound was completed using H&E staining, where cell nuclei are stained purple and the extracellular matrix (ECM) is represented in pink. The resulting histological images were analyzed for re-epithelialization and granulation tissue formation, with each image being assigned a numerical rating from 0 to 10 for each category.

1.

Figure 1. Adipose bioink being micronized with an adinizer .

2.

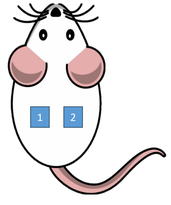


Figure 2. Implantation scheme for full thickness wound study in rats..

Results

3.

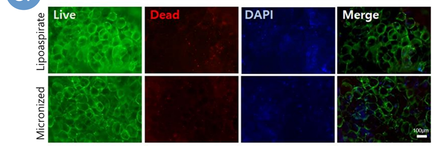


Figure 3. a) Live & dead cell assay analysis on human lipoaspirate (top row) and micronized adipose tissue (bottom row).

4.

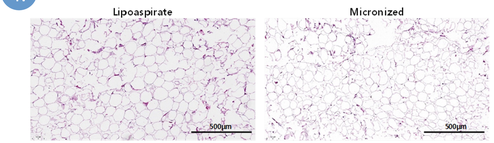
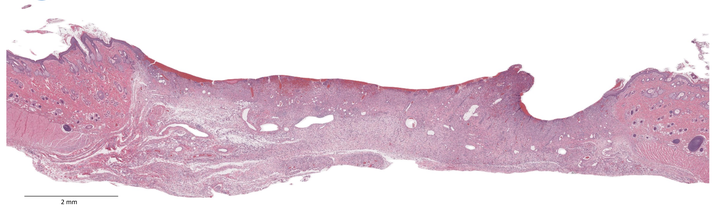


Figure 4. Morphology characterization via H&E staining of human lipoaspirate (left) and micronized adipose tissue (right).

5a.



5b.

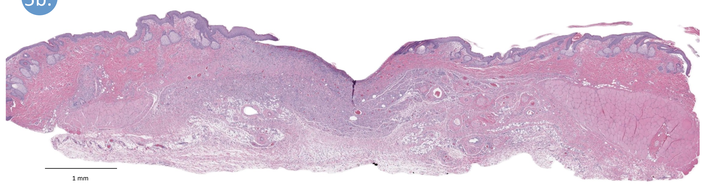


Figure 5. Histological image of adipose bioink implantation sites after 7 days (A) and 21 days (B).

6a.

6b.

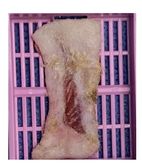
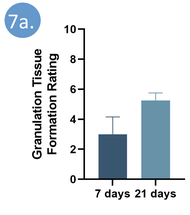


Figure 6. Explanted wound sites. (A) Adipose treated wound at 7 days. (B) Adipose treated wound at 21 days.

7a.



7a.

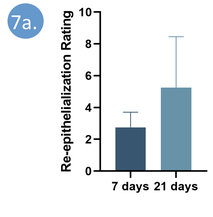


Figure 7. Granulation tissue formation ratings at 7 days and 21 days (A). Re-epithelialization ratings at 7 days and 21 days (B).

Discussion

The first experiment focused on assessing cell viability and morphology following adipose tissue micronization. Live/dead cell assays demonstrated comparable viability levels between original lipoaspirate and micronized adipose tissue (Figure 3). Microscopic analysis further confirmed similar cellular morphology between the two sample types, validating the suitability of micronized adipose tissue for scaffold bioink formulation (Figure 4).

The second experiment focused on determining the impacts of adipose bioink on healing in a full thickness rat wound model.

A skin wound is healed once the wound is re-epithelialized with keratinocytes. Notably keratinocytes never lead the charge across a healing wound. Rather, keratinocytes require a collagen extracellular matrix with supporting fibroblasts and vasculature to grow on. Thus, for typical skin wounds, a vascularized provisional granulation tissue is first deposited in the tissue deficit followed by keratinocyte migration and proliferation to close the wound (1).

At 1 week after the application of the adipose bioink graft, granulation tissue is observed filling the wound bed. The natural wound healing response is evident where granulation tissue is being generated to fill the wound bed in order to support keratinocyte migration, proliferation, wound contraction, and ultimately, healing of the wound.

By 21 days, increased granulation tissue formation and re-epithelialization have occurred. No adverse effects were observed. The adipose bioink was found to elicit a biological response, resulting in the rapid fill of the skin defects with vascularized granulation tissue.

Conclusions

- Micronized adipose tissue maintains comparable cell viability and morphology to original lipoaspirate, validating its suitability for scaffold bioink formulation.
- No adverse effects were noted following application of adipose bioink to full thickness wounds.
- The research showcases the efficacy of innovative 3D-printed adipose bioink for wound healing applications.
- Histological analysis reveals granulation tissue formation, epithelialization, and neovascularization at 7 days that is further increased by 21 days.

REFERENCES

1. Chan BP, and Leong KW, Scaffolding in Tissue Engineering: General Approaches and Tissue-specific Considerations, Eur Spine J (2008) 17 (Suppl 4):S467-79.
2. Pastar, I., Stojadinovic, O., Yin, N. C., Ramirez, H., Nusbaum, A. G., Sawaya, A., Patel, S. B., Khalid, L., Isseroff, R. R., & Tomic-Canic, M. (2014). Epithelialization in wound healing: A comprehensive review. Advances in Wound Care, 3(7), 445–464.