Novel 3D Printed Scaffolds Using Adipose Bioink: Wound Healing in a Rat and a Pig Model

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This poster was presented at the 2024 Symposium on Advanced Wound Care (SAWC) Spring Meeting

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Introduction

Wound healing remains a significant challenge in medical practice, particularly for chronic wounds where conventional treatment approaches often fall short. Regenerative medicine, with its focus on developing novel scaffolds to promote tissue repair and regeneration, has seen promising advancements in recent years. (1) Some in the field are exploring the use of adipose scaffolds. (2, 3) One such innovative approach involves the utilization of three-dimensional (3D) printed scaffolds containing autologous adipose tissue (4).

In this study, we investigate the efficacy of novel 3D printed scaffolds utilizing adipose bioink for wound healing applications. The scaffold fabrication process involves the use of an FDA approved extrusion-based 3D printer to print with bioink derived from human lipoaspirate. Notably, the bioink preparation includes meticulous micronization steps, achieved by passing the lipoaspirate through a series of adinizer blades, resulting in a bioink with optimized printing characteristics.

To evaluate the performance of the 3D printed scaffolds, we conduct a series of experiments employing relevant animal models and histological assessments. First, a rat full-thickness defect model was utilized to assess the formation and maturation of granulation tissue and re-epithelialization. Histological analysis, including hematoxylin and eosin (H&E) staining, was performed on specimens harvested at specific time points to evaluate tissue regeneration dynamics.

Subsequently, the survival and morphological integrity of adipose tissue cells following the micronization process were investigated using live and dead cell assays and H&E staining. These experiments were crucial in determining the viability and suitability of the processed adipose tissue for scaffold bioink formulation.

Finally, to mimic more closely the use of an autologous lipoaspirate, a pig full-thickness wound model was employed to assess the therapeutic efficacy of the 3D printed autologous extracellular matrix (ECM) patches. Histological examination using Masson's trichome staining was conducted to evaluate the thickness of the epidermal layer post-treatment, providing insights into the regenerative potential of the 3D printed scaffolds.

Materials & Methods

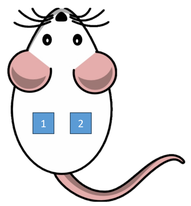
Two sets of experiments were conducted:

1 To assess the formation and maturation of granulation tissue and re-epithelialization, a full thickness defect model in a rat was used that is described elsewhere (1). H&E staining was conducted on specimens harvested at 7 and 21 days.

The adipose scaffolds were 3D Printed using an FDA approved 3D printer with a bioink made of human lipoaspirate. The bioink was prepared by passing a volume of lipoaspirate serially through four adinizer blades (4000, 2600, 600, 2000 um). To fit the defects in the model, 2 x 2 x 0.2 cm grafts were 3D Printed inside a PCL frame, onto a frozen print platform.

2. Autologous fat was used in a full thickness wound in a pig model to evaluate the thickness of the epidermal layer after 14 days of healing. Masson's trichome staining was performed on histological sections. Test groups include: Controls (n=4), at day 14 post-wounding with an application of the 3D printed autologous 3D printed graft (autologous ECM, n=4), and a control at day 14 post-wounding with no graft (control, n=4).

2a.



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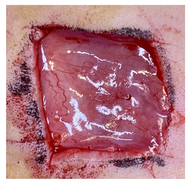

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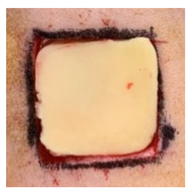

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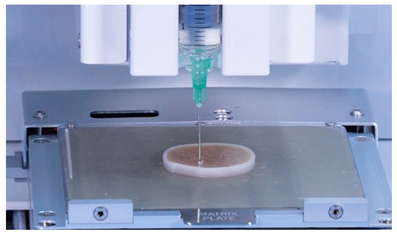
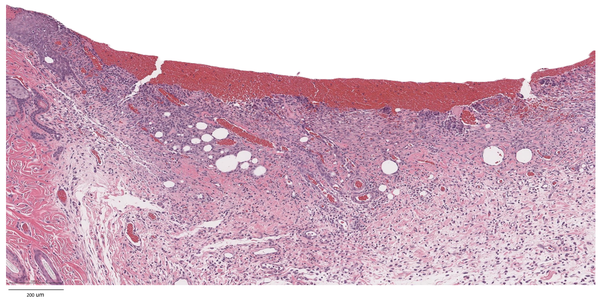


Figure 1. a) 3D printer printing adipose bioink b) dorsal location of full thickness wounds in model c) Empty 2 x 2 cm defect d) 3D printed graft placed at time of surgery

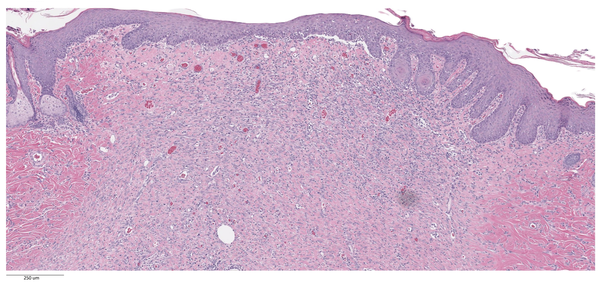
Results

Figure 3. Rat full thickness defect containing 3D printed graft at (a) 7 and (b) 21 days of healing.

3a.

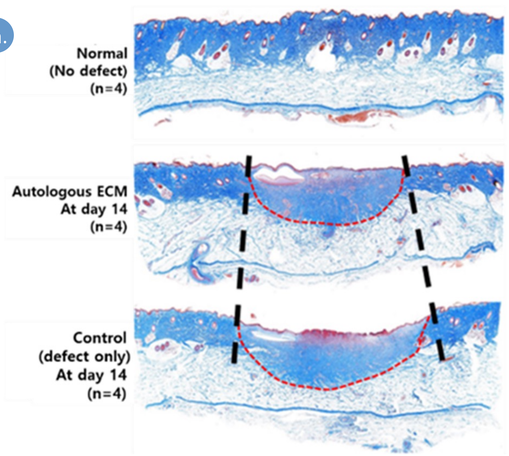


3b.



Boundary of granulation tissue

4a.



4b.

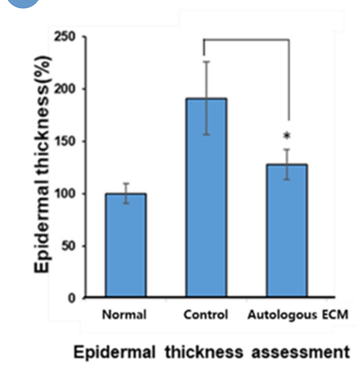


Figure 4 a) Phenotypic characterization of unwounded and wounded pig skin at day 14 post-autologous ECM treatment through a Masson's trichrome analysis b) epidermal thickness assessment.

Discussion

This study integrates various experimental approaches to assess the effectiveness of novel 3D printed grafts utilizing adipose bioink in a wound healing model, offering significant implications for advancement of the field.

In the initial experiment, typical stages of wound healing progression were observed. Initially, the wound bed filled with granulation tissue, followed by the gradual coverage of the wound by epithelial tongues. Histological examination at days 7 and 21 revealed an increasing amount of granulation tissue, which progressively densified and matured over time (Figure 3a and 3b). Moreover, signs of neovascularization were evident at later time points, indicating ongoing tissue remodeling and repair (Figure 3b).

In the final experiment, evaluation of 3D printed autologous extracellular matrix (ECM) grafts on full thickness wounds in a pig model revealed significant histological improvements (Figure 4a). Wounds treated with 3D printed autologous bioink displayed increased collagen deposition and more evenly distributed epidermal layers, resembling unwounded skin samples. Additionally, reduced granulation and diminished epidermal thickness were observed (Figure 4b), indicative of enhanced tissue maturation and structural integrity. These findings underscore the efficacy of treatment with 3D printed autologous bioink in facilitating a natural wound healing process while preserving tissue architecture, suggesting promising therapeutic potential for wound management.

Conclusions

- Overall, the comprehensive assessment of scaffold performance and treatment efficacy in these two animal studies provide valuable insights into tailored therapeutic interventions for addressing diverse wound healing challenges in regenerative medicine.
- The pig study demonstrates the effectiveness of novel 3D printed scaffolds utilizing autologous adipose bioink for wound healing applications.
- Histological analysis reveals progressive stages of wound healing, including granulation tissue formation, epithelialization, and neovascularization.
- Treatment with 3D printed autologous bioink promotes collagen deposition, enhances tissue maturation, and preserves original tissue architecture, fostering a natural wound healing process.
- These findings highlight promising avenues for the development of tailored therapeutic interventions in regenerative medicine to address various wound healing challenges.

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