Proteomic Analysis of Adipose Forms Used in 3D Printing Grafts for Healing Chronic Skin Wounds

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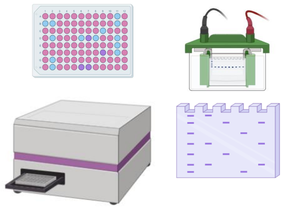
Introduction

Aplicor 3D is an FDA-cleared 3D printer that uses autologous human adipose tissue to create patient-specific wound coverings [1]. To solidify the soft adipose tissue into a graft that can be handled and applied to the wound bed, one of two minimally manipulative processing methods is employed: (i) freezing the graft, or (ii) crosslinking the graft via fibrin–thrombin chemical crosslinking. However, the effects of these different methods on the protein content of the resulting grafts remain unclear. In this study, three different assays are used to characterize and compare the protein content of grafts prepared by these methods. In addition, an Alamar Blue cell proliferation assay is performed to assess and compare keratinocyte proliferation on grafts produced using the frozen versus the fibrin–thrombin methods [2]. Results from the biomarker and cytokine analyses, as well as the cell proliferation data, provide insight into the choice between frozen or fibrin–thrombin grafts for clinical use [3].

Materials & Methods

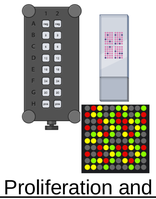
Tissue extraction and Digestion

Adipose was micronized using the Adinizer kit and crosslinked with Fibrin. Micronized adipose, Micronized adipose fibrin gel, and Non-Micronized Adipose were digested in TPer and Collagenase type I for 24 hours and centrifuged for 7 minutes at 10,000 rpm. The digested tissue was used for further studies.



Total Protein Assay and SDS-PAGE

The digested tissue was analyzed for its total protein content using Total Protein Assay where the absorbance readings were taken to assess the protein content. SDS-PAGE was later used to sperate the proteins in different samples based on size using the Invitrogen Gel tank.

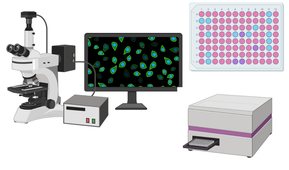


Cytokine Microarray

Angiogenic, immune-modulating and regenerative cytokines were quantified using the Human Quantibody cytokine Microarray Q4000 (RayBiotech, Peachtree Corners, GA). Using GraphPad Prism, the data was two-way ANOVA for each cytokine.

Cell Proliferation and Viability

Samples were incubated for 2, 3, 7 and 9 days. Alamar blue assay was performed to determine cell proliferation and was quantified using a BioTek Synergy Neo 2 reader (Agilent, Santa Clara, CA).



Results

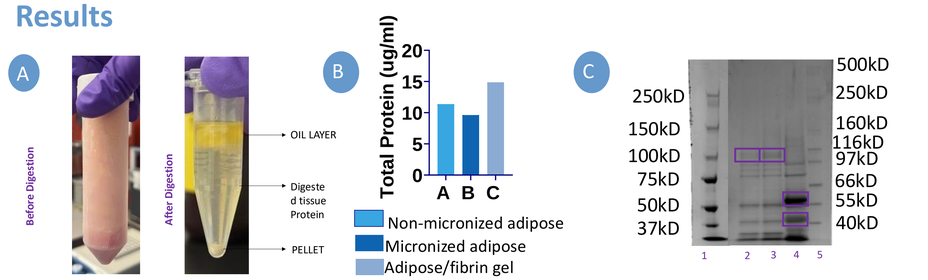


Figure 1: Tissue Extraction and Digestion and SDS PAGE: (A) The tissue samples have been digested with Collagenase type 1 and 3, and different layers (Fat layer, Digested Tissue protein and the pellet) can be observed after centrifugation. (B) Total Protein assay shows the protein concentration (ug/ml) in the digested tissue samples. (C) SDS PAGE was performed on the protein layer and separation differences can be observed on the gel. (2 is Non- micronized, 3 is Micronized and 4 is micronized fibrin gel)

Cytokine	Role in Wound Healing
TGF-β1	Stimulates collagen production, strengthens the wound.
TGF-β3	Reduces scarring by balancing collagen deposition.
TGF-β2	Controls inflammation, preventing excessive scar tissue.
PDGF-Rβ	Promotes blood vessel growth, ensuring oxygen and nutrients for healing.
FGF-4	Encourages skin and connective tissue cell growth.
FGF-7	Helps new skin form by promoting epithelial cell multiplication.
ANG-2	Works with other growth factors to form new blood vessels.
ANG-1	Strengthens newly formed blood vessels.
Angiostatin	Prevents excessive blood vessel formation.
PDGF-AA	Recruits fibroblasts to rebuild tissue.
VEGF	Stimulates new blood vessel growth, ensuring oxygen delivery.

Table 1 Wound Healing Cytokines Table showing 11 cytokines with most activity in wound-healing.

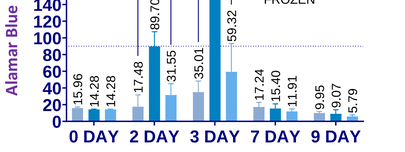


Figure 2. Keratinocyte Cell Proliferation: Keratinocyte Cell proliferation on Non-micronized, micronized and fibrin crosslinked adipose grafts.

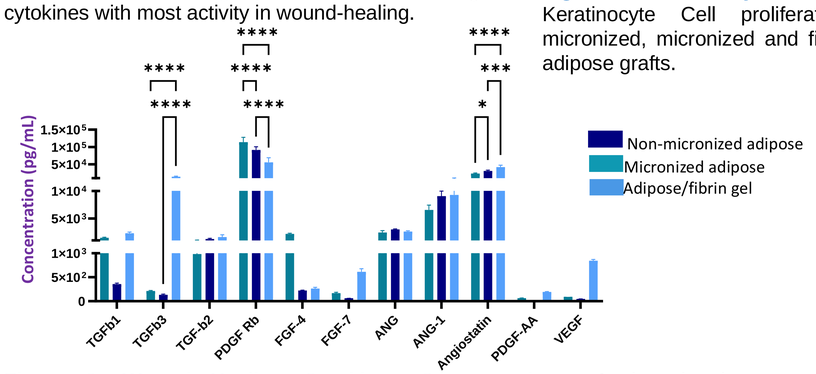


Figure 3: Wound Healing Cytokines Concentrations: Angiogenic, immune-modulating and regenerative cytokines were quantified using the Human Quantibody cytokine Microarray Q4000 (RayBiotech, Peachtree Corners, GA) was analyzed using 2-way ANOVA

Discussion

- Layer formation can be observed before and after the digestion, where upon digestion with TPer and Collagense type 1, three distinct layers (oil layer, Protein, and pellet) can be observed.
- The results of total protein assay showed the highest total protein in Fibrin gel (15ug/ml) and the lowest total protein in Micronized Adipose (9.6 ug/ml).
- SDS-PAGE shows bands at 55kDa and 40kDa in the Fibrin gel that cannot be observed in the other samples, whereas there are bands that can be observed at 97kDa in both Micronized and Non-micronized Adipose that cannot be seen in the Fibrin gel.
- Human Cytokine Array confirmed the presence of 200 cytokines, and the wound healing cytokines were abundantly present in both non-micronized adipose and adipose/fibrin gels. Cytokines PDGFRb were significantly higher in micronized adipose, while TGFb3 and Angiostatin were significantly higher in the adipose/fibrin gel samples.
- Cell proliferation was higher on the micronized frozen method compared to the fibrin method on Day 3. In addition, the keratinocyte cell proliferation showed keratinocyte reached to 100% confluency on micronized adipose at Day 3 while this value was 59% for fibrin gel.

Conclusions

- Keratinocytes Cell Proliferated most on Micronized Frozen Adipose on 3rd day.
- Human Cytokine Microarray confirmed the presence of Immune modulating, Regenerative and Angiogenic Cytokines in the Adipose tissue samples, essential for wound healing.
- This comprehensive proteomic analysis underscores the importance of manufacturing processes in optimizing adipose-derived grafts for 3D printing applications. By mapping the biomarker profiles through various stages of adipose processing and evaluating the effects of processing, this study provides valuable insights into the selection of optimal adipose forms for graft fabrication. The findings contribute to the growing body of knowledge in regenerative medicine and offer practical implications for the development of personalized wound healing therapies.

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