A Tissue Engineered Skin Model for Studying Wound Healing In Vitro

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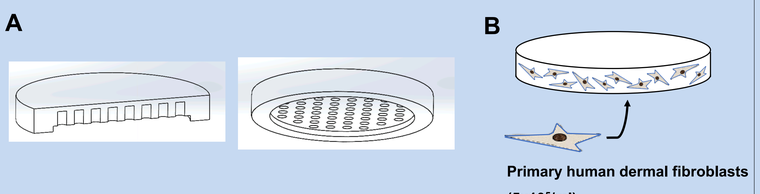
Background

Human skin has a complex array of functions and abilities such as preventing infection, regulating temperature, and healing upon physical injury. Existing tissue-engineered (TE) skin models have rarely been used to study healing, despite the continued prevalence of chronic wounds which reduce patient quality of life and often precede serious events such as amputation.

Digital light processing (DLP) bioprinting can be used to form detailed three-dimensional geometries from photo-crosslinkable hydrogels. Gelatin methacryloyl (GelMA) and hyaluronic acid methacrylate (HAMA) are biocompatible photo-crosslinkable hydrogels derived from collagen and hyaluronan respectively, both significant components of the skin's extracellular matrix (ECM).

Research Objective: Design a TE skin model that closely mimics the dermal and epidermal layers to perform systematic experiments of wound healing *in vitro*.

Tissue-Engineered Dermal Model



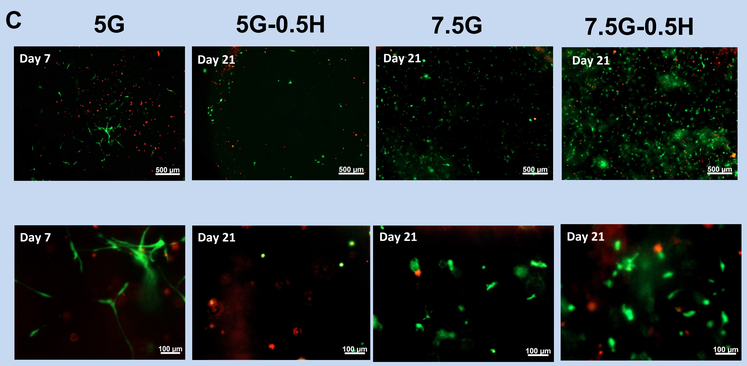


Figure 1. A) Three-dimensional (3D) geometry of the bioprinted construct. B) Schematic showing the distribution of fibroblasts within the bioprinted construct. C) Live (green) and dead (red) staining of fibroblasts laden in different hydrogel conditions after 21 days of culture.

A three-dimensional (3D) geometry for the dermal model containing partial pores for media penetration and a flat upper surface for eventual keratinocyte seeding was designed using SOLIDWORKS software (Fig. 1A). Human neonatal dermal fibroblasts were distributed in photo-crosslinkable hydrogel solutions at 5 x 10^5 cells/ml. Cell laden hydrogel was bioprinted using a DLP apparatus to form crosslinked cell laden constructs. Cell viability was evaluated in four hydrogel conditions: 5% GelMA (5G), 5% GelMA + 0.5% HAMA (5G-0.5H), 7.5% GelMA (7.5G), and 7.5% GelMA + 0.5% HAMA (7.5G-0.5H). Culture of 5G was discontinued after 7 days as most cells had died. Culture of the other three hydrogel conditions was continued to 21 days where they all still showed qualitatively high cell viability. The 7.5G-0.5H condition was chosen for further experiments due to the high viability, increase in number of cells, and presence of spread fibroblasts after 21 days.

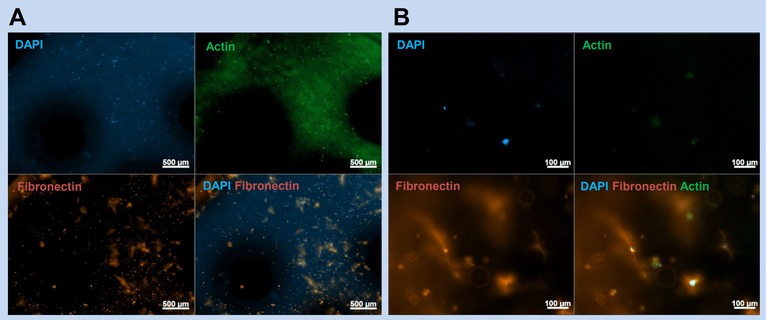
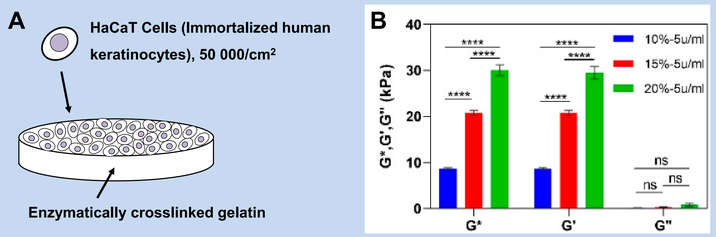


Figure 2. Immunofluorescence staining of fibroblasts in 7.5G-0.5H after 7 days of culture at low magnification (A) and high magnification (B). DAPI shows cell nuclei, actin shows the cytoskeleton, and fibronectin is a skin ECM protein deposited by fibroblasts.

It is important that the fibroblasts secrete ECM proteins and growth factors as they do *in vivo*. To evaluate this *in vitro*, we performed immunofluorescence staining of the TE dermal model after 7 days of culture (Fig. 2 A,B). Fibronectin, a skin ECM protein, was observed within fibroblasts and also in the surrounding hydrogel (Fig. 2B). To verify that fibronectin detection in the hydrogel was not the effect of non-specific binding, a control hydrogel without cells was similarly stained and showed minimal fluorescence (results not shown).

Epidermal Model



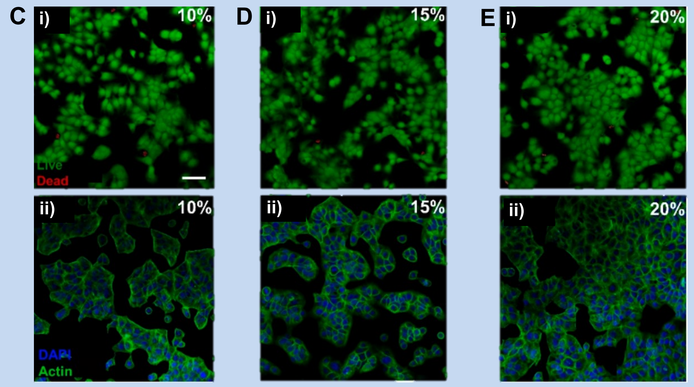
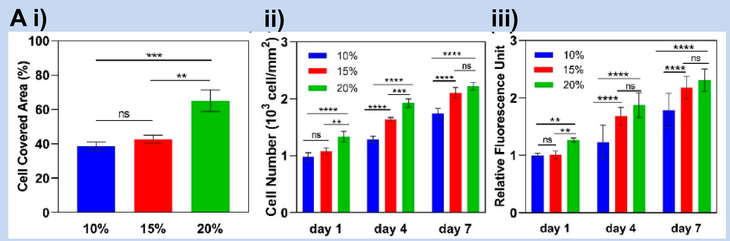


Figure 3. A) Schematic of the epidermal model. B) Mechanical properties of enzymatically crosslinked 10%, 15%, and 20% gelatin. C) Live/dead (i) and DAPI/Actin (ii) staining of HaCaT attachment to the gelatin hydrogel after 24 hours.

The epidermal model (Fig. 3A) consists of a layer of HaCaT cells cultured on enzymatically crosslinked gelatin. Mechanical testing showed that higher concentration gelatin solutions formed stiffer hydrogels (Fig. 3B). HaCaT cells show preferential attachment and proliferation on the stiffer, 20% gelatin substrate in comparison to 10% and 15% gelatin (Fig. 4A). To develop a stratified epidermis, confluent HaCaT cells on 20% gelatin (Fig. 4B) were raised to an air-liquid-interface (ALI) and cultured for 6 weeks (Fig. 4C). Filaggrin is a marker of differentiated keratinocytes of the stratum corneum, the outermost layer of the epidermis. By week 6 of ALI culture, the epidermal layer has thickened and formed the expected pattern of filaggrin expression in its superficial layers.



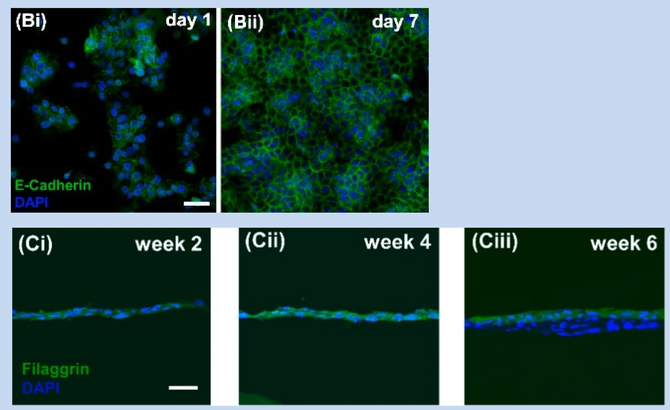


Figure 4: A) Characterization of HaCaT attachment and proliferation on 10-20% gelatin: Ai) initial cell coverage, Aii) cell number on days 1, 4, and 7, and Aiii) metabolic activity on days 1, 4, and 7. B) Immunofluorescence staining of HaCaT cells on 20% gelatin on days 1 and 7. C) Immunohistochemical staining of the construct cross-section after 2, 4, and 6 weeks of culture at the ALI.

Conclusion

We are in the process of developing a TE skin model for studying wound healing *in vitro*. A TE dermal model, with high cell viability and ECM expression, and an epidermal model that formed a stratified epidermis have both been designed and verified. Future work includes combining the dermal and epidermal models into a dermal-epidermal TE skin model that can be used to study healing *in vitro*. It is expected that the presence of fibroblasts will lead to a better organization of the epidermis.