A Tissue-Engineered Model of the Epidermis and Dermis developed using Digital Light Processing (DLP) Bioprinting

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Introduction

Human skin has a complex array of functions and abilities such as preventing infection, providing sensation, and healing upon physical injury [1]. Tissue-engineered skin models have historically been used as skin grafts or as *in vitro* models for studying drug efficacy and toxicity, cosmetics, or wound healing [2, 3]. However, many functions of skin are dependant on structures with complex three-dimensional (3D) geometries such as vasculature, sweat glands, nerves, and pilosebaceous units which are challenging to replicate in tissue-engineered skin models.

Digital light processing (DLP) bioprinting can be used to form detailed three-dimensional (3D) geometries from photo-crosslinkable hydrogels. Gelatin methacryloyl (GelMA) is a biocompatible photo-crosslinkable hydrogels derived from collagen which is the majority component of the skin's extracellular matrix (ECM). Here, we introduce the potential of using DLP bioprinting to fabricate tissue-engineered skin from a GelMA hydrogel (Fig. 1).

DLP Bioprinting for Skin Tissue Engineering

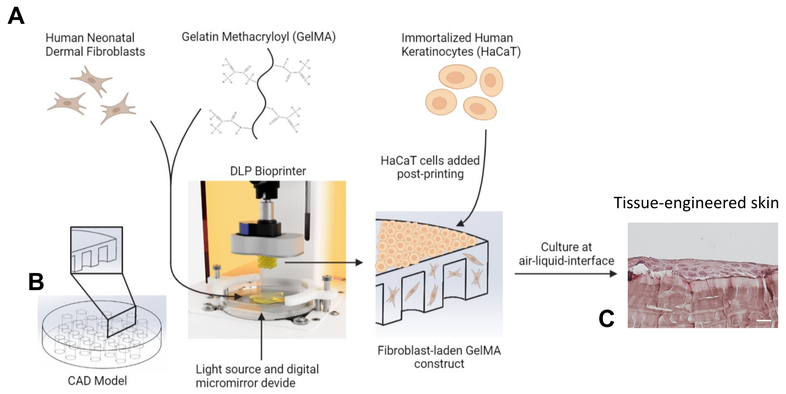

Figure 1: Schematic of the digital light processing (DLP) bioprinting workflow. (A) human primary fibroblasts and GelMA are combined and photo-crosslinked in the geometry of the input CAD file (B) by the DLP bioprinter (Lumen-X, CELLINK). HaCaT cells are added to the upper surface and cultured at an air-liquid-interface to form a stratified epidermis (C), indicating the tissue-engineered skin is mature. Scale bar = 100 µm.

To characterize the printability of small features, vertical pores with diameters of 1000, 750, 500, and 250 µm were bioprinted in 5, 7.5, and 10% GelMA (Fig. 2). All pore sizes formed in 10% GelMA while the 5% GelMA mesh had numerous defects and neither the 250 or 500um pores remained hollow. All pores sizes except for 250 µm formed successfully in the 7.5% GelMA with few defects.

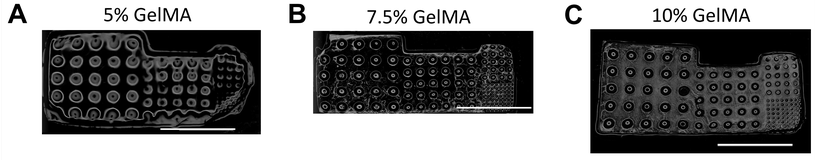

Figure 2. Printability of vertical pores in (A) 5% GelMA, (B) 7.5% GelMA and (C) 10% GelMA with DLP bioprinting. The pores have diameters of 1000 µm (left) , 750 µm (middle), 500 µm (top right), and 250 µm (bottom right). Scale bars = 10 mm.

Optimization of the Dermal Hydrogel

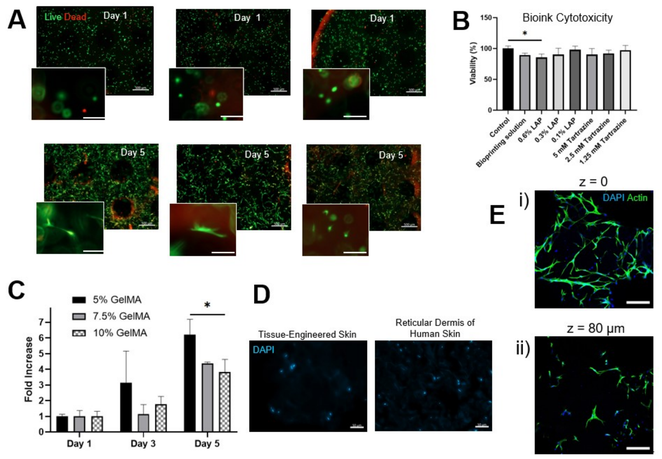

Figure 3: Optimization of DLP bioprinted GelMA hydrogel constructs to mimic the human dermis. (A) Live (green) and dead (red) stain shows dermal fibroblast viability and morphology in 5, 7.5, and 10% GelMA. Scale bars = 500 µm at low magnification and 100 µm in the inset images. (B) Quantification of the toxicity of the photo-initiator (LAP) and photo-blocking dye (tartrazine) required for DLP bioprinting. (C) Analysis of the metabolic activity of fibroblasts in 5, 7.5, and 10% GelMA over 5 days of culture. (D) Comparison of the cell density in 5 µm cross-sections of tissue-engineered skin with $0.5x10^6$ cells/ml vs. human reticular dermis. Scale bars = 50 µm (E) Confocal images of cell distribution on the lower surface (i) and interior (ii) of fibroblast-laden 7.5% GelMA constructs. Scale bars = 100 µm.

Primary human dermal fibroblasts DLP bioprinted in 5, 7.5, and 10% GelMA showed high viabilities 1 and 5 days after printing (Fig3A). The cytotoxicity influence of LAP and tartrazine at 0.3% (w/v) and 2.5mM respectively on fibroblasts after 2 hours was negligible (Fig 3B). Fibroblast proliferation in all three GelMA concentrations was quantified over 5 days of culture (Fig. 3C), showing exponential cell growth in all conditions. Both the cell-laden hydrogel and human reticular dermis were sliced into 5 µm cross-sections and stained with DAPI to show cell nuclei (Fig. 3D). The number cells in each are similar, verifying the relevance of the chosen fibroblast density of 0.5 x 10^6/ml. After 5 days, confocal images show spread fibroblasts on the bottom surface and interior of 7.5% GelMA constructs.

Verification of Epidermis Formation

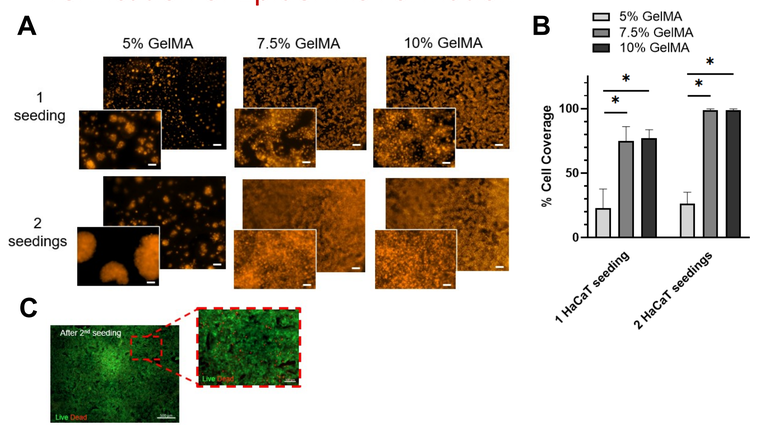

Figure 4: Suitability of DLP bioprinted GelMA constructs as a substrate for tissue-engineered epidermis. (A) HaCaT cell attachment to 5, 7.5, and 10% GelMA after 1 and 2 seedings of 0.5 x 10^6 cells/cm². (B) Quantification of HaCaT attachment. (C) Live and dead stain of the confluent HaCaT layer after 2 seedings. Scale bars = 500 µm at low magnification and 100 µm in inset images.

HaCaT attachment to 5, 7.5, and 10% GelMA constructs was characterized and quantified (Fig. 4A,B), showing that a confluent layer was formed on 7.5% and 10% GelMA after 2 seedings on consecutive days. A live and dead stain shows that the cells in the epidermal layer are almost exclusively alive (Fig. 4C). 7.5% GelMA was chosen for further experiments due to its sufficient printability, high fibroblast viability and proliferation, and suitability to form a confluent HaCaT layer after 2 seedings.

Maturation at the Air-Liquid-Interface

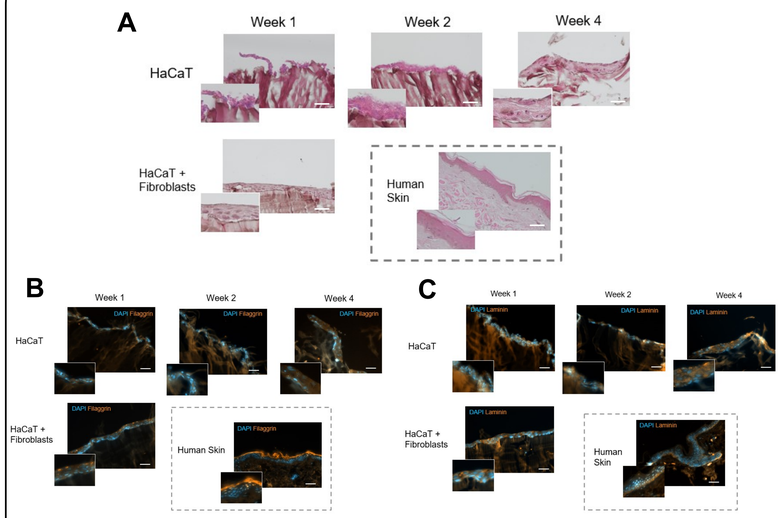

Figure 5: Maturation of the tissue-engineered (TE) epidermis cultured for weeks at the air-liquid-interface (ALI) with and without dermal fibroblasts in the DLP bioprinted constructs. Identical staining of human skin shown for comparison. (A) H&E histological stain of the TE epidermis in the absence (1, 2, and 4 weeks of culture) and presence (1 week of culture) of fibroblasts. (B) Immunohistochemistry (IHC) staining for filaggrin in constructs without (1, 2, and 4 weeks) and with fibroblasts (1 week of culture). (C) IHC staining for laminin-I in constructs without (1, 2, and 4 weeks of culture) and with fibroblasts (1 week of culture). All scale bars = 50 µm

Tissue-engineered skin constructs were then lifted to the ALI on Transwell inserts and cultured for weeks to allow for stratification of the epidermal layer. H&E stains show that after 1 week, the constructs with fibroblasts have the most organized and stratified epidermal morphology (Fig. 5A). Immunohistochemistry (IHC) was used to compare the expression of filaggrin and laminin-I in the tissue-engineered skin vs native human skin (Fig. 5 B,C). Tissue-engineered skin with fibroblasts after 1 week of ALI culture shows significant filaggrin expression in its apical layers, however this is much sparser than what is seen in human skin.

Conclusion

DLP bioprinting is a promising biofabrication technique to produce tissue-engineered skin and its complex structures *in vitro*. In this work, we chose a suitable hydrogel concentration of 7.5% GelMA based on printability and dermal fibroblast proliferation and viability. After verifying that an confluent HaCaT layer was formed after 2 cell seedings, we cultured DLP bioprinted tissue-engineered skin at the ALI for up to 4 weeks. Filaggrin expression in the condition with fibroblasts was seen after 1 week and it is expected that in our future experiments with longer ALI culture times that the epidermis will further differentiate.

References

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