3D Bioprinted Vascularized Skin-on-a-Chip Model for Drug Testing and Wound Healing Studies

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INTRODUCTION

Skin, the largest organ of the human body, serves crucial functions such as protection, thermoregulation, and immunity. Creating artificial skin models presents opportunities for *in-vitro* drug testing and wound healing applications. Among various techniques, 3D bioprinting has gained popularity due to its ability to recreate controlled cellular microenvironments and serve as an alternative to animal experimentation. To reach physiologically relevant sizes, bioprinted tissues require vascularization as a means to transport nutrients e remove waste products from all encapsulated cells. Sacrificial bioprinting has emerged as a successful strategy to produce vasculature within 3D constructs. Choosing suitable biomaterials is critical, with options including natural hydrogels and synthetic hydrogels. In this study, we present a method for fabricating a perfusable 3D vascularized skin model using GelMA 8% (dermis, G8), GelMA 15% (epidermis, G15), and Pluronic F127 40% (sacrificial material, P40). These biomaterials combine biocompatibility and favorable properties for cell adhesion and migration. Neonatal foreskin fibroblasts (BJs), human epidermal keratinocytes (HEKs), and endothelial cells (HUVECs) were selected as primary cell lines for this model.

MATERIALS AND METHODS

GelMA was synthesized by dissolving gelatin in a carbonate-bicarbonate buffer and adding methacrylic anhydride. The GelMA solution was then dialyzed, filtered, freeze-dried, and stored until final use. The optimized bioinks contained GelMA (8% w/v for the dermis and 15% w/v for the epidermis), and 0.1% LAP as photoinitiator dissolved in 1xPBS. A sacrificial ink with Pluronic F-127 40% w/v in cold 1xPBS was prepared for vascular network formation. GelMA hydrogels were fully characterized using mechanical and rheological tests. Cell-laden hydrogels were loaded into syringes and sequentially co-printed using an extrusion bioprinter (BioX). GelMA was then crosslinked with UV light (405 nm), and the sacrificial ink was removed through refrigeration. Vascular channels were endothelialized by injecting HUVECs and BJs ($10 \cdot 10^6$ cells/mL in a ratio of 70:30), followed by incubation and removal of unattached cells. A perfusion circuit composed of a peristaltic pump and custom bioreactor enabled culture medium flow through the endothelialized channel. Flow rate was adjusted to optimize shear stress and induce cells alignment in the direction of flow. Immunofluorescence assays were performed to characterize the different layers of the model.

RESULTS AND DISCUSSION

The aim of this study was to create a vascularized skin model using sacrificial 3D bioprinting with three bioinks: GelMA 8% for the dermis, GelMA 15% for the epidermis, and Pluronic F127 40% as the sacrificial ink. The model consisted of two layers, the dermis and epidermis, with a serpentine-shaped vascular channel running through the dermis. The goal was to establish a stiffness gradient between the two layers to mimic the physiological values of *in-vivo* skin.

The bioinks were optimized by testing various printing conditions, including temperature and pressure, to achieve the desired patterns. Gelatin-based hydrogels were printed using temperature-controlled printheads, and the printability parameter (Pr) was calculated by measuring void spaces in single-layer grids. The ideal printing temperatures were 23±0.5°C for G8, 25±0.5°C for G15, and room temperature for P40, with recommended printing pressures of 50±10kPa, 70±10kPa, and 90±5kPa, respectively. For the dermis layer, fibroblasts (BJ) and endothelial cells (HUVEC) were co-cultured in GelMA 8%. Cell viability, proliferation, morphology, and the production of collagen and elastin were evaluated, showing optimal results. The stratification of keratinocytes in GelMA 15% was achieved by exposing them to air, resulting in the complete formation of the basement membrane between the epidermis and dermis after 3 weeks of culture.

To facilitate endothelialization of the vascular network, a co-culture of endothelial cells and fibroblasts was seeded within the channel, optimizing cell densities and duration for endothelialization.

CONCLUSIONS

Our study optimized bioink printing parameters for GelMA-based and Pluronic hydrogels, achieving smooth and well-defined structures. The co-culture of fibroblasts and endothelial cells within the dermal layer, along with the stratification of keratinocytes in the epidermal layer and the endothelialized vascular channel successfully recreated a perfusable vascularized 3D skin model. The evaluation of cell viability, proliferation, and production of extracellular matrix components indicated the model's physiological relevance. The controlled environment and realistic cell interactions enable studying skin tissue response and advancing tissue engineering and regenerative medicine.

The entire bioprinted skin model matures after approximately three weeks of culture, making it suitable for drug and/or cosmetic tests as well as wound healing trials (*Industrial Invention Patent Application No. 102023000011436*).