Integration of a perfusable 3-layered 3D bioprinted skin construct within a millifluidic chip platform

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OBJECTIVES

The aim of this study was to develop a perfusable, 3-layered 3D bioprinted skin construct integrated into a customizable and cost-effective millifluidic chip platform. Unlike conventional static or bilayer models, this system integrates hypodermal, dermal, and epidermal layers, including a simulated vasculature directly 3D bioprinted within a fluidic chip, enabling dynamic perfusion and improved physiological relevance.

METHODS

The system consists of two main components: **a millifluidic chip** and a **bioscaffold.**

Millifluidic chip design and fabrication:

- Designed using CAD software (Autodesk Fusion 360) and fabricated by photopolymerization-based 3D printing
- The cylindrical insert fits standard 6-well plates
- It enables direct bioprinting, external perfusion, and live imaging

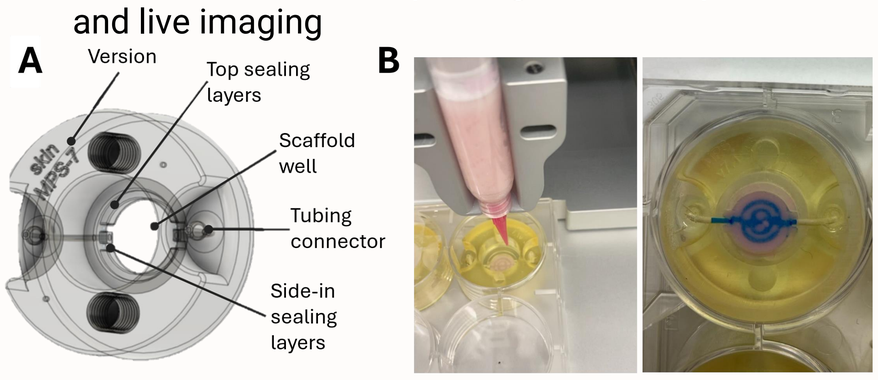


Figure 1. (A) Millifluidic chip design (Fusion 360). (B) Direct 3D bioprinting of scaffolds for skin equivalents.

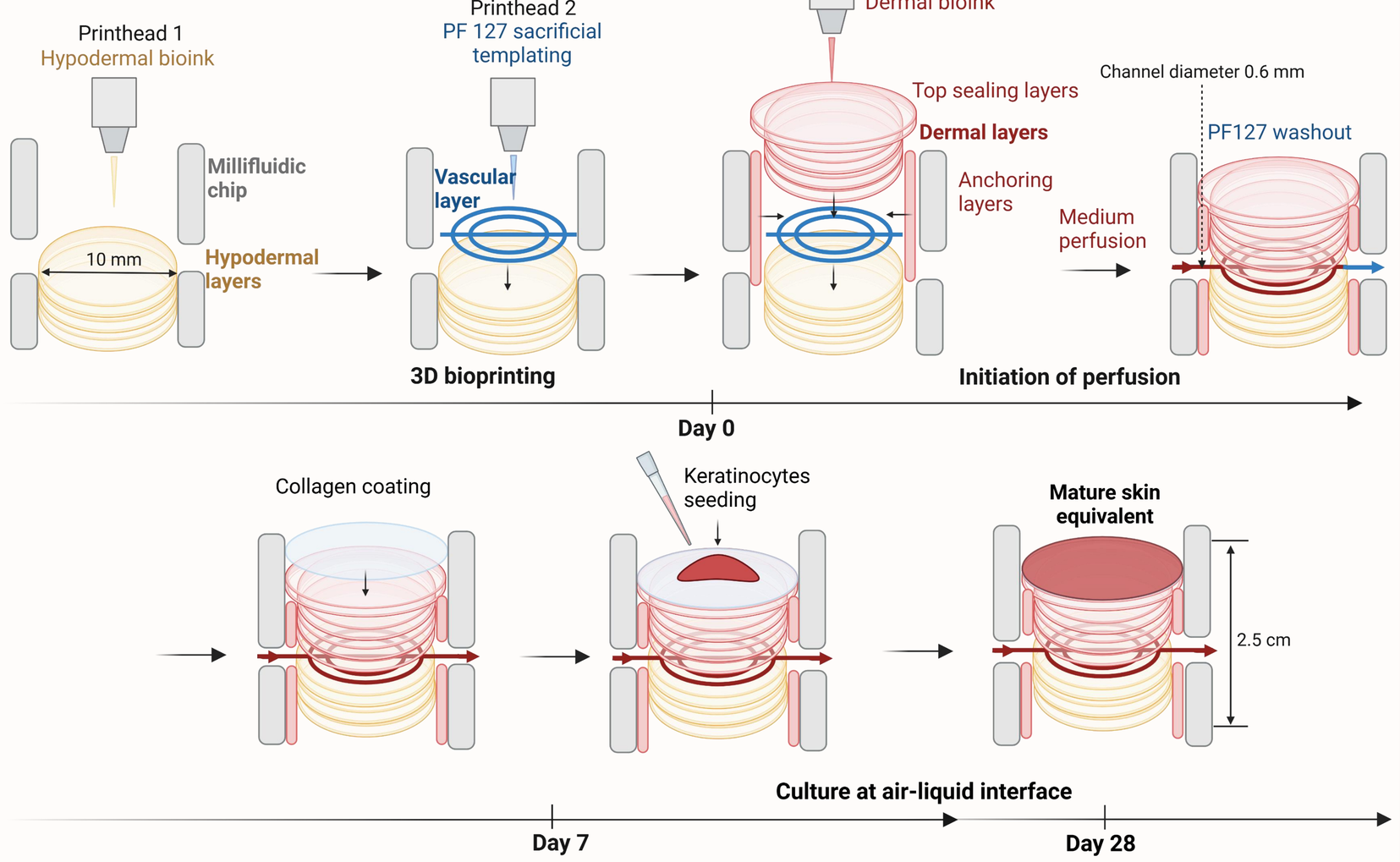


Figure 2. Experimental workflow for skin equivalent fabrication (Biorender.com).

Bioscaffold design and fabrication:

- Skin constructs were fabricated using a triple-head 3D bioprinter (Vitaprint)
- Hypodermal and dermal bioinks (alginic acid, methyl cellulose, PEG, nanofibrillated cellulose) were loaded with adipocytes and fibroblasts
- Perfusion channels were patterned using Pluronic F-127 (PF 127) as a sacrificial material
- After 7 days of perfusion, the constructs were coated with collagen and seeded with keratinocytes
- Constructs were cultured under continuous flow for three weeks to promote construct maturation

RESULTS

Chanel integrity and perfusion

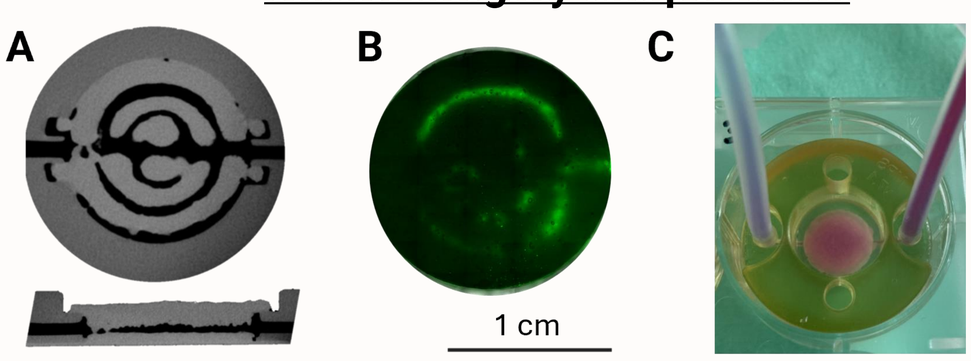


Figure 3. (A) Microtomography at 48 h post-printing (ZEISS Xradia Versa 620). (B) Real-time imaging of GFP-HUVEC perfusion (Leica DMI 6000). (C) Perfusion of colored Milli-Q water through the 3D-printed scaffold.

Cell viability

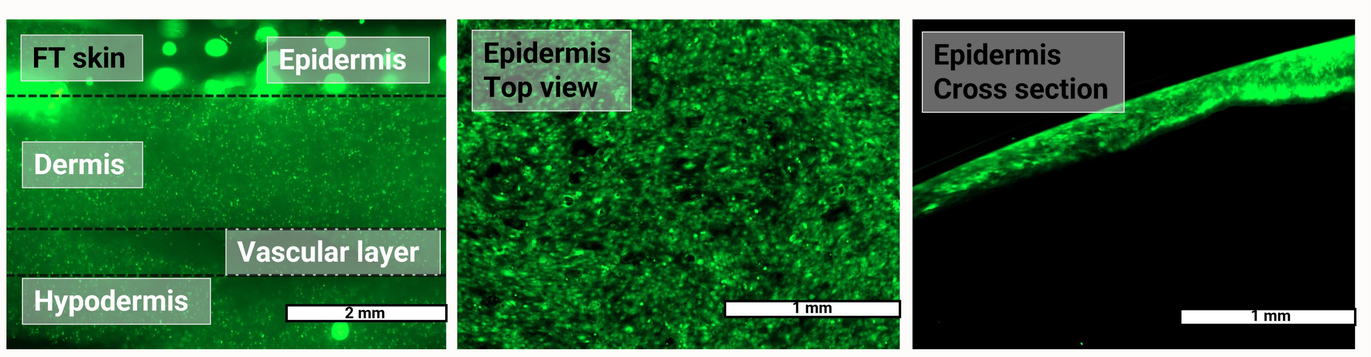


Figure 4. Live/Dead staining of the full-thickness (FT) skin equivalent after 4 weeks of continuous perfusion (cross-sectional view, left panel). Live/Dead staining of the epidermis cultured on a collagen coating, 1 week after seeding, shown as a top view (middle panel) and cross-sectional view (right panel) (EVOS FL Cell Imaging System).

Dermis characterization

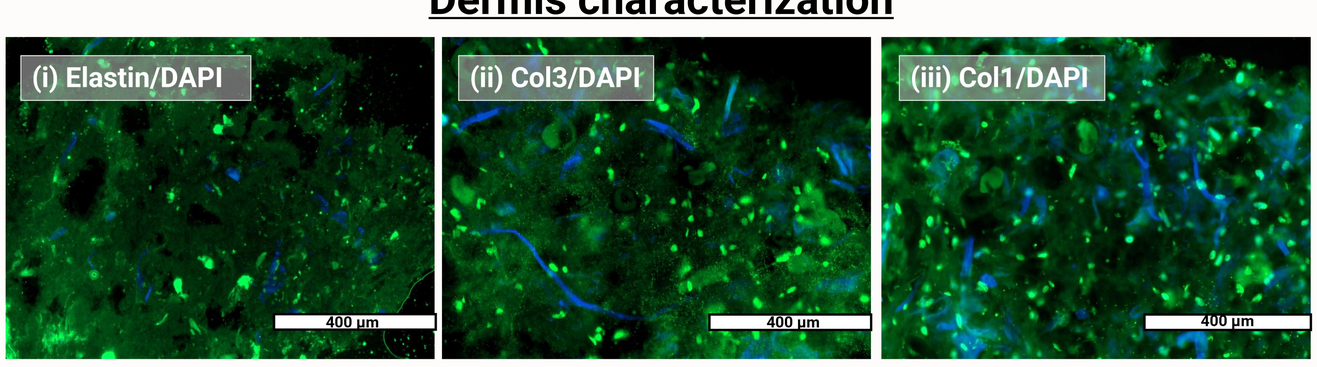


Figure 5. Immunohistochemical staining of scaffolds cultured under perfusion for 3 weeks. (i) elastin/DAPI, (ii) collagen III/DAPI, and (iii) collagen I/DAPI. Nuclei are shown in blue (DAPI), and target proteins are shown in green (EVOS FL Cell Imaging System).

Hypodermis characterization

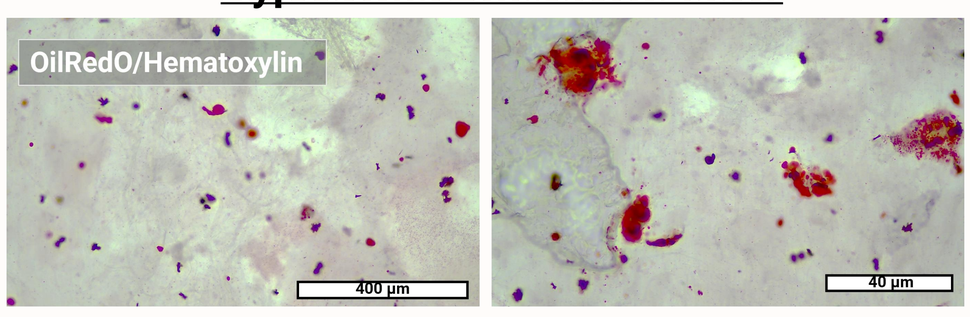


Figure 6. Histological staining with Oil Red O and hematoxylin (Nikon Eclipse E200).

DISCUSSION & CONCLUSON

- Cost-effective, modular millifluidic platform with a highly customizable design
- Enables engineering of perfusable skin constructs with sustained long-term perfusion
- Millifluidic chip allows real-time imaging and monitoring
- Maintains long-term viability of embedded cell types, including adipocytes and skin fibroblasts
- Adipocytes and fibroblasts exhibit appropriate layer-specific marker expression
- Supports the formation of an epidermal layer

ACKNOWLEDGEMENTS

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