Development of a Hydrogel-Based Dermal Equivalent for a 3D-Bioprinted Melanoma Skin Model

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Introduction

The human skin (Fig. 1) is a stratified organ comprising a dermal compartment populated by fibroblasts that actively synthesize extracellular matrix (ECM), and an epidermal compartment primarily composed of keratinocytes that engage in dynamic cross-talk with melanocytes. Melanocytes are the cell of origin for melanoma, one of the most aggressive and treatment-resistant malignancies, characterized by rapid invasion and early metastatic dissemination. Despite rising global incidence, existing in vitro melanoma models fail to recapitulate critical aspects of native skin architecture, including physiological dermis–epidermis topography and the directed infiltration of melanoma cells from the epidermal layer into the underlying dermis. Physiologically relevant, three-dimensional skin models are therefore needed to advance our understanding of tumor biology, support drug development pipelines, and reduce dependence on animal experimentation. This study presents part one of a stepwise strategy to build such a model: the development of a hydrogel-based dermal equivalent (DE) with physiological topography, ECM production, and biomechanical properties.

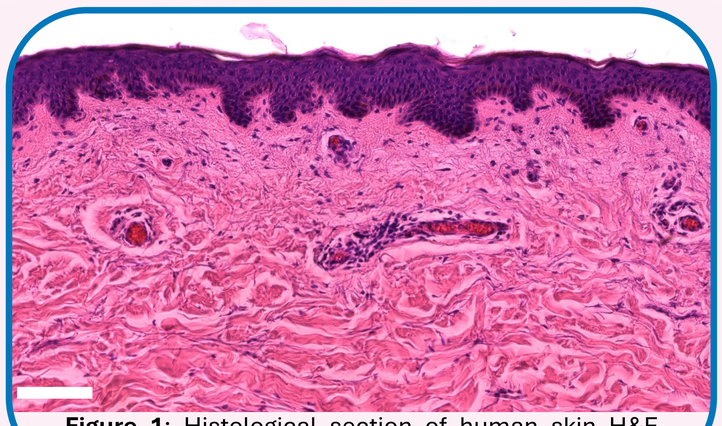


Figure 1: Histological section of human skin H&E staining. Scale bar 100 µm.

Methods

1. **Hydrogel Optimization.** GFP-expressing primary neonatal human dermal fibroblasts (HDFn) were encapsulated in a composite hydrogel containing gelatin-methacryloyl (GelMA), elastin-methacryloyl (ELMA), and hyaluronic acid (HA). The composition was optimized via Design of Experiments (DoE) to maximize viability, spindle-like morphology, and ECM deposition while ensuring structural stability.
2. **Rete Ridge Mold Fabrication.** Physiological rete ridge topography was designed in Autodesk Inventor and fabricated at micrometer resolution via two-photon polymerization (2PP), yielding a master mold (Fig. 2a).
3. **Agarose Casting & Topography Transfer.** Liquid agarose was cast over the 2PP master and demolded, transferring the negative rete ridge topography into the agarose bed (Fig. 2b).
4. **3D-Bioprinting.** The HDFn-laden bioink was deposited into the structured agarose bed using a droplet-based RegenHU bioprinter. 10 seconds of ultraviolet (UV) curing (λ = 365 nm) crosslinked GelMA and ELMA, stabilizing the construct with a patterned surface simulating physiological dimensions (Fig. 2c, d).
5. **Rheological Characterization.** Shear-thinning behavior was assessed by viscosity profiling (plate-to-plate rheometer, Fig. 3a). UV-crosslinking kinetics were monitored via real-time oscillatory measurements of G' and G" (Fig. 3b).
6. **14-Day Culture & Characterization.** DEs were cultured for 14 days and characterized by MTS viability assay and immunohistochemical (IHC) staining for fibronectin and fibroblast morphology.

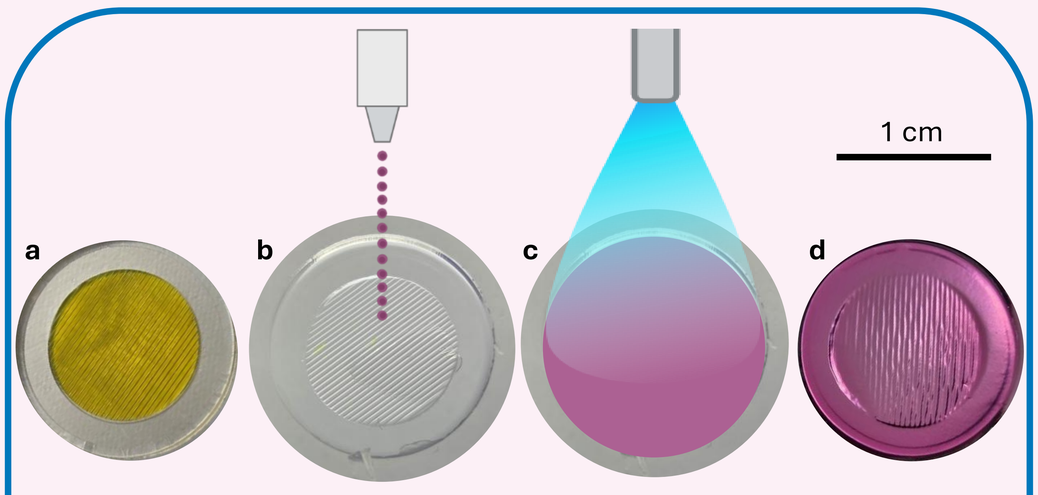


Figure 2: Fabrication of dermal equivalent (DE). **a** 2PP master with µm-precision rete ridges geometry. **b** Negative topography transferred to agarose bed, 3D-bioprinting of DE-bioink into structured mold. **c** UV-curing for GelMA and ELMA crosslinking. **d** DE with physiological topography after 1 day in DMEM with 10% FBS, 1% Pen/Strep.

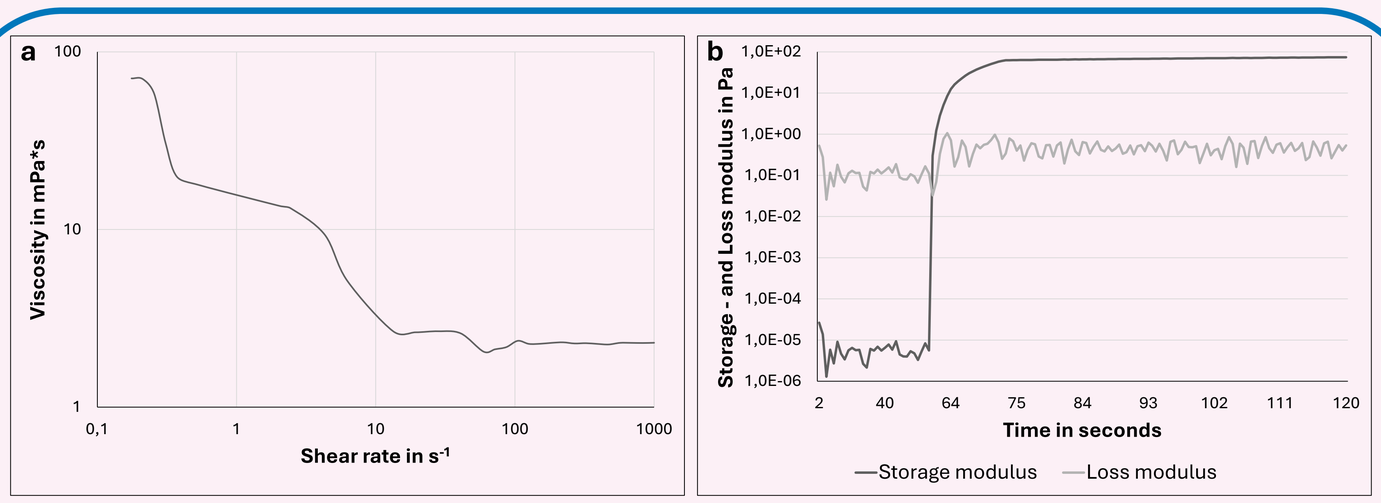


Figure 3: Rheology of hydrogel composed of 7% GelMA, 2% ELMA, and 0.3% HA. **a** Viscosity vs. shear rate in mPa*s. **b** Storage- and loss modulus in pascal before, during and after photo-crosslinking (10 s UV-curing at time point 60 s).

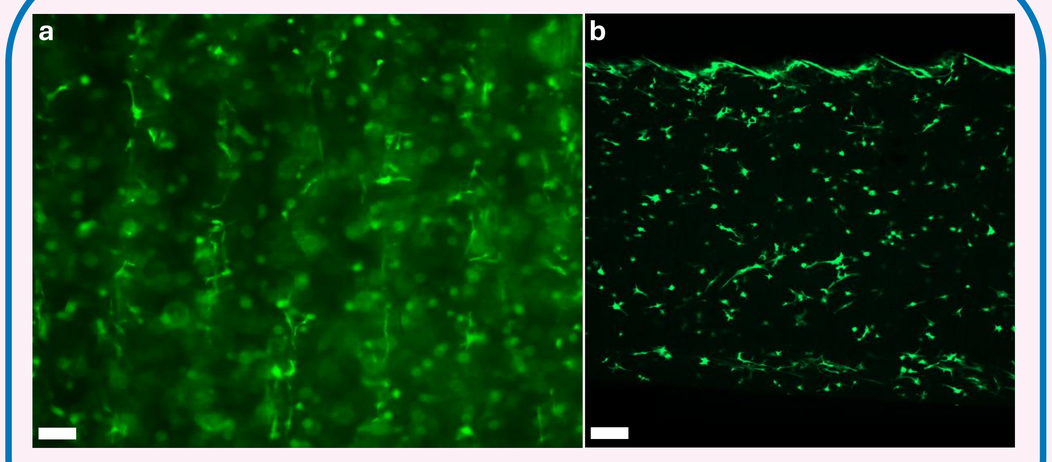


Figure 4: Dermal equivalent (DE) cultivated for 14 days with spindle-shaped GFP-HDFn and topography. **a** Surface view. **b** Histological section. Scale bar 200 µm.

Results

DoE-guided optimization identified a composition of 7% GelMA, 2% ELMA, and 0.3% HA as the optimal hydrogel formulation. GelMA provided the structural backbone and shear-thinning behavior enabling printability (Fig. 3a). GelMA and ELMA formed a UV-crosslinked elastic network (confirmed by G'/G" crossover shown in Fig. 3b) conferring long-term dimensional stability and physiological elasticity. HA enhanced early hydration and cell compatibility before being gradually resorbed and replaced by fibroblast-secreted ECM. After 14 days, HDFn exhibited spindle-like morphology (Fig. 4) and active fibronectin deposition (Fig. 5c), reflecting a physiologically active phenotype. The DE maintained mechanical properties within the physiological dermal range throughout the culture period. Rete ridge topography was successfully transferred via the structured agarose bed, and the DE surface supported keratinocyte adhesion and basal epidermal layer formation.

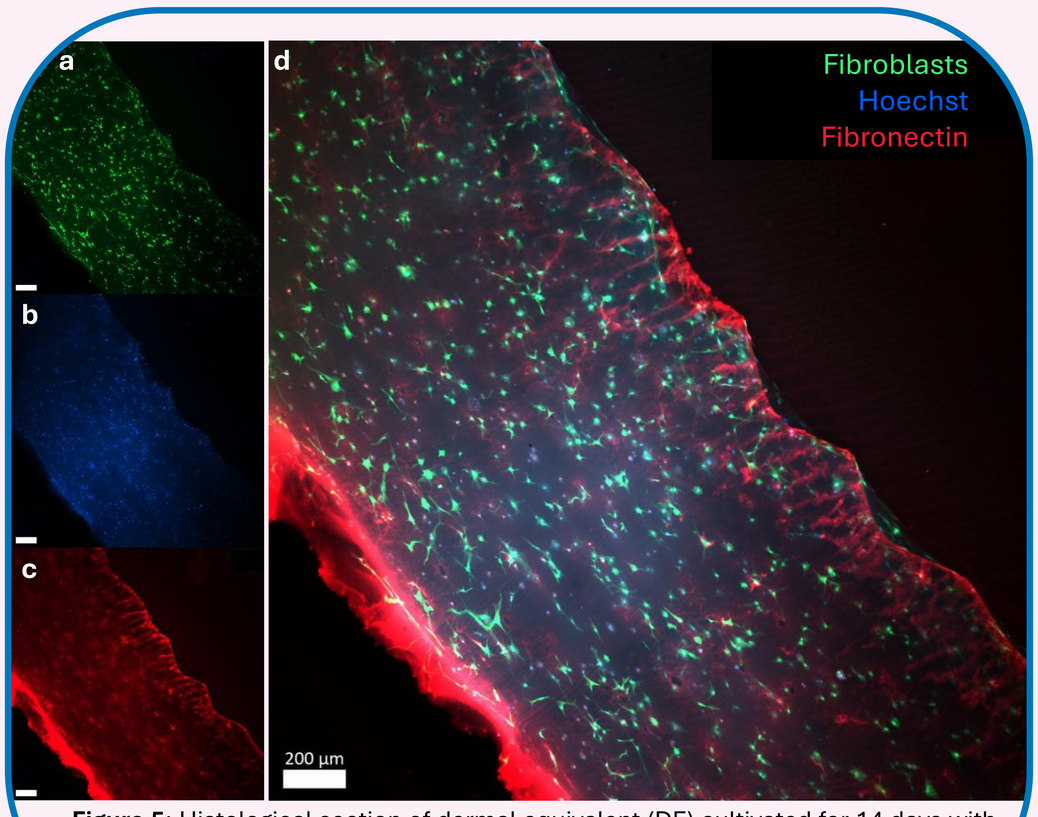


Figure 5: Histological section of dermal equivalent (DE) cultivated for 14 days with spindle-shaped GFP-HDFn and topography. **a** GFP-HDFn. **b** Hoechst nuclei staining. **c** Alexa Fluor 647 fibronectin staining. Scale bar a-c: 200 µm. **d** merged

Conclusion

The optimized hydrogel promotes spindle-shaped fibroblast morphology, ECM production, and physiological dermal stability. Shear-thinning properties enable bioprinting, and UV-induced G' > G" crossover confirms robust gelation and dimensional stability for 14 days. The 2PP-based agarose casting workflow reproducibly transfers rete ridge micro-architecture, replicating the in vivo dermal-epidermal junction, and is adaptable to other topographies (e.g. aged or diseased skin). Keratinocyte adhesion and basal epidermal layer formation confirm the DE as a suitable dermal foundation. The DE mimics key properties of native dermis and provides a promising platform to study melanoma invasion, stromal remodeling, and tumor microenvironment interactions under controlled 3D conditions. Next steps include co-culture of melanocytes and keratinocytes on the DE to establish a full-thickness 3D melanoma skin model for tumor invasion studies.

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