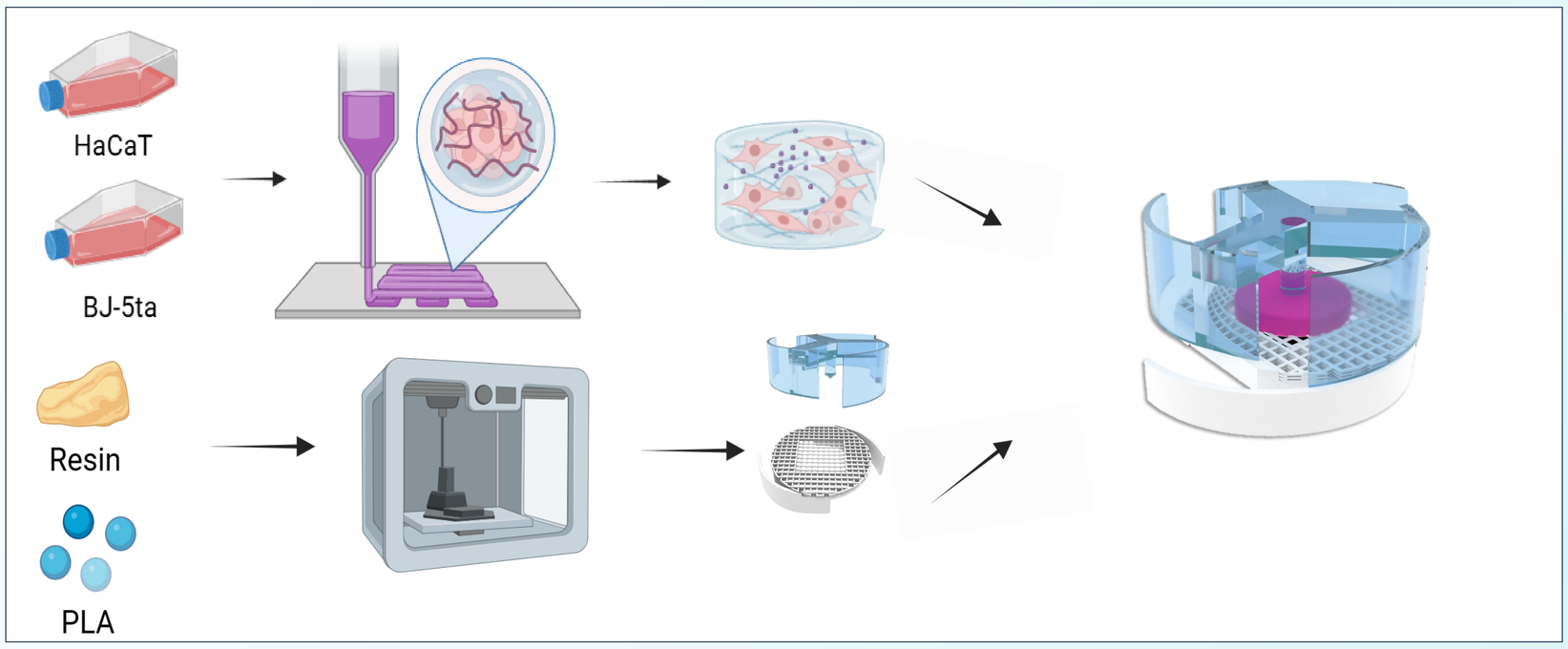
3D-printed insert platform for controlled wound healing and tissue growth assessment in 3D skin models

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Abstract

Human skin models are essential for advancing research and clinical applications, but traditional 2D monolayer models fail to fully replicate the complex physiological properties of skin. To bridge this gap, there is a need for more accurate in vitro models that mimic skin's full functionality. Acute and chronic wounds heal through matrices that facilitate skin re-epithelization. Nevertheless, the absence of appropriate in vitro models has hindered the systematic preclinical evaluation of keratinocyte migration on these matrices.[1,2] To address these challenges, we introduce a novel 3D-printed transwell system designed to improve the air-liquid interface (A/L) for enhanced skin maturation and provide a reproducible platform for wound healing assays. The system supports the A/L interface for better skin model handling and maturation, and incorporates a conical pin mechanism for controlled wound creation, mimicking real skin injury. This cost-effective system enables precise study of keratinocyte migration, differentiation, and wound healing in a 3D model, offering a reliable tool for systematic preclinical analyses of skin repair mechanisms.



Methods

The wound healing insert systems are composed by two independent elements. The first one is a insert that allows to establish the air/liquid interface essential for the skin differentiation. It is made by three pieces: a grid, a 3 μm membrane pore size and ring used to secure the membrane under the grid. The second one is made by a bridge mechanism with a conical pin. This tool could be remained inside the 3D skin for 21 days of maturation, allowing the air/liquid interface to be established. The wound is created tearing just two of the three 3D-bioprinted skin layers. CAD-designed components are 3D-printed with polylactic acid (PLA) filament by FLM printer, while the bridge and pin are made using digital light processing (DLP) with biocompatible resin.

The skin equivalent was printed with BIOX (cellink) using cellink skin bioink. The epidermis component was created by spontaneously Transformed Human Keratinocyte cell culture (HaCaT)and the dermis by the BJ5TA immortalized foreskin fibroblasts.

Cytotoxicity assay is performed according to ISO 10993-5 using a MTT assay. The Live/Dead assay, histological examination, and immunofluorescence analysis were conducted following the standardized protocols established by Cellink. Histological analysis and immunofluorescence methods are described in the captions.

Conclusions

Preliminary testing shows the system reliably forms the A/L interface and allows precise and reproducible wound creation, ensuring reproducibility across experiments. The next steps involve promoting wound closure by filling the wound with fibroblast-activating factors, facilitating re-epithelialization, and enhancing collagen deposition.

Results

Construction of wound-healing insert system

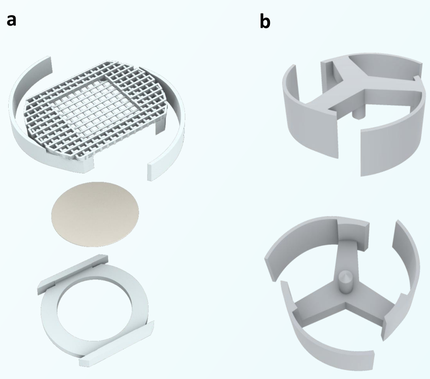


Fig. 1 a) Rendered insert construction. Diagram illustrating the device, showing the upper and lower sections of the inserts with the customized membrane positioned in between. b) Rendered wound healing device. Schematic of the isometric top view and bottom view of the flipped object, with a focus on the conical pin.

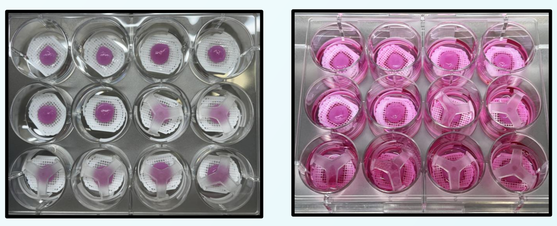


Fig. 2 3D printed insert systems with and without wound healing device in cell culture 12 well plate with 3D tissue on the first day of A/L interface

Cytotoxicity assay

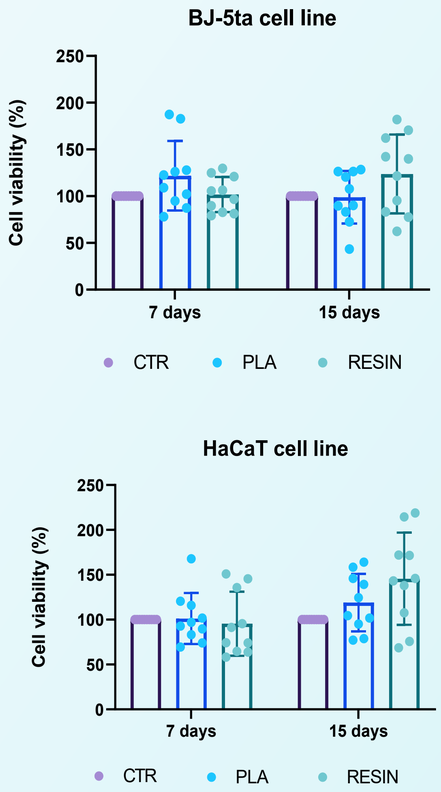


Fig. 3 Cell viability according to ISO 10993-5 of HaCaT and BJ-5ta. Viability was measured via MTT assay. PLA and resin do not exhibit detrimental effects on cell viability at 7 or 15 days.

Characterization of 3D bio-printed skin model

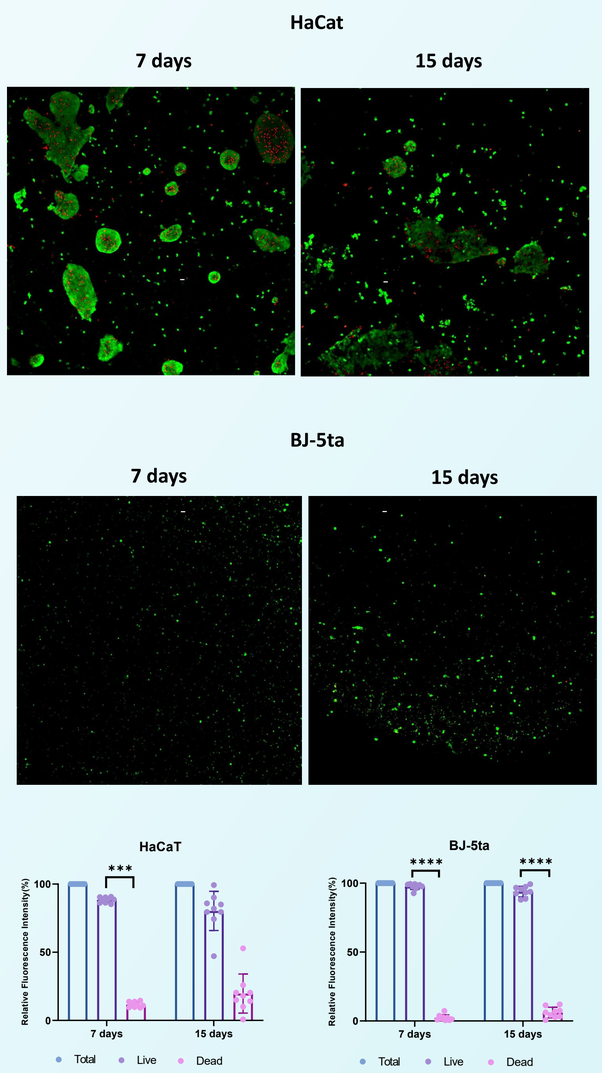


Fig. 4 Live/dead Assay staining with calcein and propidium iodide of the single layer of HaCat and BJ-5ta with cell density of 10 x 10^6 and 8 x10^6 respectively after 7 and 15 days of A/L interface (Scale bar 50 μm).

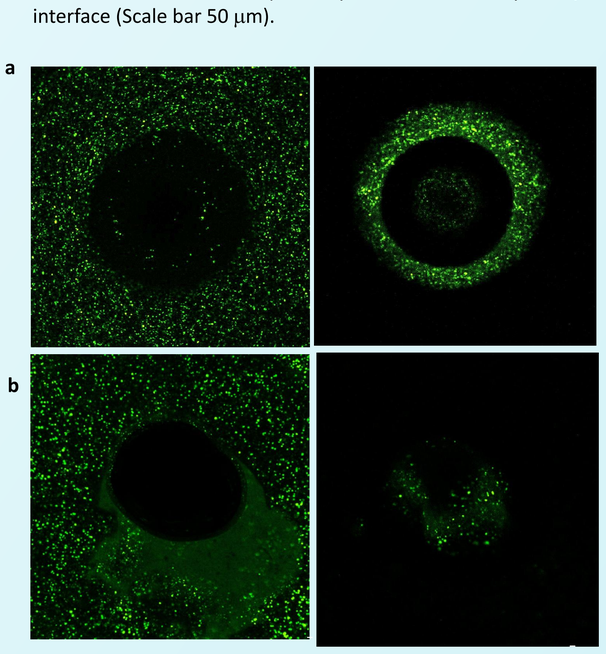


Fig. 5 Live/dead staining of the whole 3D skin construct after the wound made by 3D-printed device (a) and 2 mm biopsy punch biopsy (b). The shape of the 3D-printed device wound appears more regular and circular compared to the one made with the biopsy punch (Scale bar 50 μm).

Histological analysis and Immunofluorescence

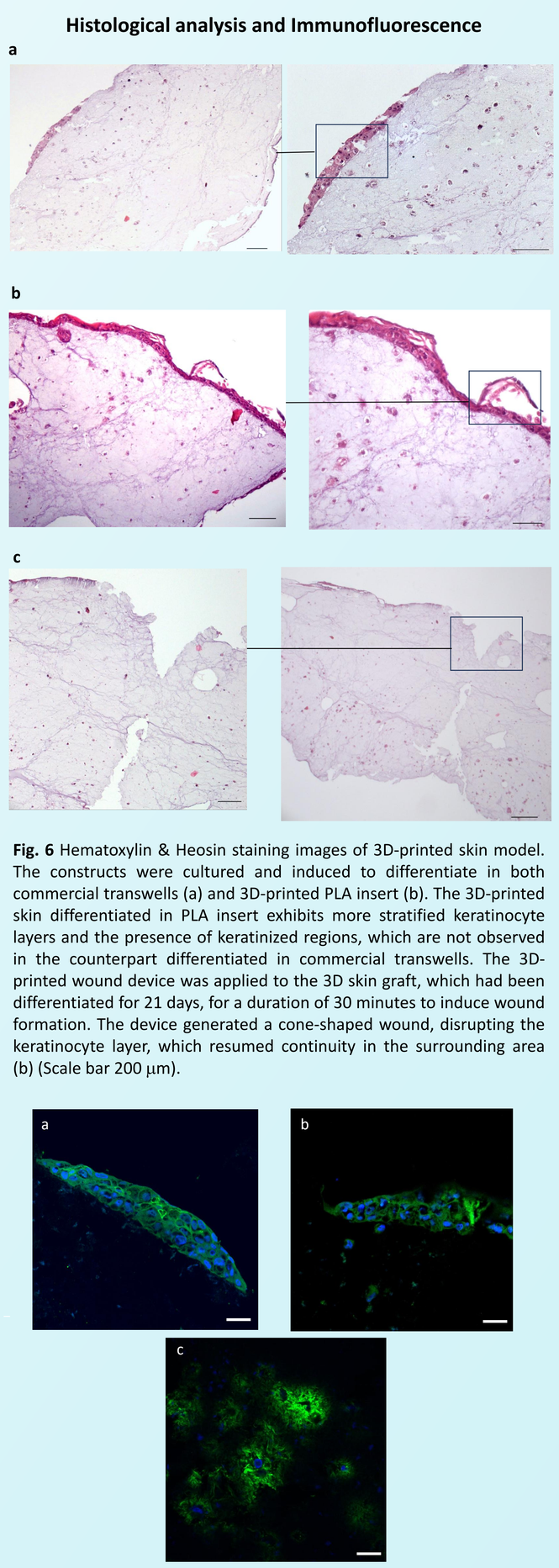


Fig. 6 Hematoxylin & Heosin staining images of 3D-printed skin model. The constructs were cultured and induced to differentiate in both commercial transwells (a) and 3D-printed PLA insert (b). The 3D-printed skin differentiated in PLA insert exhibits more stratified keratinocyte layers and the presence of keratinized regions, which are not observed in the counterpart differentiated in commercial transwells. The 3D-printed wound device was applied to the 3D skin graft, which had been differentiated for 21 days, for a duration of 30 minutes to induce wound formation. The device generated a cone-shaped wound, disrupting the keratinocyte layer, which resumed continuity in the surrounding area (b) (Scale bar 200 μm).

Fig. 7 Immunofluorescence staining of 3D-printed skin model**.** BJ5TA fibroblasts and HaCaT keratinocytes were bio-printed in three layers. Gel has been crosslinked using 50 Mm $CaCl_2$ for 5 minutes and thrombin overnight (10 U/mL) and dermal components submerged for three days, then allowing the epidermal differentiation for 21 days. Alexa fluor 488 Keratin 10 (a), elastin (b), collagen type I (c) and DAPI staining (Scale bar 50 μm).

Acknowledgements

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