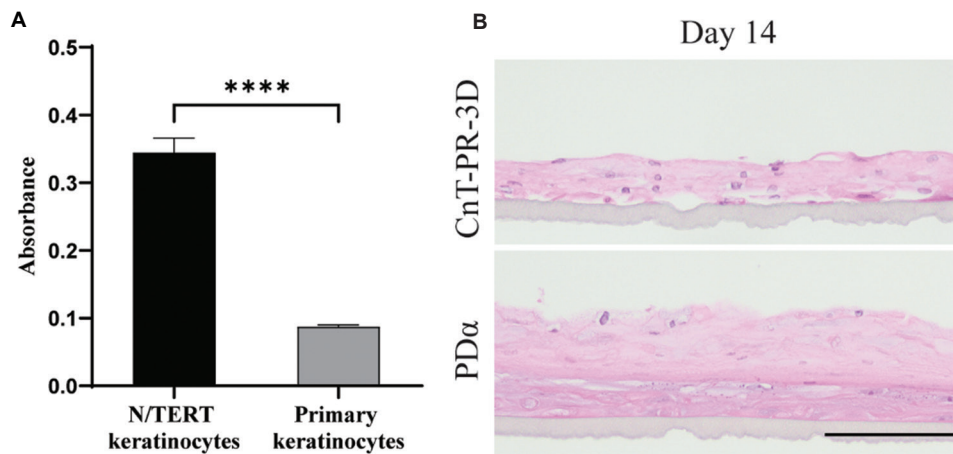


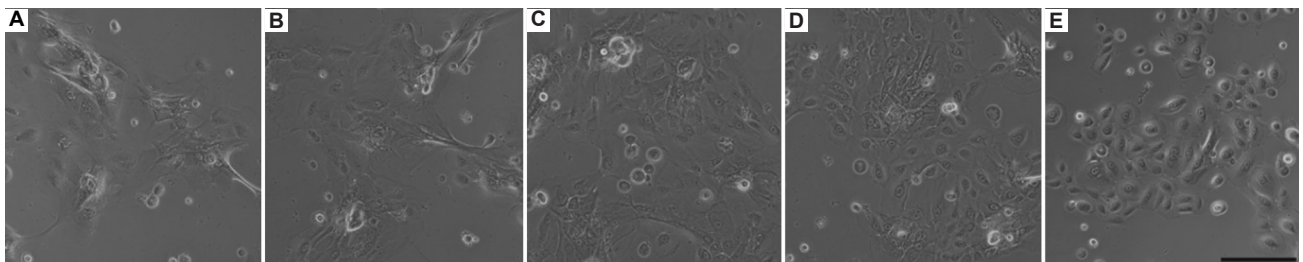
RESEARCH ARTICLE

# Developing a bioink for single-step deposition and maturation of human epidermis

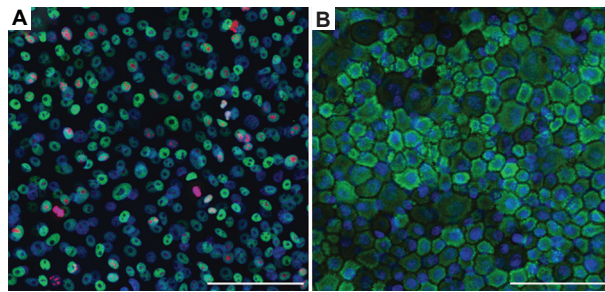
## Supplementary File



**Figure S1.** Primary keratinocytes do not grow well in PD $\alpha$  media. (A) There was significantly less proliferation of primary keratinocytes compared to N/TERT keratinocytes cultured in PD $\alpha$  media (\*\*\*\* $P < 0.0001$ ). (B) Primary keratinocytes were unable to form a proper stratified epidermis when cultured in PD $\alpha$  media. Therefore, there was a need to formulate a single-step media optimized for primary keratinocytes. Scale bar: 100  $\mu$ m.



**Figure S2.** Primary keratinocyte cultured in various media formulations. Morphological cues of cells in 2D cultures were used to optimize and develop the PD $\beta$  media. (A) PD $\alpha$  media. (B, C, and E) Various media formulations. (D) PD $\beta$  media. Scale bar: 200  $\mu$ m.



**Figure S3.** Immunofluorescence staining of primary keratinocytes cultured in PD $\beta$  media at high density. (A) When primary keratinocytes were cultured at high density, Ki67 (red) expression decreased while p63 (green) expression remained high and was present in all cells. (B) When primary keratinocytes were cultured at high density, K10 (green) expression increased. However, there was still no FLG expression (no visible red). This suggests that the cells can transition to a differentiated phenotype when they have proliferated sufficiently to reach the requisite density. Nuclei are stained blue. Scale bar: 100  $\mu$ m.

**Videoclip S1.** Video showing the hydrophobic epidermal barrier properties of the c-HSE constructs. (Refer to uploaded videoclip).