

Supplementary Information for:

**Fabrication of human keratinocyte cell clusters
for skin graft applications by templating water-
in-water Pickering emulsions**

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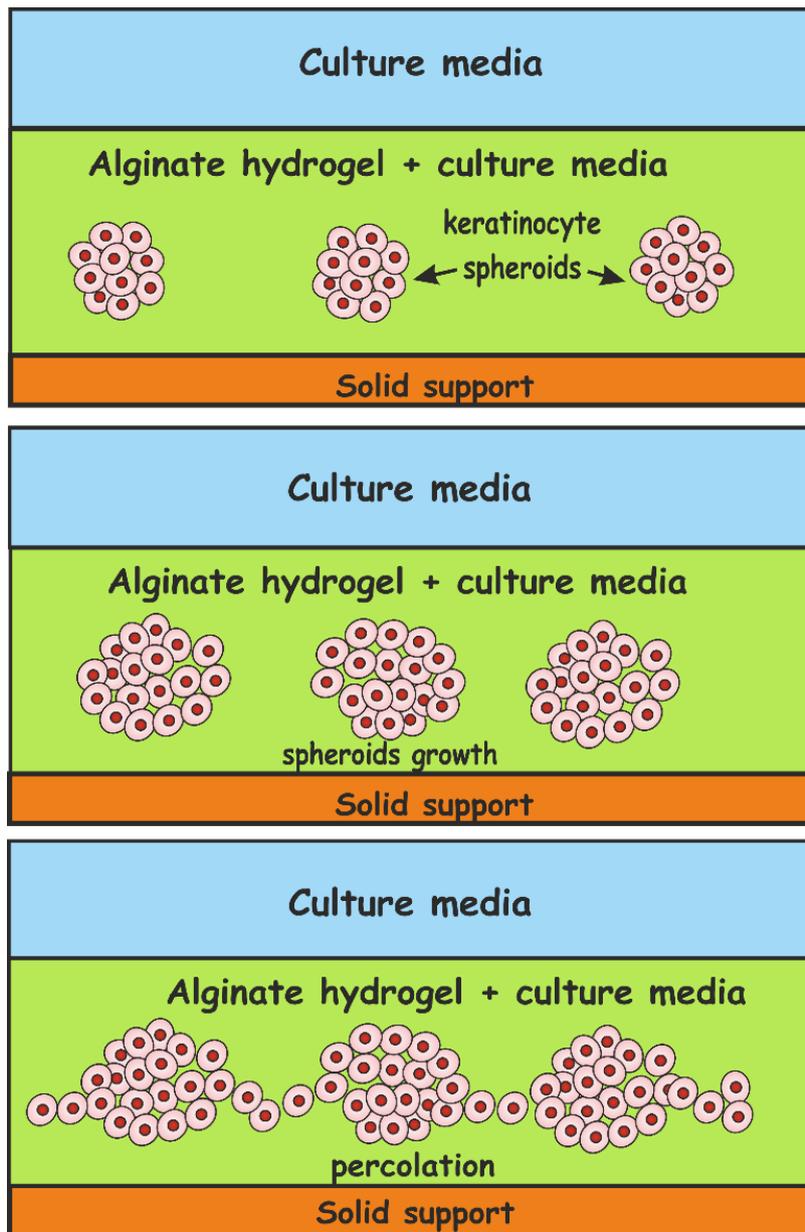


Figure S1. Schematics of the formulation of keratinocyte clusteroids in cross-linked alginate films and the evolution of their growth.

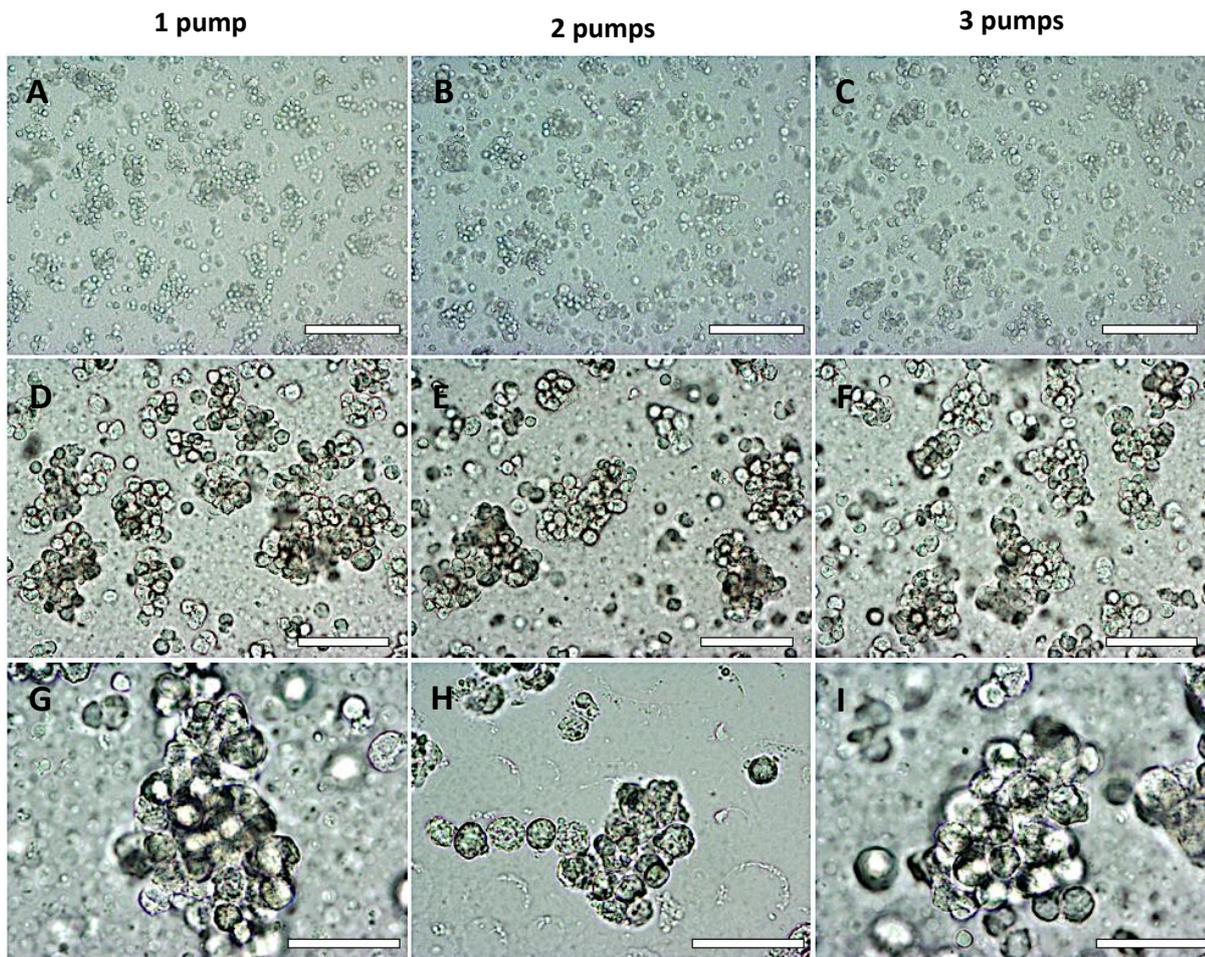


Figure S2. Optical microscopy images of HaCaT cell droplets (PEO 5.5 wt%/DEX 5.5 wt%) stabilized by 2wt% WP particles, at different stage of homogenization: (A, D, G) 1 pump, (B, E, H) 2 pumps and (C, F, I) 3 pumps. Scale bars are (A to C) 200 μm , (D to F) 100 μm and (G to I) 50 μm .

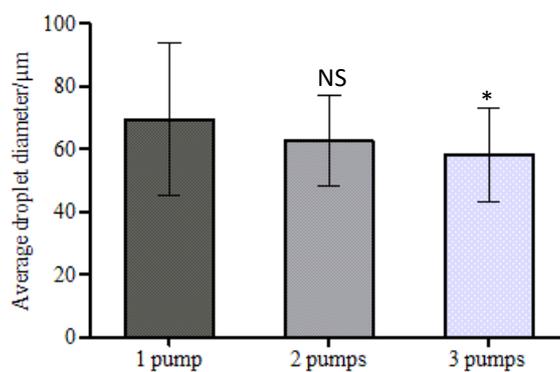


Figure S3. Average DEX droplet diameter for the formulation of WP/NaCl 300mM solution at pH 5.8, PEO 5.5 wt%/DEX 5.5 wt% homogenized by 1, 2 or 3 pumps. The data were obtained by optical microscopy measurements of the emulsion droplets for each micrograph with ImageJ Software. (Student's t-Test, NS: non-significant, *: $P < 0.05$)

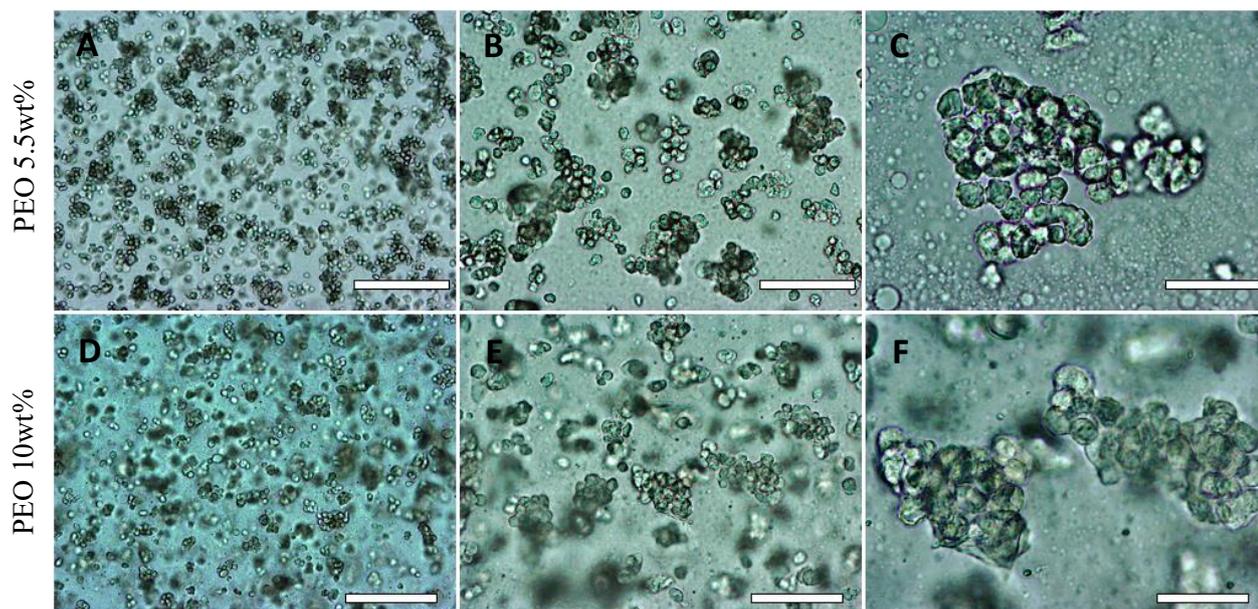


Figure S4. Optical microscopy images of (A to C) HaCaT cell ($\phi = 0.2$) droplets (PEO 5.5 wt%/DEX 5.5 wt%) and (D to F) HaCaT cell spheroids (PEO 10 wt%/DEX 5.5 wt%) stabilized by 2wt% WP particles. Scale bars are (A, D) 200 μm , (B, E) 100 μm and (C, F) 50 μm .

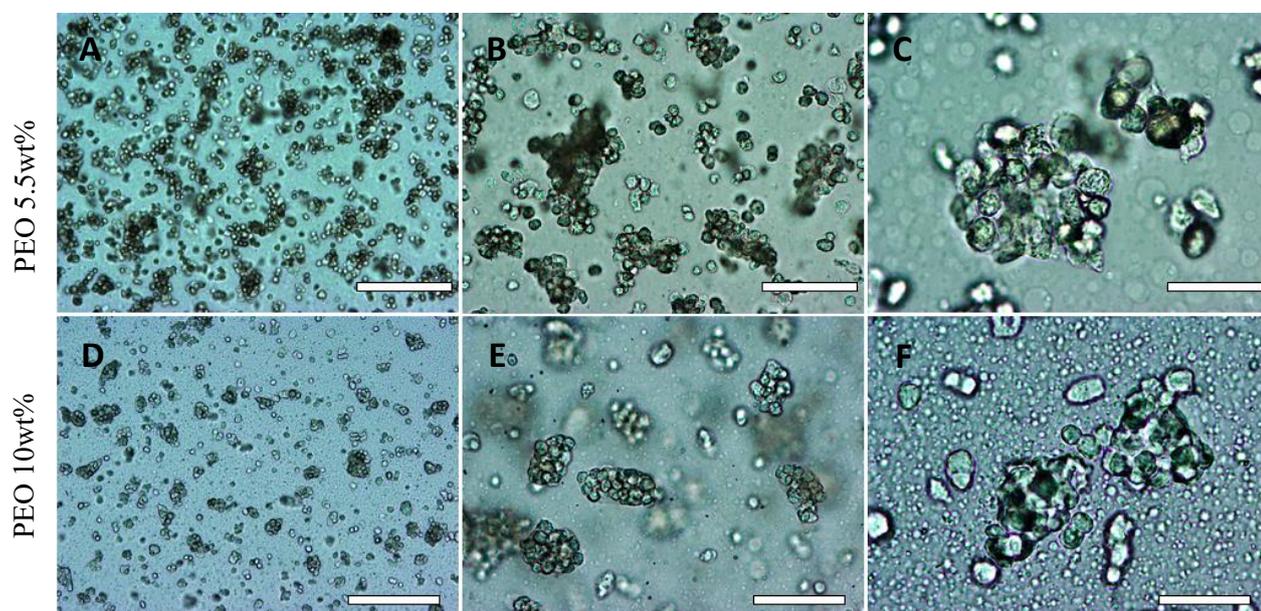


Figure S5. Optical microscopy images of (A to C) HaCaT cell ($\phi = 0.25$) droplets (PEO 5.5 wt%/DEX 5.5 wt%) and (D to F) HaCaT cell spheroids (PEO 10wt%/DEX 5.5 wt%) stabilized by 2wt% WP particles. Scale bars are (A, D) 200 μm , (B, E) 100 μm and (C, F) 50 μm .

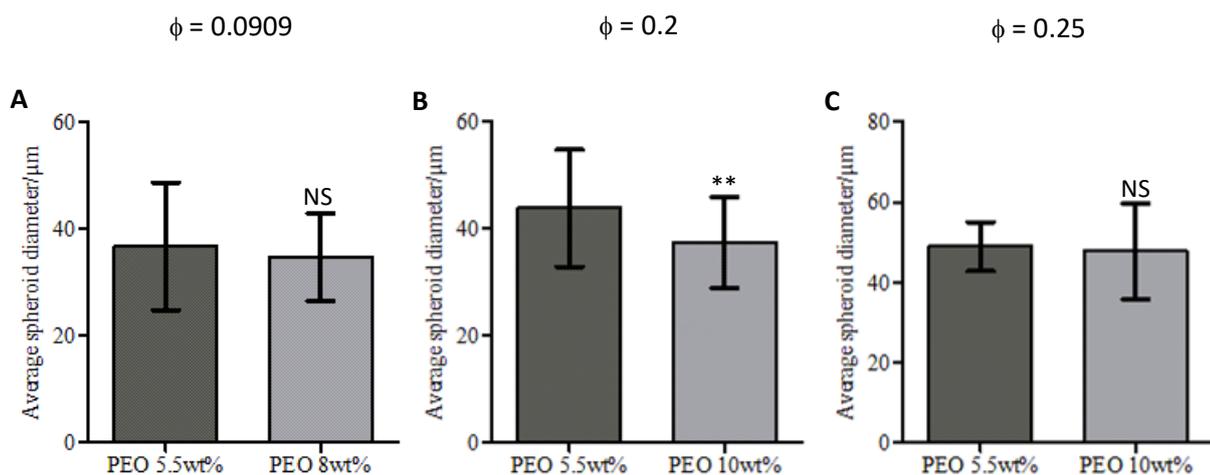


Figure S6. Average HaCaT spheroid diameter for emulsions (A) PEO 5.5wt or PEO 8wt% $\phi_{\text{HaCaT}} = 0.0909$, (B) PEO 5.5 wt% or PEO 10 wt% $\phi_{\text{HaCaT}} = 0.2$ and (C) PEO 5.5 wt% or PEO 10wt% $\phi_{\text{HaCaT}} = 0.25$. Data were obtained by optical microscopy measurements of droplets for each micrograph with ImageJ Software. (Student's t-Test, NS: non-significant, **: $P < 0.01$).

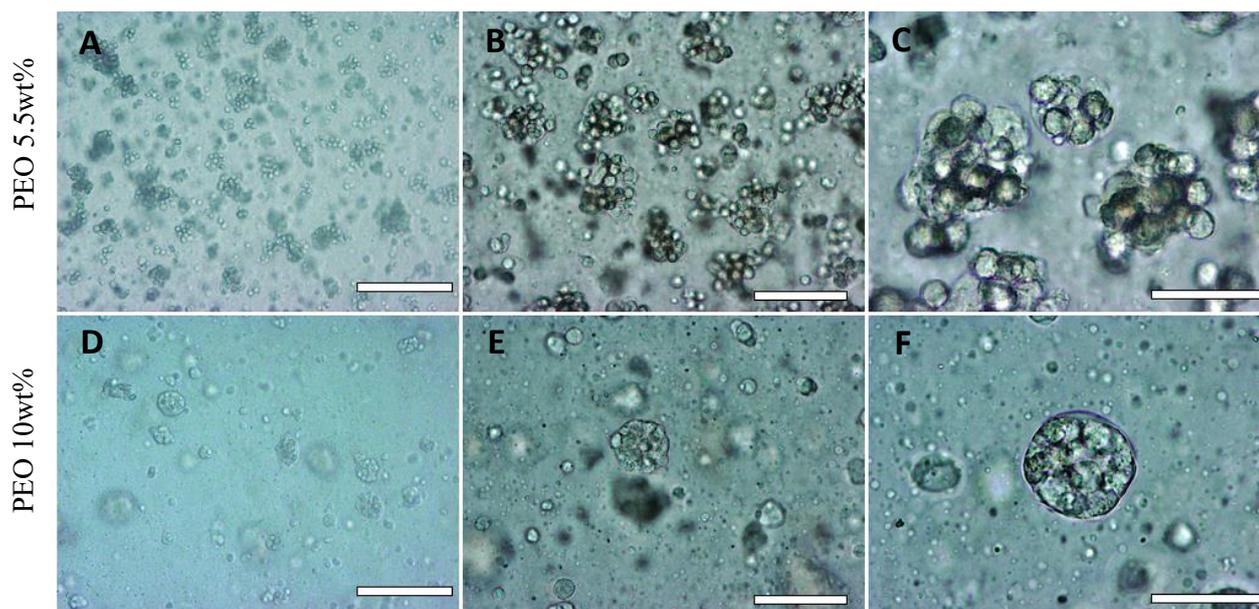


Figure S7. Optical microscopy images of (A to C) HaCaT cell droplets (PEO 5.5 wt%/DEX 5.5 wt%) and (D to F) HaCaT cell spheroids (PEO 10 wt%/DEX 5.5 wt%) stabilized by 2 wt% WP particles. $\phi_{\text{HaCaT}} = 0.25$ and $\phi_{\text{Dex}} = 0.2$. Scale bars are (A, D) 200 μm , (B, E) 100 μm and (C, F) 50 μm .

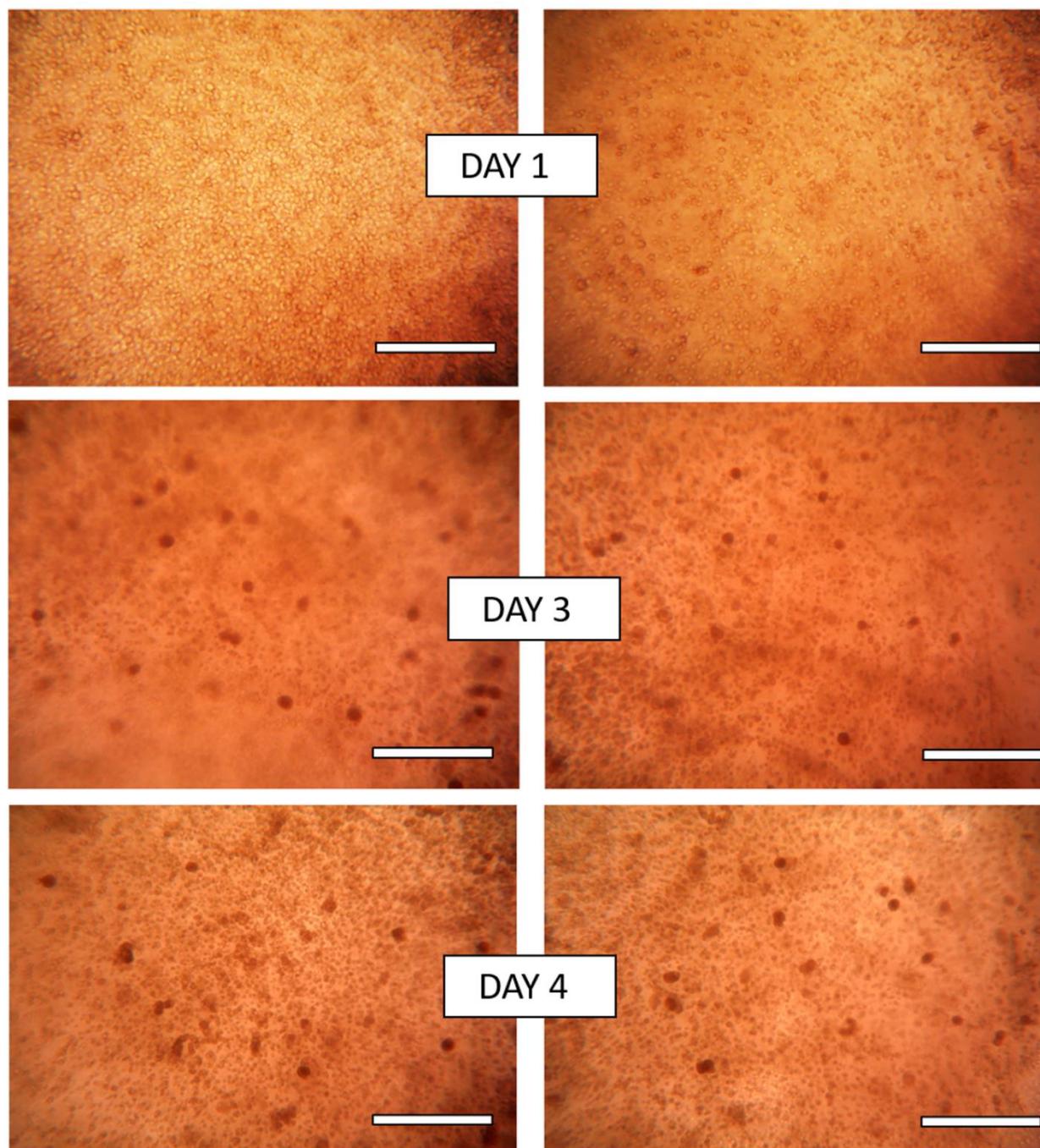


Figure S8. Growth of individual HaCaT cells incorporated with 0.75wt% sodium alginate in DMEM media followed by cross-linking with 1 M CaCl_2 . The HaCaT cells were cultured in the alginate film for 4 days under DMEM media and images were taken from each well. Scale bars are 200 μm . Note that a very small fraction of the individual HaCaT cells develop into spheroids and growth over the course of 4 days.

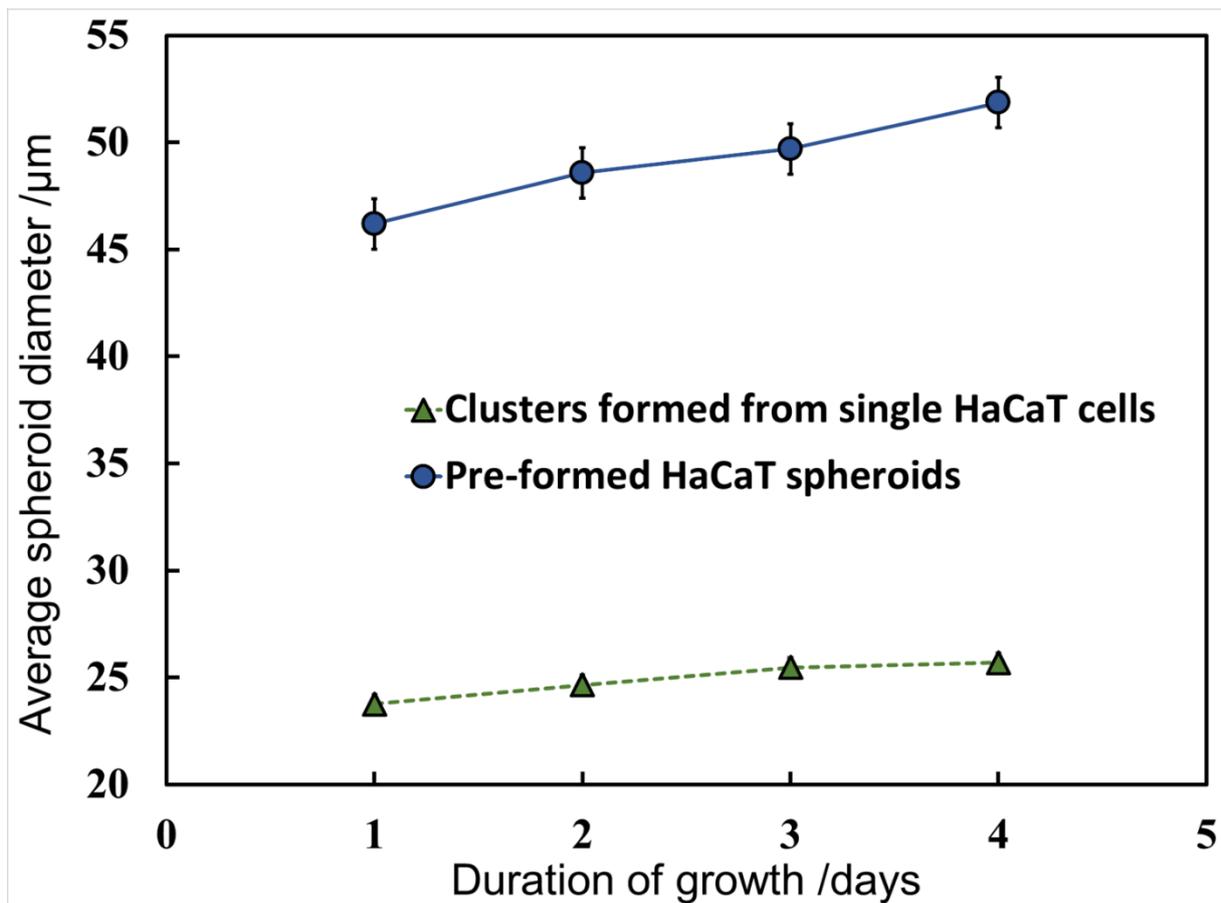


Figure S9. Growth curves of spheroids spontaneously formed from individual HaCaT cells (triangle symbols) and pre-formed HaCaT spheroids by our method (circle symbols) both incorporated within a film of 0.75wt% sodium alginate in DMEM media followed by cross-linking with 1 M CaCl₂. The HaCaT cells were cultured in the alginate film for 4 days under DMEM media and images were taken from each well. Note that a lower rate of growth of the individual HaCaT cells that develop into spheroids over the course of 4 days – see typical images of the alginate films with the individual cells growth in Figure S7 (The error bars are within the symbol size).