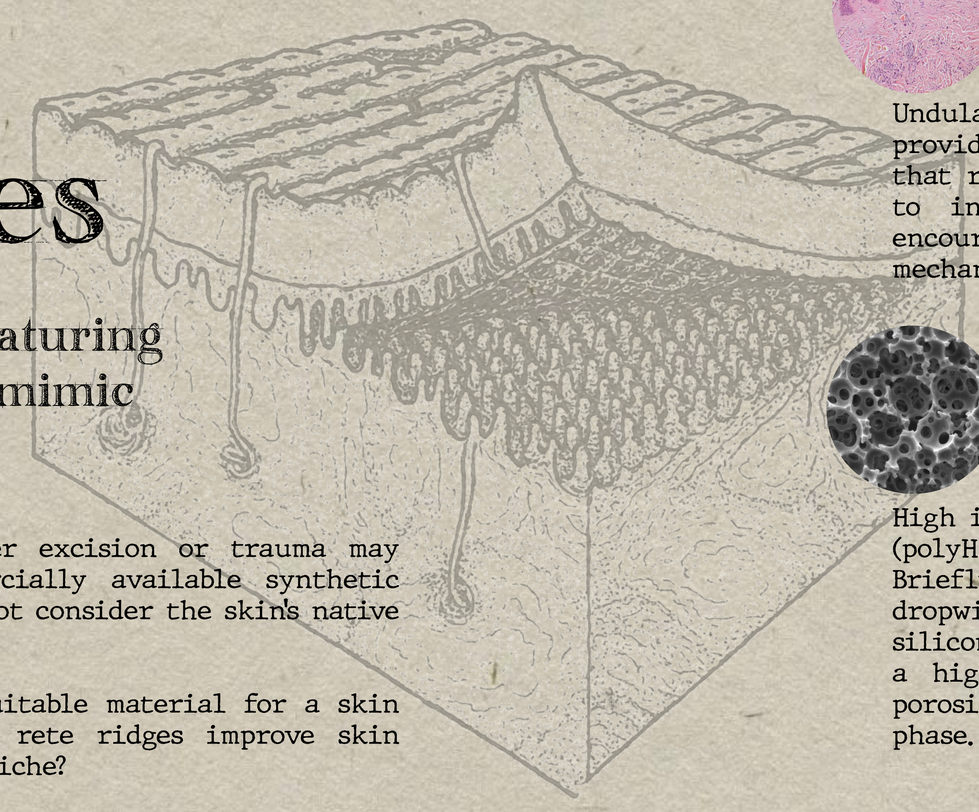


Amy Morgan[1,2*]
Prof Frederik Claeyssens[1], Dr Joey Shepherd[2], Dr Ílida Ortega Asencio[2]

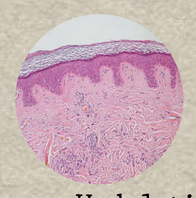
Recreating Rete Ridges

A polyHIPE dermal scaffold featuring ridged topography to faithfully mimic the dermal-epidermal junction.



Full thickness skin wounds caused by skin cancer excision or trauma may require support to regenerate neo-dermis. Commercially available synthetic dermal constructs are clinically successful but do not consider the skin's native microarchitecture, for example, Rete Ridges.

Is methacrylated polyglycerol sebacate (PGS-M) a suitable material for a skin regeneration matrix? Can the addition of pseudo rete ridges improve skin regeneration by creation of an artificial stem cell niche?



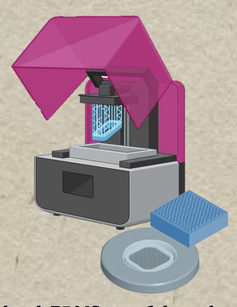
Rete ridges?

Undulations at the dermal-epidermal junction provide a microenvironment for stem cells that replenish the epidermis. They also help to increase surface area between layers encouraging cross-layer cell signalling and mechanical strength.

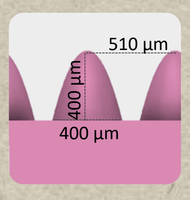
PolyHIPE?

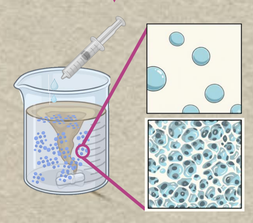
High internal phase emulsion polymerisation (polyHIPE): an emulsion templating technique. Briefly, a polymer (oil) phase is mixed with dropwise addition of water and UV cured in a silicone mould. The water is removed to leave a highly porous interconnected matrix. A porosity >74% is deemed a 'high' internal phase.

Moulding pseudo-rete ridges

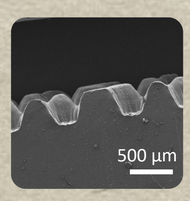


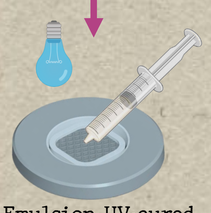
Ridged PDMS mould made with 3D printed template



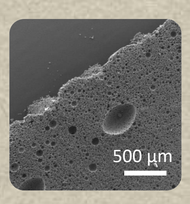


Water-in-oil emulsion is formed by dropwise addition of water into PGS-M solution





Emulsion UV-cured in ridged mould



Near-native ridges



Figure 1 Top surface optical and SEM images of human dermis (A) and ridged polyHIPE (B) showing similar 'sulci' topography.

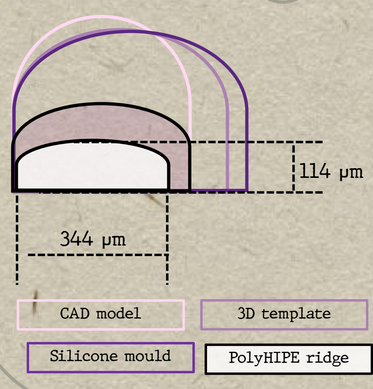
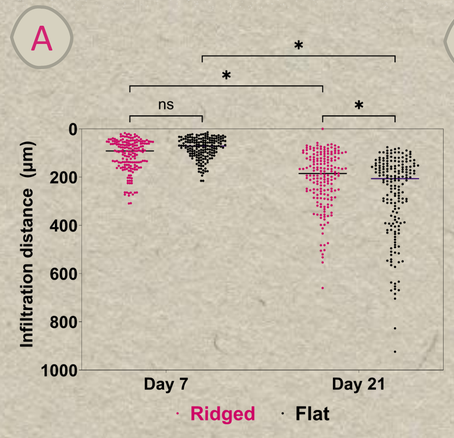
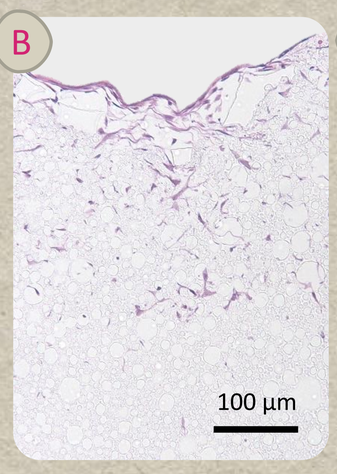
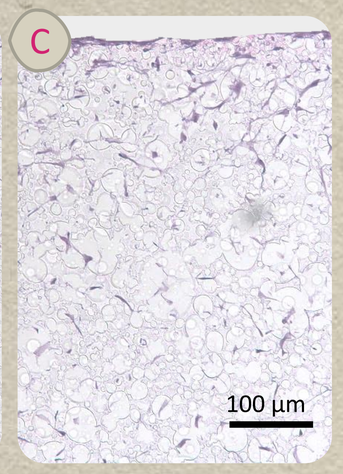


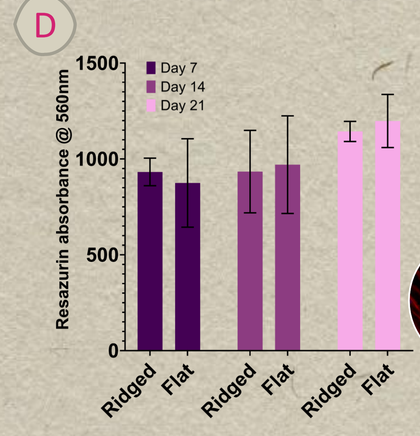
Figure 2 Schematic of ridge dimensions. Rete ridge dimensions are described by a range in literature; height 50-200 µm and width 50-400 µm (Shen et al 2023). Near-native ridge dimensions are achieved here by casting polyHIPE into ridged moulds

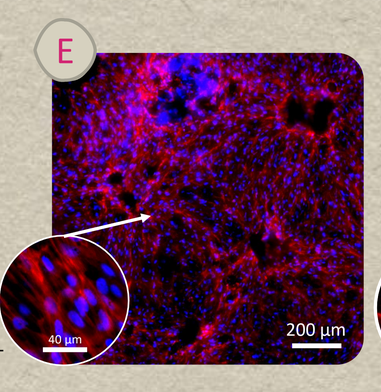
Fibroblast infiltration











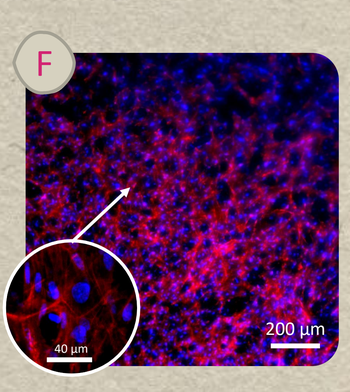
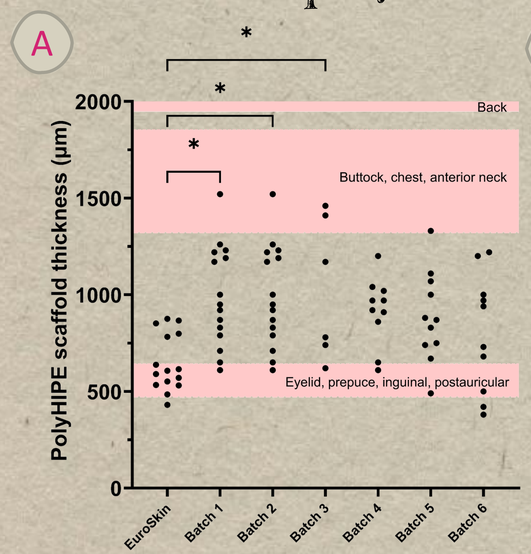
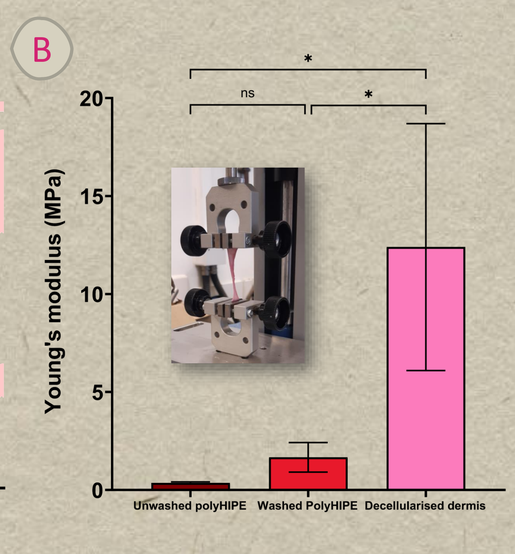
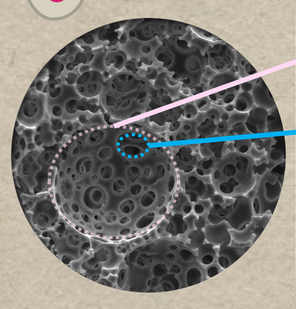


Figure 4 **(A)** Maximum infiltration distance of human dermal fibroblasts (HDFs) into the polyHIPE bulk of ridged and flat polyHIPE. * $p < 0.05$, ns = not significant. **(B & C)** H&E-staining of HDFs infiltrating ridged (B) and flat (C) polyHIPE. **(D)** Resazurin metabolic assay of HDFs on polyHIPE at days 7, 14 and 21. **(E-F)** DAPI/Phalloidin-TRITC staining of HDFs on ridged (E) and flat (F) polyHIPE at day 21.

PGS-M polyHIPE as a dermal matrix







Mean pore diameter (d)	29.0 ± 11.0 µm
Mean window diameter (D)	5.8 ± 2.1 µm
Interconnectivity (D/d)	0.2

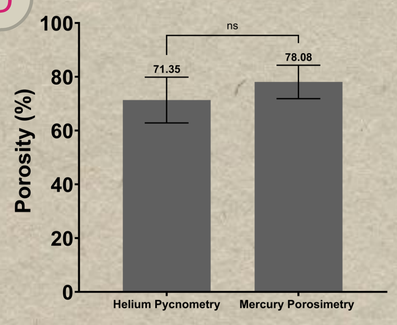
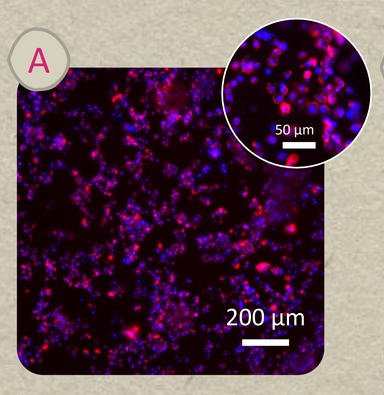
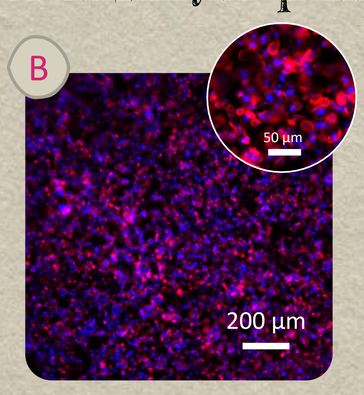


Figure 3 **(A)** PolyHIPE scaffold thickness across batches showing reproducibility of cast/cure method. Pink bands represent ranges of native dermal thicknesses (Lee & Hwang 2002). **(B)** Young's moduli of polyHIPE versus human cadaver dermis. Washing polyHIPE doubles the Young's modulus. * $p < 0.05$. ns = not significant **(C)** Mean pore and window diameters and interconnectivity **(D)** Porosity as measured by helium pycnometry and mercury porosimetry. Mean porosity = 74.7%

Keratinocyte patterning





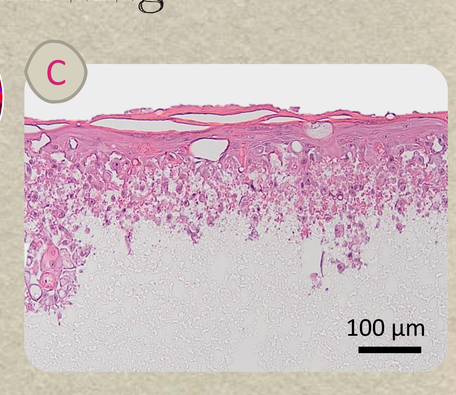


Figure 5 (A-B) DAPI (blue) and Phalloidin-TRITC (red) fluorescence images showing N/TERT-2D keratinocyte monolayer on ridged (A) and flat (B) surfaces at day '0' of ALI. Cells are guided into ridge troughs (C) H&E-stained image of N/TERT-2D keratinocytes on ridged polyHIPE after 10 days at ALI. N/TERT-2Ds have invaded the dermal bulk, causing stunted stratification.

Conclusions

1. Near-native rete ridge dimensions achieved using 3D printed templates and moulding
2. Fibroblasts infiltrate and reside in the synthetic dermal matrix
3. Keratinocytes are guided into ridge troughs but are invading the dermis

Future Work

Develop of a synthetic basement membrane equivalent to prevent ingress of keratinocytes

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Shen et al. (2023) *Acta Biomaterialia*
Lee & Hwang. (2002) *Surg Radiol Anat.*
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