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# Modulating Pore Size in Thermally Cross-linkable Collagen 3D Biomimetic Matrices for Dermal Tissue Engineering

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## Background

Dermal regeneration of wounds with truncated wounds, such as those found in diabetic ulcers require biomimetic matrices that act as molds to the wound<sup>3</sup>. However, little has been done demonstrating the effects of modulating pore size and the effect on cell proliferation, with most of the focus being on structural effects<sup>2,4</sup>.

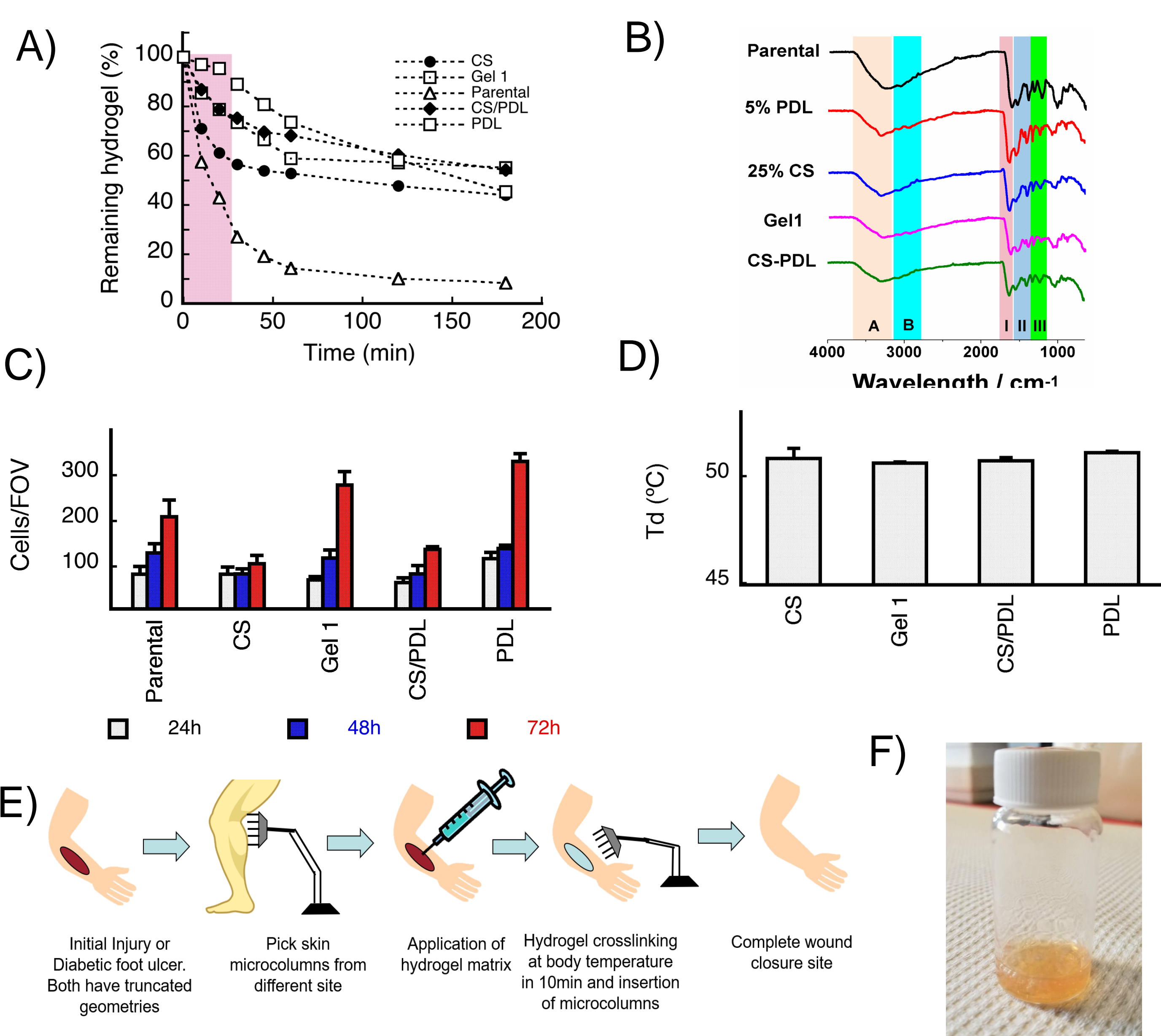
Collagen based hydrogels have been widely used for tissue engineering, including dermal regeneration<sup>1,3</sup>. We have developed such a hydrogel with thermally cross-linkable properties. This study aims to understand the effect of varying pore size by air injection on the proliferation human dermal GFP+ fibroblasts. Modulating pore size will also give more information on the inflammatory response created by the biomimetic matrices.

## Methods

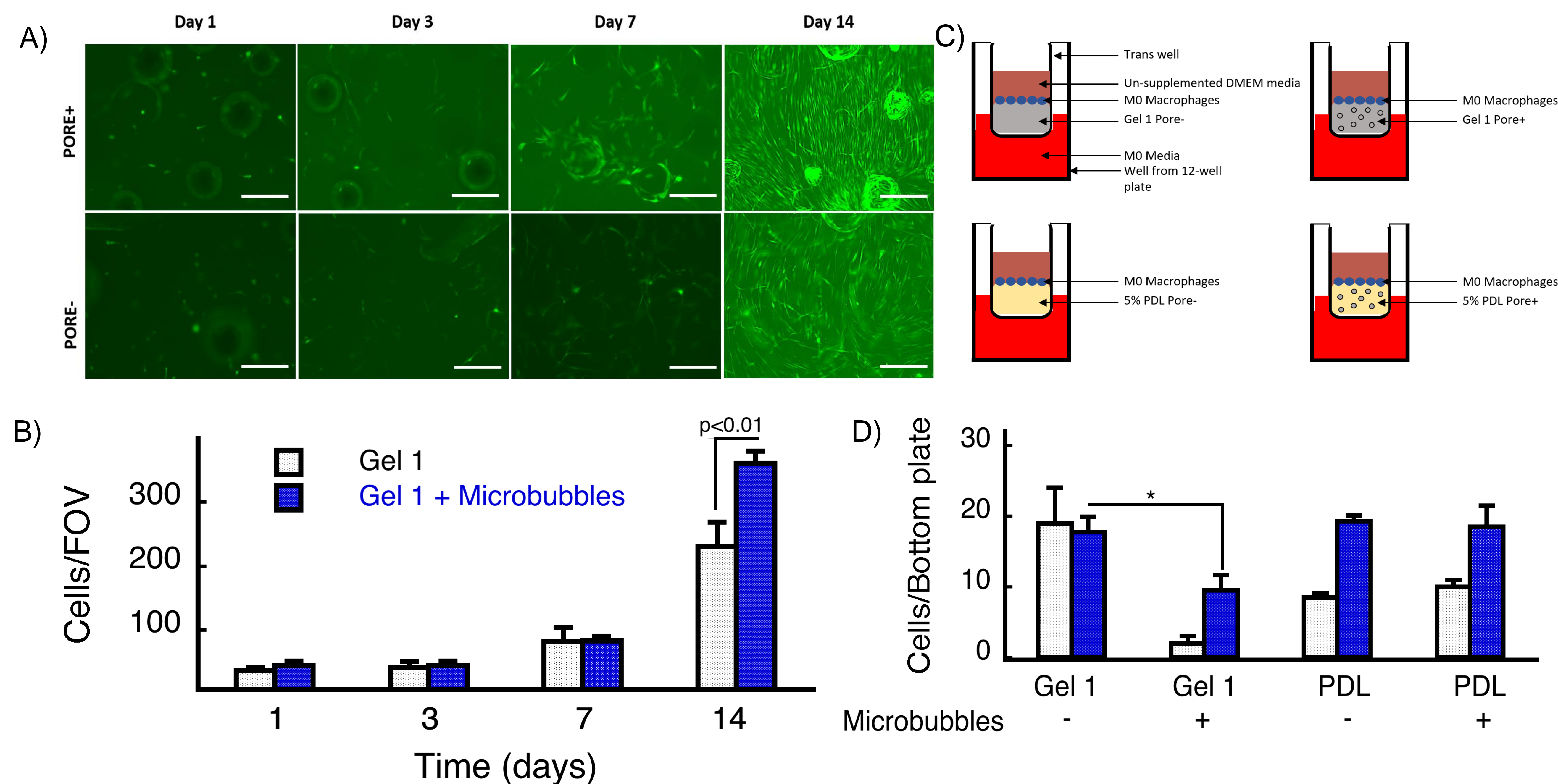
The best collagen-based hydrogel formulation was selected using characterization studies. The selected 'Gel 1' hydrogel matrix was synthesized using a procedure previously developed by E. Alarcon *et al.* 200,000 Human Dermal GFP+ Fibroblasts were injected into the hydrogel formulation and solution was cross-linked using air injection by pipette in the Pore+ hydrogels. All hydrogels were washed with cell media (DMEM, 10% FBS, 1% Penicillin/Streptomycin) and the proliferation assay was performed over 14 days with fluorescent images taken on days 1, 3, 7 and 14.

Macrophage migration study was also performed using undifferentiated M0 mice macrophages. 5% Poly-D-Lysine and Gel 1 hydrogel formulations were synthesized and pores were induced in Pore+ samples. 100µl Hydrogel samples were crosslinked in trans wells for 10 minutes at 37°C and 200,000 M0 macrophages were seeded on top of the hydrogel with 100µl un-supplemented DMEM media. The bottom well contained 300µl M0 macrophage media with L929. Images of migration were taken at Day 2 and 6 after seeding.

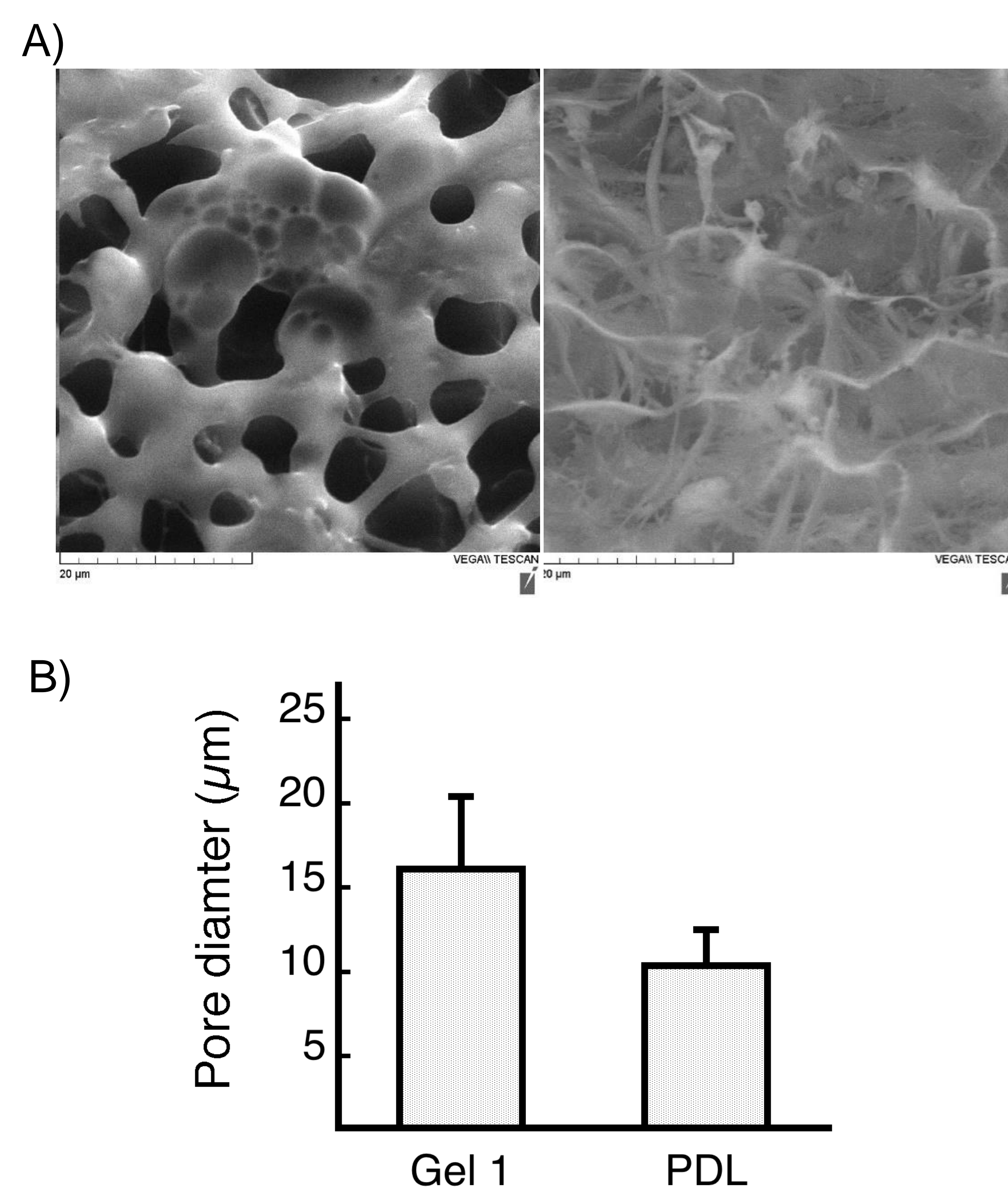
## Results



**Figure 1:** A) Collagenase Degradation Assay results. Note CS = Chondroitin Sulfate B) FTIR spectroscopy results C) Pilot fibroblast culture assay done over 72h. D) DSC characterization results. No statistical significance between all gels. E) Schematic clinical representation of the use of hydrogel. F) Image of Pore- Gel solution



**Figure 2:** A) Fluorescence images of GFP+ fibroblasts cultured in Pore+ and Pore- Gel 1 hydrogel formulations. Scale bar = 150µm B) Proliferation plot for GFP+ human dermal fibroblasts up to day 14. Confluency reached in Pore+ by Day 14 C) M0 Macrophage migration experimental set up D) Migration results. \* indicates  $p < 0.01$  between Day 6 Gel 1 Pore+ and Day 6 Gel 1 Pore-



**Figure 3:** A) Comparative Cryo-SEM images of Pore- Gel 1 (Left) and Pore- 5% PDL (Right) surfaces. B) Comparative graph is depicted below ( $p < 0.01$ )

## Conclusion

Hydrogel formulations containing modulated pore size (Pore+) performed better with the growth human GFP+ dermal fibroblasts *in vitro*. Pores within the hydrogel were fully closed by Day 14 while non-pore modulated hydrogels (Pore-) did not reach same level of confluency as Pore+. Proliferation rates remained statistically similar until Day 14 between Pore- and Pore+ hydrogels. A larger M0 migration response was seen in Pore+ formulations when compared to Pore- formulations at Day 2. Although extra experimentation is required *in vitro* for skin micro-column viability, this study demonstrates the potential of such collagen based hydrogels for dermal tissue engineering.

## Acknowledgements

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## References

- Busra, F. *et al.* Attachment, proliferation, and morphological properties of human dermal fibroblasts on ovine tendon collagen scaffolds: a comparative study. *Malays J Med Sci.* **24**, 33–43. (2017)
- Pensalfini, M. *et al.* Factors affecting the mechanical behaviour of collagen hydrogels for skin tissue engineering. *Journal of Mechanical Behaviour of Biomedical Materials* **69**, 85-97 (2017)
- Wong, V. W. *et al.* Engineered Pullulan-Collagen Composite Dermal Hydrogels Improve Early Cutaneous Wound Healing. *Tissue Engineering Part A* **17**, 631-644 (2011)
- Zhang, J. J. *et al.* Synthesis and characterization of hyaluronic acid/human-like collagen hydrogels, *Mater. Sci. Eng. C* **43** (2014) 547–554