# Development of Technologies to Support the Robust and Reproducible Growth of Complex Bioengineered Human Skin Equivalents

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## Introduction

The dermis is the underlying submucosal compartment of the skin and is responsible for supporting the overlying epidermis both structurally and trough trophic signals (Hsieh & Lin, 1999). It provides biophysical support through the extracellular matrix (ECM) secreted by the fibroblasts (Sorrell & Caplan, 2009). ECM primarily consists of proteoglycans and fibrous proteins (e.g. collagen, elastin, fibronectin and laminin) (Padhi & Nain, 2020). In reconstructed human skin models the ECM plays a crucial role in preventing keratinocyte infiltration and promoting epidermal development. Can Dulbecco's modified eagle medium (DMEM) with serum be used to promote increased fibroblast proliferation and ECM deposition in the dermal model? Could this lead to a reduction in culture time of a robust dermal compartment? Finally, can this dermal compartment support a complex and stratified epithelium?

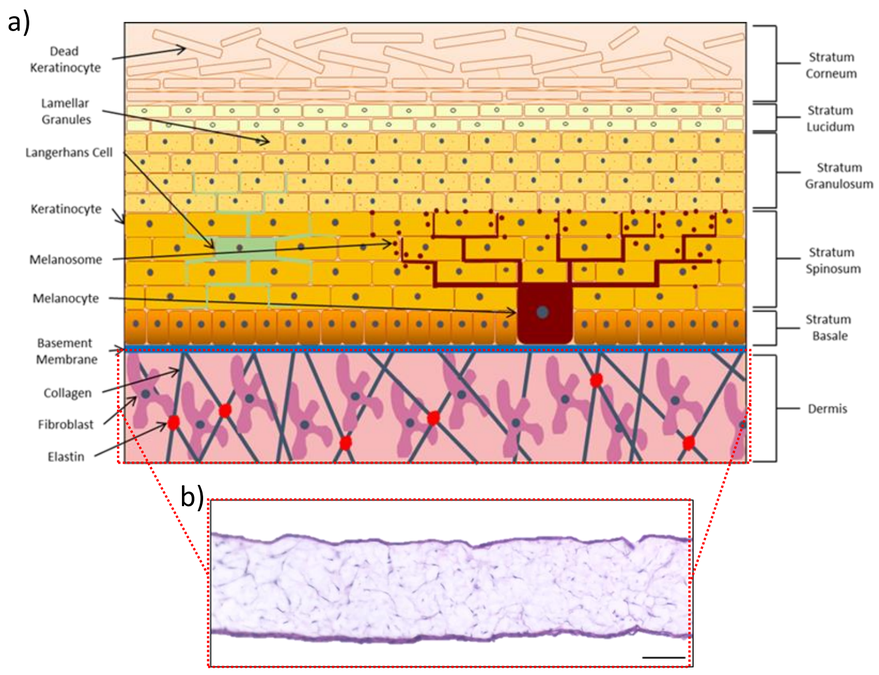


Figure 1: (a) Diagram of native human skin including ECM deposition in dermal compartment which provides crucial support to the overlying epidermis. And what is project aims to replicate *in vitro*. (b) Dermal model cultured for 4 weeks in M106. Analysis of model by H&E. Scale bar: 100µm.

## 2: Improved 2D fibroblast culture in DMEM-based media

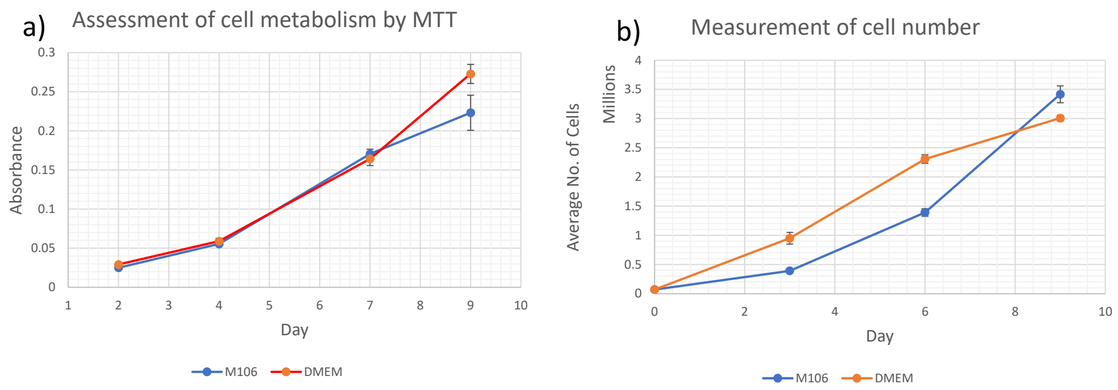


Figure 2: DMEM fibroblasts show higher metabolic rates after 7 days and increased initial proliferative capacity in 2D, thus supporting the hypothesis that DMEM could improve dermal robustness in 3D. (a) Characterisation of the number of actively metabolising cells (and indirectly cellular viability) in each media condition. (b) Cellular proliferation per media condition. Growth curve originates at the initial seeding density of 71,000 cells in 2D. Error bars constructed using the standard error of the mean (SEM), n=3.

## 3: DMEM improves dermal morphology

Dermal compartments were generated by seeding neonatal human dermal fibroblasts (HDFn) into Alvetex® (an inert microporous polystyrene scaffold) and allowed time to infiltrate and attach to the Alvetex®. This was then submerged in either low serum M106 or DMEM containing 10% non-heat treated foetal bovine serum (NHT-FBS) in order to determine model robustness over time and if the culture period could be reduce by the addition of supplements (Transforming growth factor-β1 (TGF-β1) & ascorbic acid (AA).

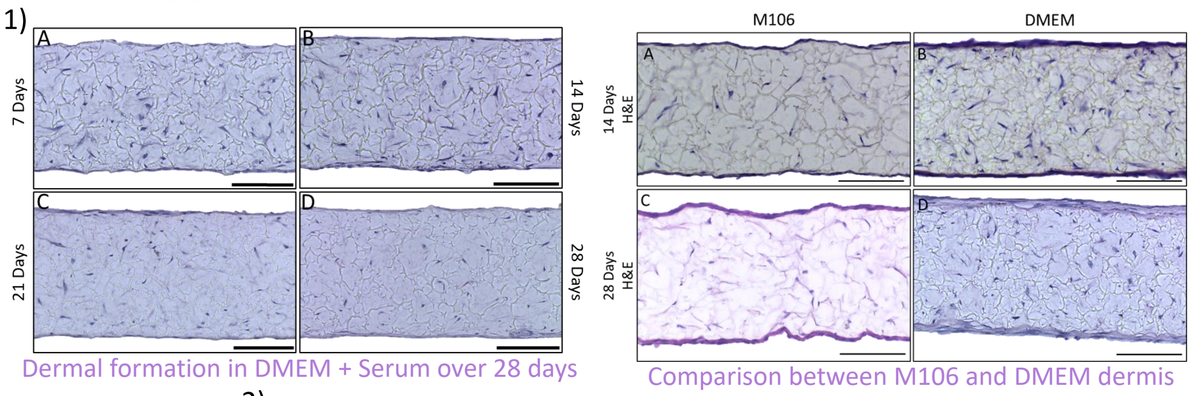


Figure 3: DMEM promotes increased cellular proliferation within scaffold and cell layering on top of scaffold, both are critical factors in the ability to support a stratified epidermis. (1) Dermal development over 28 days (TGF-β and AA included). (2) Dermal formation with (2A) and without (2B) AA and TGF-β. All models stained with H&E. Scale bars = 100µm.

## 4: Deposition of Collagen is enhanced in DMEM

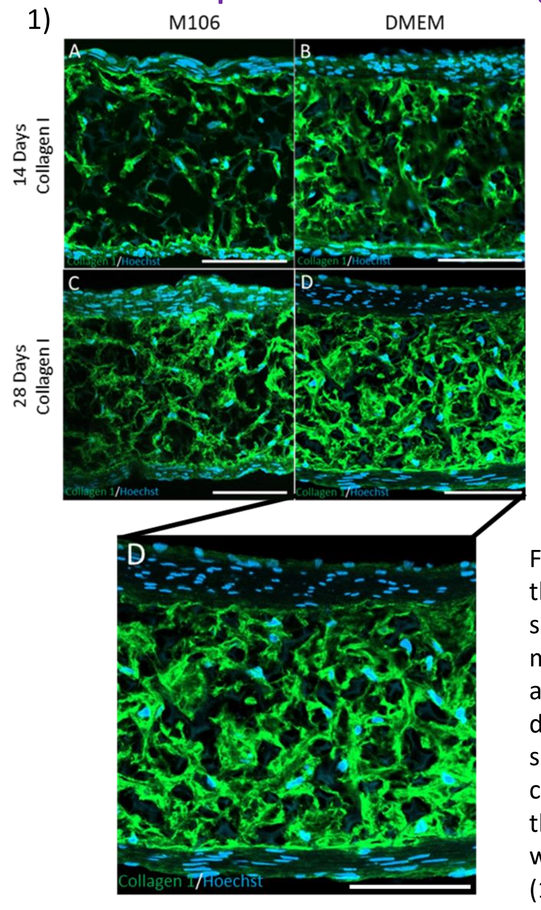


Figure 4: Collagen deposition can be seen throughout the dermal compartment and on the surface of the scaffold membrane. DAPI staining shows fibroblast multi-layer on top of scaffold membrane. Both of these aspects are critical in the ability to support the development of an epidermis due to increased physical support. DMEM shows a distinct visual increase in collagen deposition compared to M106. This supports the hypothesis that dermal culture time can be reduced without keratinocyte infiltration. Green = collagen 1 (1A-D) and collagen 3 (2A-D). Blue = DAPI staining of cellular nuclei (1-2A-D). Scale bars = 100µm.

## 5: Enhanced epidermal support in DMEM-based media

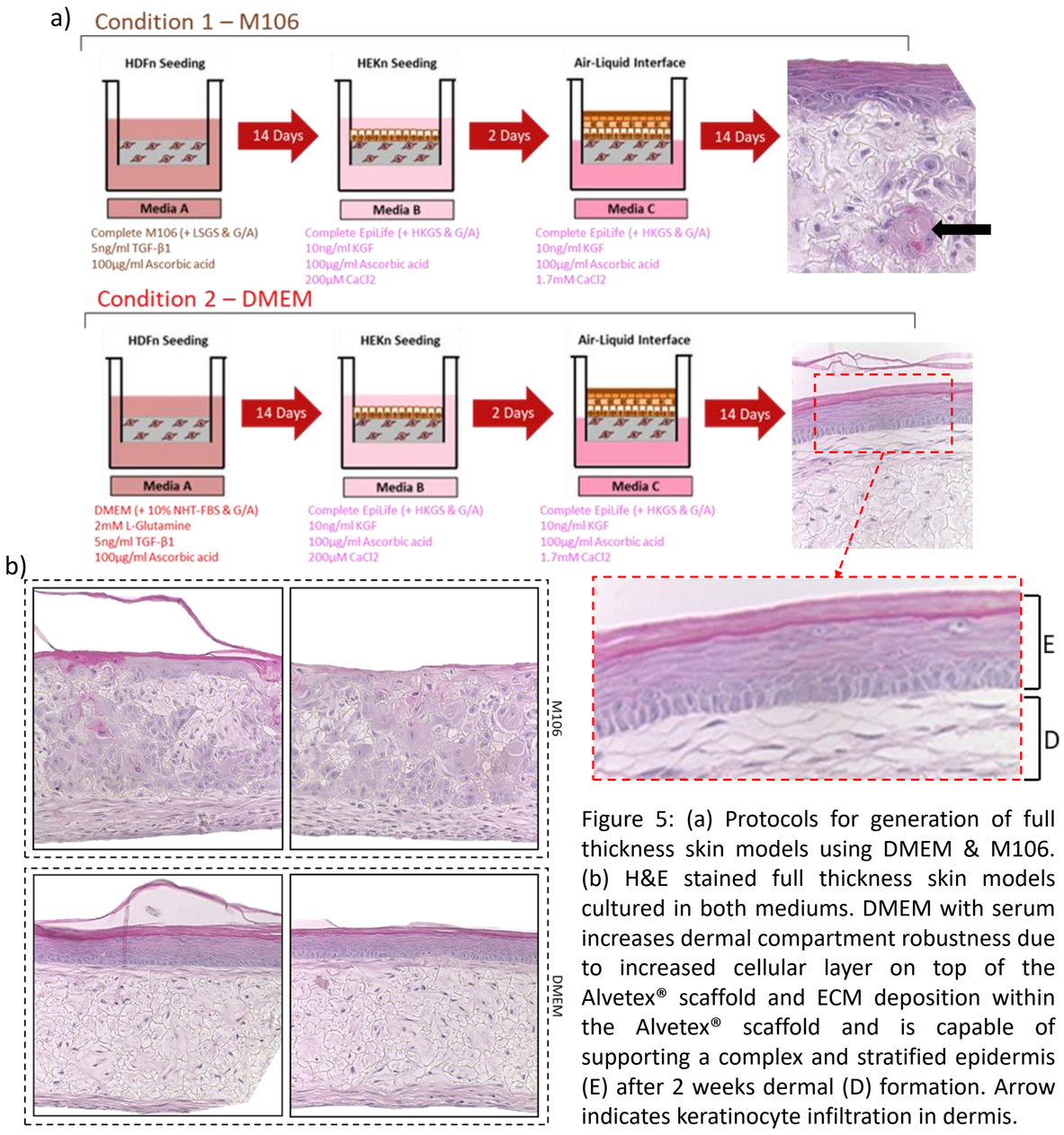


Figure 5: (a) Protocols for generation of full thickness skin models using DMEM & M106. (b) H&E stained full thickness skin models cultured in both mediums. DMEM with serum increases dermal compartment robustness due to increased cellular layer on top of the Alvetex® scaffold and ECM deposition within the Alvetex® scaffold and is capable of supporting a complex and stratified epidermis (E) after 2 weeks dermal (D) formation. Arrow indicates keratinocyte infiltration in dermis.

## 6: Conclusions and future directions

**Conclusions:**

- Deposition of Collagen 1/3 appears to be increased in the DMEM condition after 2 week in comparison to the M106 condition.
- Dermal formation in DMEM over 2 weeks was sufficient to support epidermal formation and prevent keratinocyte infiltration, whereas the M106 2 week condition was not.

**Future Directions:**

- Further analysis of ECM deposition by immunohistochemistry, western blot and dedicated ECM assay for elastin, fibrinogen, fibronectin and glycosaminoglycan's.
- Further analysis of epidermal formation and infiltration by immunohistochemistry for epidermal differentiation markers (K10 & K14). This includes further immunohistochemical characterization of basement membrane formation in comparison to native human skin.
- Preparation for the future inclusion of additional skin cell populations (melanocytes & immune cells).

## 7: Bibliography

Hsieh, S. T., & Lin, W. M. (1999). Modulation of keratinocyte proliferation by skin innervation. *The Journal of Investigative Dermatology*, *113*(4), 579–586. https://doi.org/10.1046/j.1523-1747.1999.00737.x

Padhi, A., & Nain, A. S. (2020). ECM in Differentiation: A Review of Matrix Structure, Composition and Mechanical Properties. *Annals of Biomedical Engineering*, *48*(3), 1071–1089. https://doi.org/10.1007/s10439-019-02337-7

Sorrell, J. M., & Caplan, A. I. (2009). Fibroblasts-a diverse population at the center of it all. *International Review of Cell and Molecular Biology*, *276*, 161–214. https://doi.org/10.1016/S1937-6448(09)76004-6