A digital reconstruction of the dermal-epidermal junction (DEJ) of the skin in three dimensions

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Background

The dermal–epidermal junction (DEJ) is a basement membrane which separates the dermis and epidermis within the human skin. The DEJ has a unique topography, its surface undulates and forms a dense interconnecting collagen network. This network is formed of interconnecting projections; papillae (dermis into the epidermis) and rete ridges (epidermis into the dermis). The DEJ requires further study, it's primarily required to maintain skin integrity but it is also known to assist skin homeostasis, layer adhesion and function. Tissue engineering (TE) allowed the development of skin regeneration strategies and TE scaffolds for clinical use. However, the currently available TE skin equivalents neglect to replicate the DEJ anatomical structures.

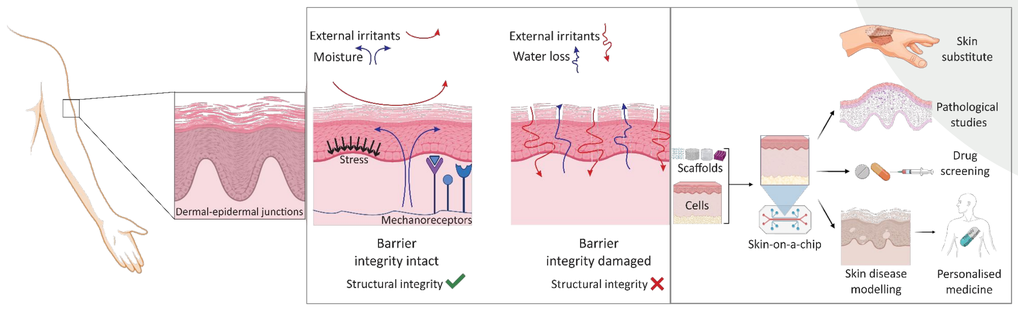

Figure 1. Left, depicts dermal-epidermal junctions (DEJ). Central, show the barrier and structural integrity of the DEJ intact (central-left) and damaged (central-right). Right, depicts the applications of DEJ development [1].

Introduction

This study explores the digital reconstruction of the DEJ via computer aided design (CAD) to form 3D models using Autodesk Fusion 360, a 3D modelling software. The 3D models were produced using additive manufacturing and laser-based patterning using SLA 3D printers. The main aim of this study is to develop 3D models which mimic the DEJ topography at a cellular level. This study presents high resolution 3D models with sinusoidal waveform topography, specifically defined by cellular dimensions. In this process 3D printing paraments were optimised, grey resin was used for greater resolution and print orientation was explored. Additionally, cubic patterns of cellular dimension were modelled in 3D and a more accurate 3D model of the human DEJ created using a stack of 400 optical coherence tomography (OCT) images of the isolated DEJ was developed to further assess the effectiveness of topography and dimensions of each model on cells during cell culture.

Methods

In this study, Autodesk Fusion 360 was used to form 10 stl files, 5 of which are repetitive cubic patterns and 5 sinusoidal waveform which more closely match the topography of the DEJ. The dimensions of both pattern types were modelled to match the range of dimensions in which cells are found in the DEJ and one measurement beyond this range (1200 depth 600 width), Table 1.

Depth / µm	Width / µm
125	50
50	225
200	225
125	400
1200	600

Table 1. Dimensions used to design 3D models, first 4 rows reflect DEJ cellular dimensions, final row is dimensions outside of this range.

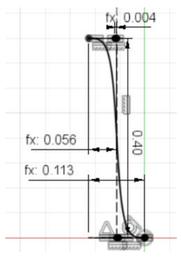

Figure 2. Waveform sketch of dimensions: 200 µm depth (400µm peak-peak) and 225µm width (wave-length)

Amira, a data visualization, processing and analysis software was used to output .stl files of the human DEJ. OCT images of skin tissue from the forearm, cheek and chin within the range of 50-225 µm in depth and 50-400 µm in width were used in this study. Each set of OCT images holds 400 chronologically stacked images, from this Amira forms a 3D representation of the human DEJ via volume rendering.

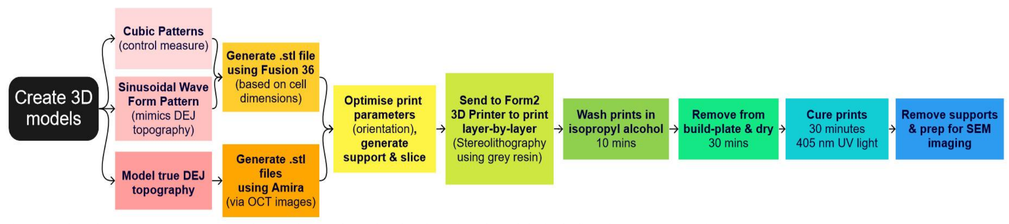

Figure 3. Flowchart to represent steps taken in this study to develop 3D printed models, including the post-processing steps.

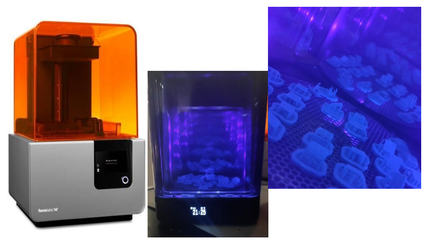

Figure 4. Left Form2 SLA 3D printer. Center and right automated post-curing FormCure.

The 3D models designed in Fusion 360 were developed using a 6 step process for models with cubic patterns and a 13 step process for models with a waveform pattern, Figure 3. Models were sliced in Preform, a 3D printing software, print parameters were optimised, layer resolution set to 25µm and print orientation was explored between 0° and 60° in single and multiple axes. The models were printed using Grey resin via Form2 liquid resin 3D printers and quality of print was assessed via a Scanning Electron Microscope (SEM).

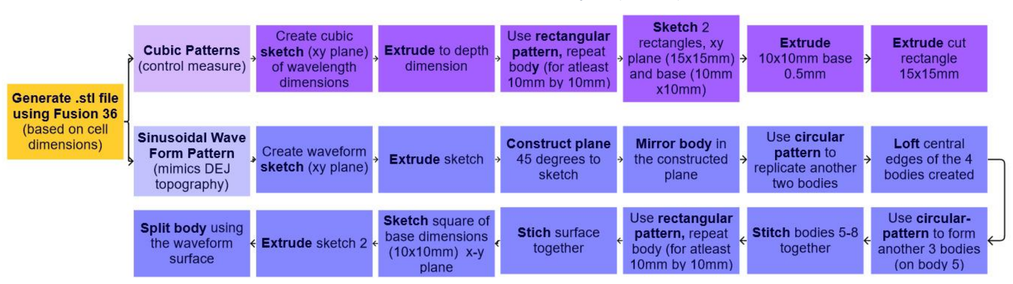

Figure 5. Flowchart to represent steps taken to design 3D models of both cubic and waveform pattern type. Functions used in Autodesk 360 in each step are in bold.

Results and Discussion

CAD Models

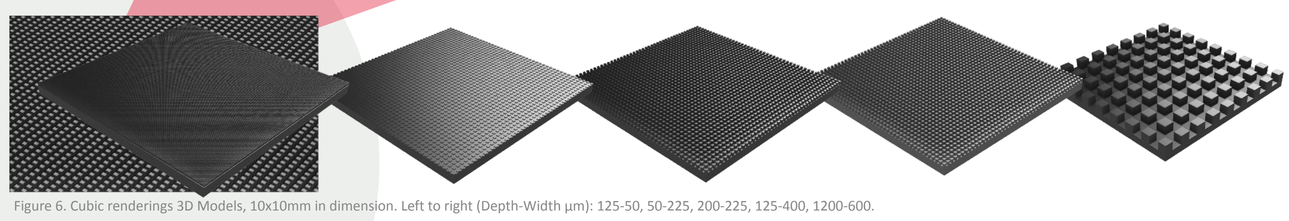

Figure 6. Cubic renderings 3D Models, 10x10mm in dimension. Left to right (Depth-Width µm): 125-50, 50-225, 200-225, 125-400, 1200-600.

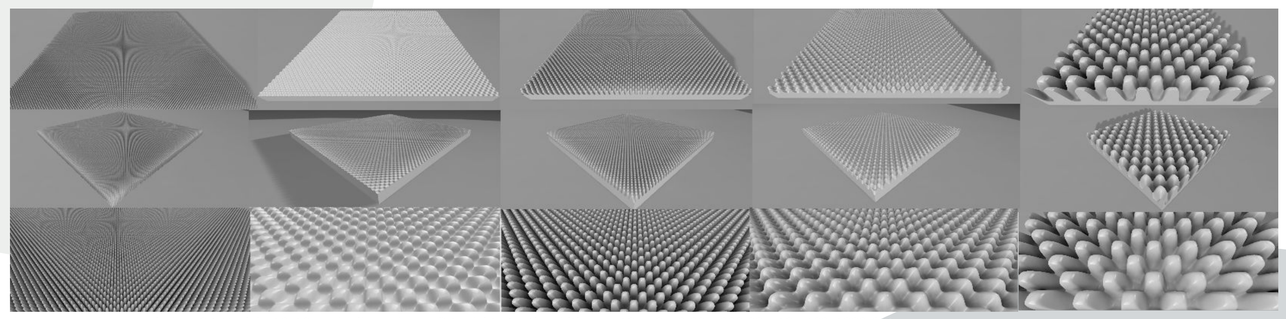

Figure 7. Waveform renderings 3D Models, 10x10mm in dimension. Left to right (Depth-Width µm): 125-50, 50-225, 200-225, 125-400, 1200-600.

3D Printed Models

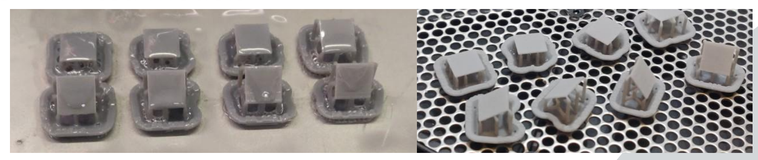

Figure 8. 3D Printed Models after rinsing isopropyl alcohol via FormWash (left), post-curing in UV in FormCure (Grey resin, Form2 SLA printer).

SEM Images

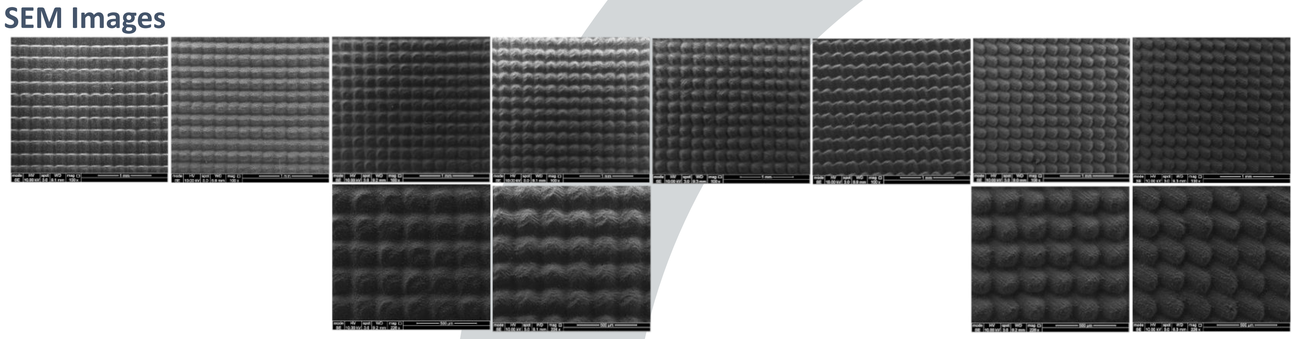

Figure 9. SEM of 50µm (depth) 225µm (width) cubic 3D models. Left to right (Orientation in x and y axis, degrees): 0, 5, 10, 20, 30, 40, 50, 60.

Due to the high resolution and high number of parts involved in the 3D design of models high performance computers were required to output the desired .stl files. Alternative CAD software should be explored in the future. In this study, 10 models were designed and 80 models were 3D printed. Through print parameter optimization it is determined that with greater orientation the print quality also increases, however when orienting in both the x and y axis the cubic topography is replaced with an oblong one, hence orienting to 50-60° in one axis is most suitable. From SEM analysis it is determined the Form2 is unable to provide suitable print quality, the edges of cubic patterns are rounded and the negative space is not as defined as expected. In comparison the OCT images provide a non-continuous 3D surface, filters must be applied to extrapolate this missing data.

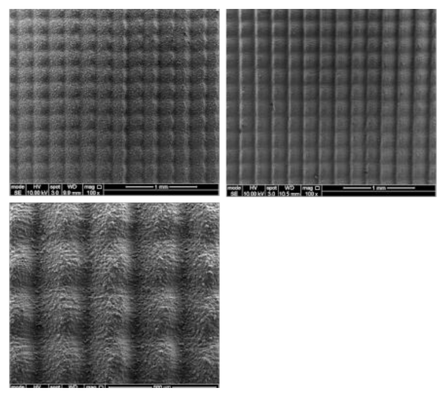

Figure 10. SEM of 50µm (depth) 225µm (width) cubic 3D models. (Orientation only in x axis).

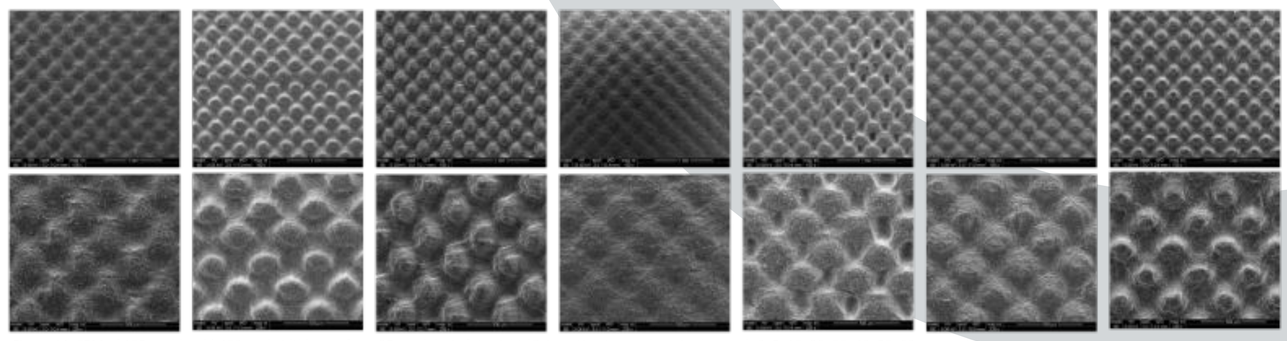

Figure 11. SEM of 125µm (depth) 400µm (width) waveform 3D models. Left to right (Orientation in x and y axis, degrees): 0, 5, 10, 20, 30, 40, 50, 60.

DEJ from OCT Images via AMIRA

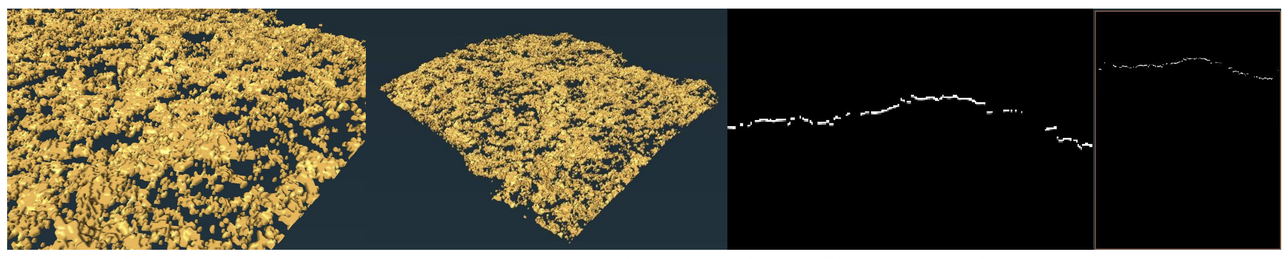

Figure 12. Left and Central-left: 3D volume rendering using AMIRA software of human DEJ. Right and Central-right: Slice 255 of 400 OCT images of the human DEJ used to form the volume rendering (left).

Conclusions

In conclusion, 10 unique 3D models, have been designed and produced, hence in the future the designed topographies will be accessed via cell culture. Due to micron range limitation a high resolution printer is required, for example the Micro Kudo 3D printer, which has a resolution of 15 µm or a 2-photon laser 3D printer. The Form2 SLA printers used in this study have a layer resolution of 25 µm however due to the laser spot-size of 140 µm this printer struggled to output greater print quality.

The effects of the designed physical topographies and dimensions will be investigated via cell culture and hence used to form negative moulds or directly 3D printed to develop novel 3D skin models or platforms which utilise this scaffold. In particular, these 3D models will be used to form moulds for novel biodegradable, biocompatible and highly elastomeric copolymers; poly(glycerol sebacate)-co-polyethylene glycol (PGS-co-PEG) polymers. Using these biopolymers to create precise 3D scaffolds which considers DEJ topography could be a promising alternative for in vivo models.

Acknowledgements

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Figure 2 and 3 were created using miro.com.

References

[1] Aleemardani, M., Trikić, M. Z., Green, N. H., Claeyssens, F. (2021). The Importance of Mimicking Dermal-Epidermal Junction for Skin Tissue Engineering: A Review. *Bioengineering (Basel, Switzerland)*, *8*(11), 148. https://doi.org/10.3390/bioengineering8110148
[2] Vogt, L.; Ruther, F.; Salehi, S.; Boccaccini, A. R. Poly (Glycerol Sebacate) in Biomedical Applications—A Review of the Recent Literature. Adv. Healthc. Mater. 2021, 10 (9), 2002026.
[3] Liu, P., Zhu, J.-Y., Tang, B. and Hu, Z.-C. (2018), Three-dimensional digital reconstruction of skin epidermis and dermis. Journal of Microscopy, 270: 170-175. https://doi.org/10.1111/jmi.12671

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