Recreating the Human Limbus

Ryan Dimmock

Keele University

Engineering and Physical Sciences Research Council

Introduction

- The limbal epithelial stem cell (LESC) niche is responsible for supplying stem cells to regenerate the cornea.
- The limbus is located at the interface between the cornea and the conjunctiva surfaces and has an undulating anatomic structure.

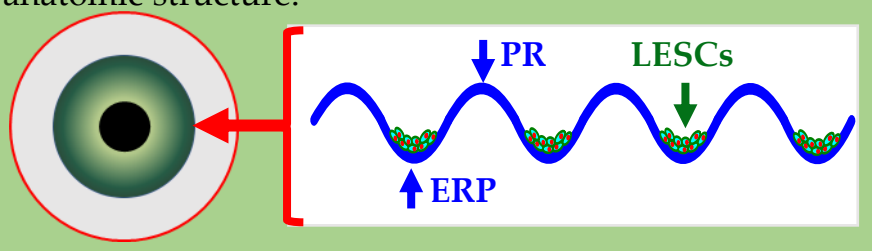

Figure 1: *Location and schematic of the Limbus structure. The crests are the Palisade Ridges, the troughs the Epithelial Rete Pegs. The limbal epithelial stem cells reside in the basal regions [1].*

- LSCD is a degenerative disease which can cause blindness, it is attributed to limbus structure degradation and LESC loss.
- Polymer wrinkling techniques provide a convenient and cost effective way to replicate limbal structure [2].
- Current work is moving towards creating a dynamic model, exploiting polymer wrinkling in a novel bioreactor to study LESC behaviour on these substrates to better understand LSCD development.

Results

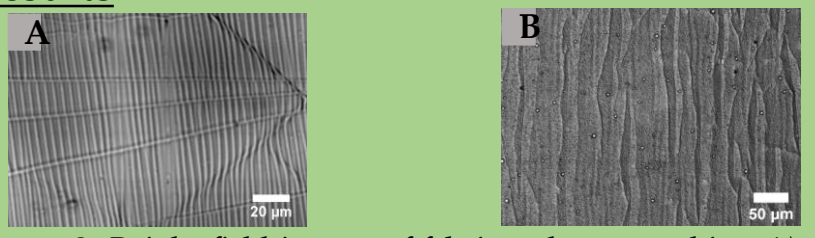

Figure 3: *Bright field images of fabricated topographies, A) Oxygen plasma treated and B) acid oxidised topographies.*

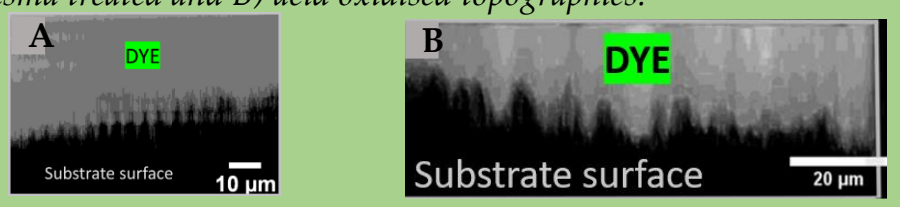

Figure 4: *Cross-section reconstruction of topographies using confocal microscopy using FITC fluorescent dye. A) Plasma and B) acid oxidised PDMS.*

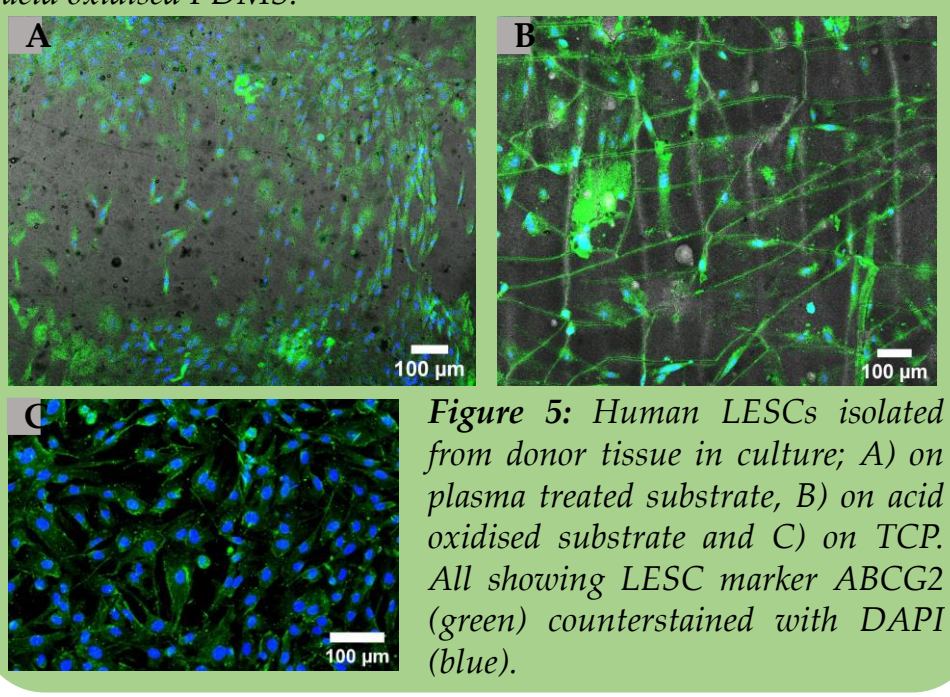

Figure 5: *Human LESCs isolated from donor tissue in culture; A) on plasma treated substrate, B) on acid oxidised substrate and C) on TCP. All showing LESC marker ABCG2 (green) counterstained with DAPI (blue).*

Methods

- Polydimethylsiloxane (PDMS) was formulated with 5% crosslinker.
- Surface modification of PDMS to create topographical substrates was performed using 2 methods:

1) Exposure to 3:1 sulfuric: nitric acid mixture that was thermally decomposed.
2) Exposure to low temperature oxygen plasma.

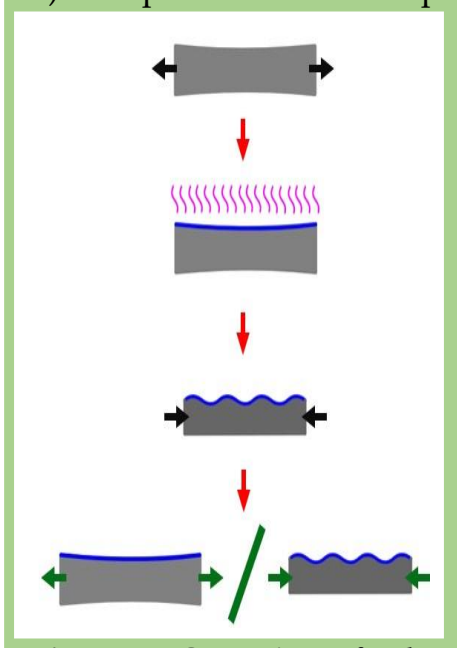

Figure 2: *Overview of substrate fabrication.*

- Limbal stem cells were obtained from human donor tissue, limbal sections were dissected and digested overnight in Collagenase IV.
- Confocal microscopy was used to image cells and substrates.

Discussion and Conclusions

- The cell isolation protocol was able to successfully extract and isolate cells which present the ABCG2 marker, a marker which is canonically associated with being present in limbal epithelial stem cells [3].
- The employed polymer wrinkling techniques were able to influence cell behaviour by changing their shape and distribution on the surfaces.
- The cells retained the ABCG2 marker after being cultured on the surface, demonstrating the conservation of this stem cell marker.
- The different wrinkling methodologies both produced wrinkling with different frequencies and geometries.
- The acid oxidised surface also demonstrates post treatment tuneability whereby the surface topography can be altered through re-application of stretching.
- This ability is being taken forwards into further work to dynamize the culture system, mimicking the progressive topographical changes associated with LSCD [4].

References

[1] Haagdorens, M. et al., A method for quantifying limbal stem cell niches using OCT imaging, *British Journal of Ophthalmology*, 2017, 1250-1255, 101(9).

[2] Dimmock, R. et al., Biomedical Applications of Wrinkling Polymers, *Recent Progress in Materials*, 2020, 1-31, 2(1).

[3] Gouveia, R. et al., YAP, ΔNp63, and β-Catenin Signaling Pathways Are Involved in the Modulation of Corneal Epithelial Stem Cell Phenotype Induced by Substrate Stiffness, *Cells*, 2019, 347, 8(4).

[4] Banayan, N. et al., Spectral-domain Optical Coherence Tomography in Limbal Stem Cell Deficiency. A Case-Control Study, *American Journal of Ophthalmology*, 2018, 179-190, 190