Stem Cells (Human Corneal Epithelial)

Student: Raheel Ahmed, Supervisor: Dr Sajjad Ahmad

Contacts: raheel.ahmed@ncl.ac.uk s2ahm@doctors.org.uk

Institute of Human Genetics

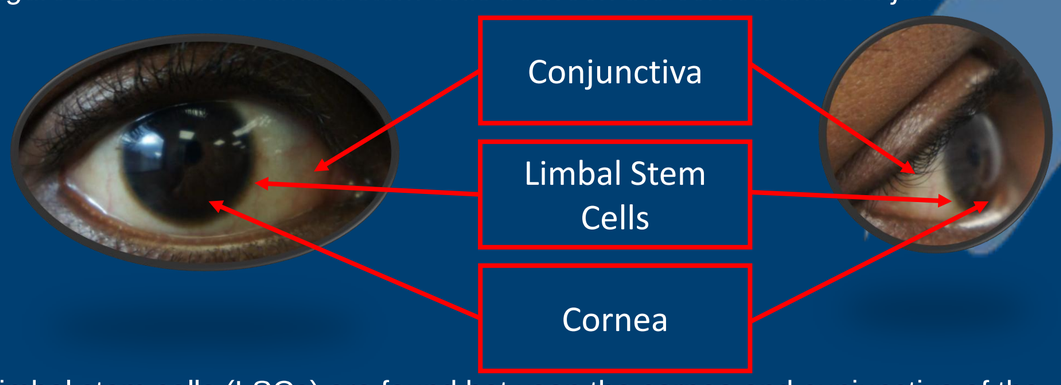
1 Aims

- Culturing human corneal epithelial stem cells (*limbal stem cells*)
- Finding the difference in the rate of cell growth of *limbal stem cells* when grown in 2 different types of plates: IWAKI (normal plastic) and Synthemax

2 Introduction

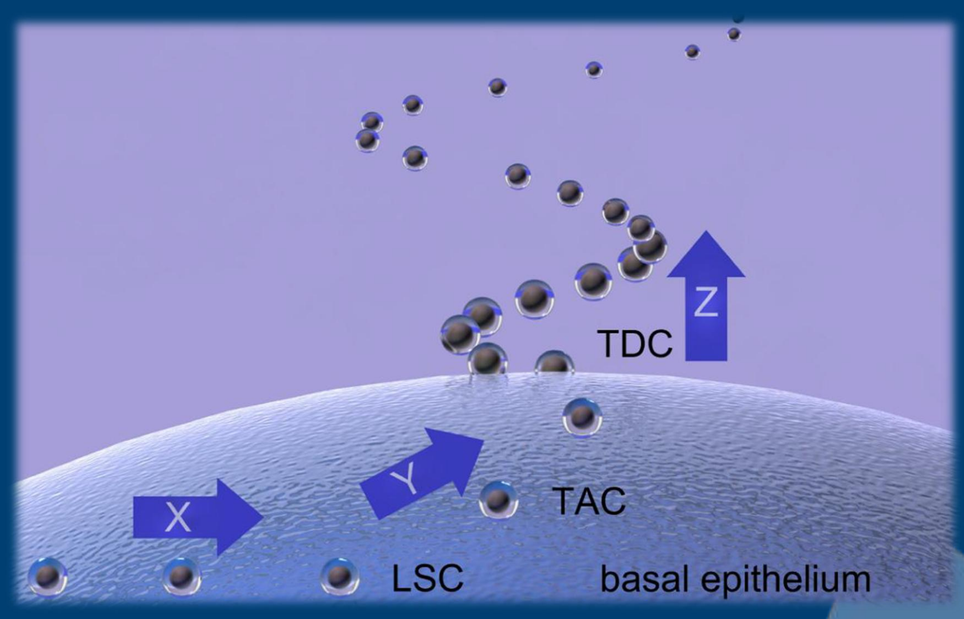
Stem cells are biological cells that are found in human beings and other multicellular organisms. Mammalian stem cells are sub-divided into embryonic and adult stem cells. These cells can divide and differentiate into any other specialised cells. Moreover, they can self -renew producing additional stem cells. They can be artificially grown in cell culture and transformed into specialised cell types. Newcastle University is at the forefront of such stem cell research.

Figure 1: Location of limbal stem cells between the cornea and Conjunctiva



Limbal stem cells (LSCs) are found between the cornea and cunjunctiva of the human eye (see figure 1). Limbal stem cell deficiency (LSCD), usually caused by chemical burns, results in loss of visual detail and pain. Normally, the management of such patients involves transplantation of whole or cultured limbal epithelium[1]. This project dealt with the latter source of management by differentiating and investigating the cultures of limbal epithelium.

Figure 2: A schema showing how limbal stem cells (LSC) move from basal membrane (X) to the outer cornea (Y and Z) to replace the corneal cells lost.



The process outlined in Figure 2 is not possible in LSCD. Therefore, investigating whether the cells could be cultured faster in the Synthemax plate, manufactured by Corning (an American company selling scientific applications) ,would help to find a faster way to transplant the lost limbal stem cells in LSCD.

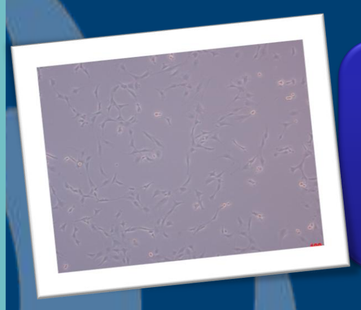
5 Conclusion

Schrehardt and colleagues (2005) have previously shown that unlike other limbal markers, ABCG2 is the best at distinguishing limbal stem cells from progenitor cells[3]. In the light of this, one may claim that *there is preferential proliferation of limbal stem cells in synthemax plates.* However, more research is needed to validate this conclusion.

6 So What?

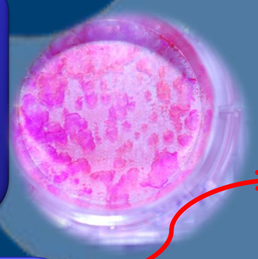
The results from this project may help in a PhD product on the development of an in vitro limbal stem cell wound healing assay. This project is part of an ongoing collaboration with Corning. Dr Sajjad and his group are also in the process of writing a manuscript and so the data from this project may well be put into that manuscript.

3 Methodology[2]

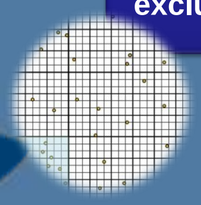


1. Human limbal stem cells were cultured in vitro using mitotically inactivated Swiss mouse fibroblast feeders (3T3s).
2. The cultures were incubated and grown for up to 14 days until they occupied more than 90% of the well (became confluent).
3. The two wells pooled together. Half of the cells put into the IWAKI plate and the other half into the Synthemax plate.

4. **LSC's colony forming efficiency (CFE)** calculated by: (No. of cells formed/number of cells plated) x 100



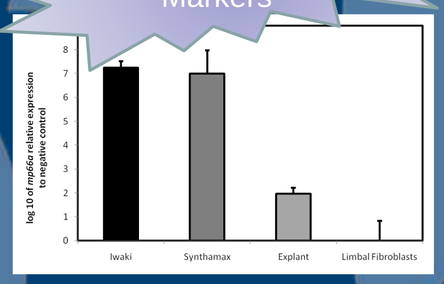
5. Cell viability was calculated using a **haemocytometer** and **Trypan blue exclusion assay**

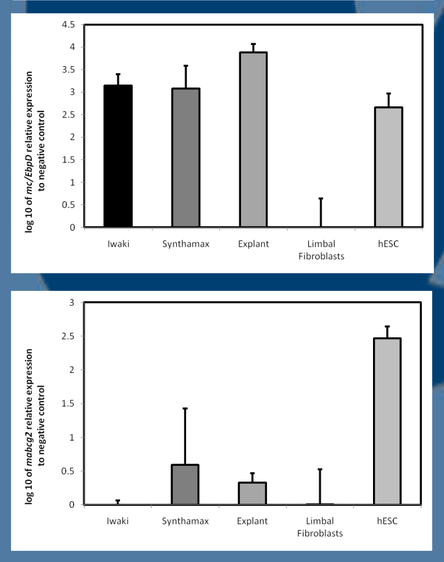


6. The IWAKI and Synthemax plates were incubated at 37^0 C. Media changed every second day
7. RNA Extraction
8. Reverse transcription to give complementary DNA
9. Real time Polymerase Chain Reaction on specific DNA from Limbal Stem Cells
10. Statistical Analysis

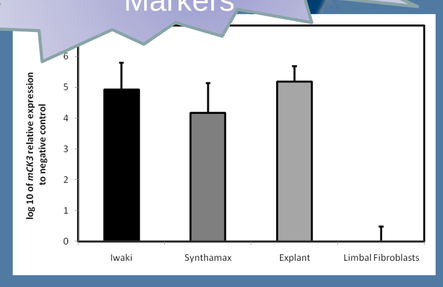
4 Results and analysis

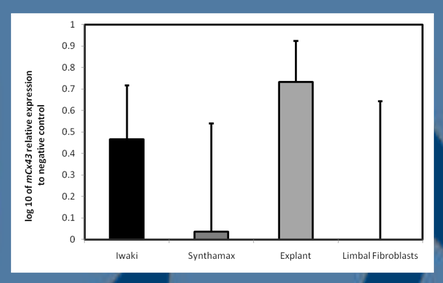
Positive Limbal Markers





Negative Limbal Markers





The assays and primers used in this experiment have previously been validated by the group. It was found that generally there was no difference in expression of limbal markers or morphology of cells grown in IWAKI and Synthemax plates. However, the expression of ABCG2 was much higher in synthemax compared to IWAKI plate.

7 References

1. Ahmad et al. 2006 "Corneal epithelial stem cells: characterization, culture and transplantation" Stem Cells 1(1): 29-44

2. FE Kruse and SC Tseng. 1991 "The Limbus epithelium in vitro" Fortschr Opththalmol 88(2): 107-112

3. Schlötzer-Schrehardt and F Kruse. 2005 "Identification and characterization of limbal stem cells" Exp. Eye Res. 81(3): 247-64

Newcastle University

8 Acknowledgement

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