Understanding the pathophysiology of the mammalian cornea through *in vitro* culture systems, animal models and novel sight-restoring stem cell therapies to treat patients with blindness

Honours and PhD Research Projects

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PROJECT 1

Identifying novel markers of corneal stem cells to assist in their identification, isolation, purification and transplantation

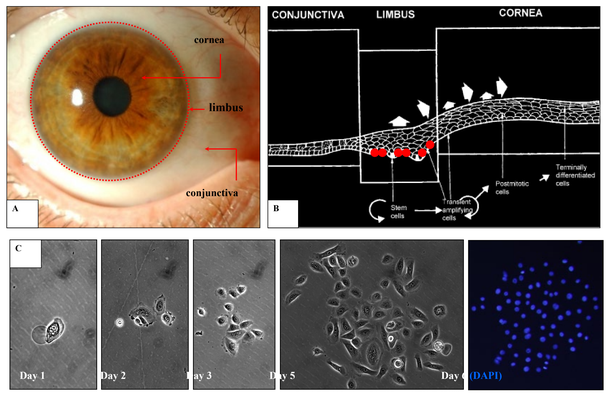


Figure. (A) The normal ocular surface includes conjunctiva which merges into the cornea (the clear window to the world). These two regions are separated by the limbus, a narrow zone that contains the corneal stem cells (B, red dots) that maintain the cornea in a healthy and transparent state throughout life. Stem cells in the limbus migrate into the cornea when corneal epithelial cells are damaged, diseased or shed (B, arrows). Because we have a good idea where the corneal stem cells reside, we can harvest small biopsies (~1mm^2) from the limbal zone and we can isolate and expand the stem cells population for therapeutic applications. Panel C demonstrates a single stem cell that has the replicative capacity to generate a clone of cells after 6 days in culture.

AIMS:

1. Identify better isolation and expansion conditions.
2. Conduct single cell RNA sequencing studies to identify a transcriptomic signature for these cells.
3. Determine whether these cells are unipotent or multipotent.
4. Define the conditions that will allow us to grow organoids for drug discovery and disease modelling
5. Develop novel therapeutic strategies for transplantation.

PROJECT 2

Develop novel stem cell transplantation to restore vision in patients with blinding corneal disease

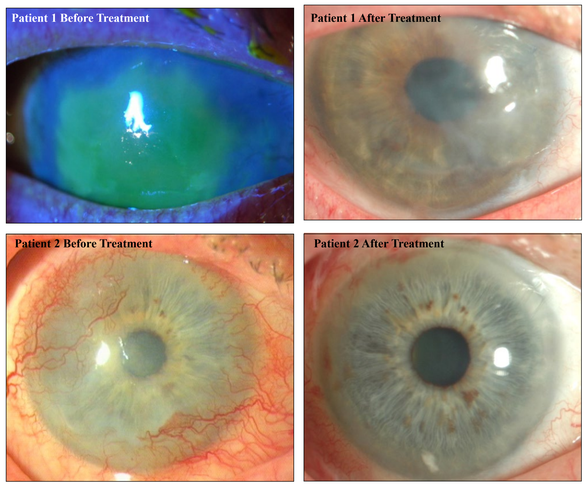


Figure. Patients with severe corneal blindness due to severe and persistent corneal ulceration (upper left) or from corneal haze and blood vessel in-growth (lower left) have poor ocular health and vision. We harvested stem cells from their conjunctiva (patient 1) or from the limbus in their healthy opposite eye (patient 2), grew these cells on a contact lens and placed the lens on each patient's affected eye. Within months of this treatment there were significant improvements in eye health and vision was partially restored (upper and lower right panels)[refs].

AIMS:

1. Test novel biological and synthetic carrier what facilitate graft longevity, integration and restoration of eye health and vision.
2. Trial this strategy in pre-clinical animal models
3. Conduct clinical trials to improve health outcome of patients with blinding corneal disease.

Di Girolamo N, et al. A contact lens-based technique for expansion and transplantation of autologous epithelial progenitors for ocular surface reconstruction. *Transplantation.* 2009;87:1571-1578.
Bobba S, Chow S, Watson S, Di Girolamo N. Clinical outcomes of xeno-free expansion and transplantation of autologous ocular surface epithelial stem cells via contact lens delivery: a prospective case series. *Stem Cell Res Therapy.* 2015;6:23.

PROJECT 3

A new transgenic mouse model to monitor corneal stem cell dynamics in live animals during aging, wound-healing and disease

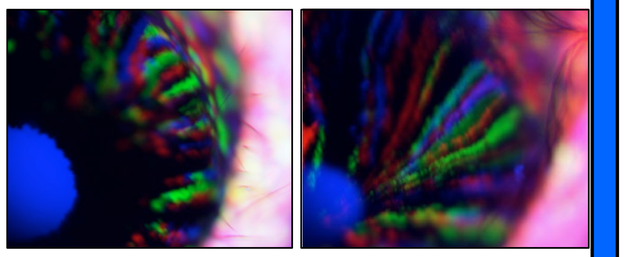


Figure. Multi-colour genetic labelling of stem cells and their progeny and following their destiny as they migrate towards the central cornea under steady-state.

Questions: When do corneal stem cells develop? Where is their principal residence? How do they maintain the cornea under steady-state? How do they respond to trauma (chemical/ physical)? What happens to them in chronic ocular surface disease? Can they be tracked, where do they go, how long do they survive, and how do they function after transplantation?

Answers: These questions are difficult to answer in humans so we developed a unique transgenic lineage tracing model. For the first time we are now able to track the fate of progenitor cells and their progeny in real-time in live animals.

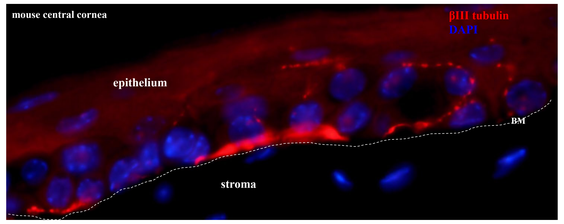
AIMS:

1. Determine how these cells develop embryologically and how they replenish the cornea under steady-state (physiological aging)
2. Determine their fate during wound-healing, disease and transplantation.
3. Develop mouse models for interrogation studies.

Di Girolamo N, et al. Tracing the fate of limbal epithelial progenitor cells in the murine cornea. *Stem Cells.* 2015;33:157-169.
Lobo E, Delic NC, Richardson A, Raviraj V, Halliday GM, Di Girolamo N, Myerscough M, Lyons G. Self-organized centripetal movement of corneal epithelium in the absence of external cues. *Nature Commun.* 2016;7:12388.
Park M, Richardson A, Pandzic E, Lobo EP, Lyons JG, Di Girolamo N. Peripheral (not central) corneal epithelia contribute to the closure of an annular debridement injury. *Proc Natl Acad Sci.* USA. 2019;116:26633-26643.

PROJECT 4

Measure corneal nerve features as a diagnostic and therapeutic outcome tool



- Ageing
- Wound-healing
- Chronic ocular surface disease
- Systemic metabolic disease (e.g. diabetes)
- Corneal nerves as a marker or peripheral neuropathy
- Relationship between corneal nerves - epithelia
- Developing algorithms with imaging experts

PROJECT 5

Understanding how the cornea heals in order to discover factors that expedite this process to prevent infection and vision loss

Questions: Do corneolimbal epithelial stem cells partake in wound healing? What soluble factors can be delivered to the ocular surface to stimulate reepithelialisation?

We recently discovered that a mild-to-moderate corneal debridement wound resolves by migration of epithelial cells with concomitant proliferation of limbal progenitors. Mapping this dynamic process with precision may have clinical ramification in devising novel therapies for patients with persistent epithelial defects. We have also identified an ECM and soluble factor called vitronectin which when added to injured cells in a dish, repair the damage in a timely manner. Our goal here is to devise a topical formulation and deliver this (and other factors) in preclinical models of corneal wounding.