Localizing and *tracking* **proliferative** activity in the rabbit **corneal endothelium**

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On September 10, 2020, Mette Hedegaard Correll defended her thesis "Localizing and tracking proliferative activity in the rabbit corneal endothelium" at the Faculty of Health and Medical Sciences, University of Copenhagen.

This PhD project was conducted at the department of ophthalmology, Rigshospitalet, Copenhagen, in collaboration with the Corneal Graft Biology, Engineering and Imaging Laboratory (BiiGC) in Saint Etienne, France. Her supervisors were Steffen Heegaard, Professor, MD, DMSc; Morten Dornonville de la Cour, Professor, MD, DMSc; Jens Folke Kiilgaard, Associate professor, MD, PhD, Department of ophthalmology, Rigshospitalet – Glostrup, University of Copenhagen, Denmark

Introduction

Corneal endothelial cells (ECs) cover the inner surface of the cornea and maintain corneal clarity due to their pump- and barrier function. Despite this crucial role, the regenerative capacity of the endothelium is limited, and ECs that are lost after cataract surgery or due to Fuchs' corneal endothelial dystrophy are not sufficiently replaced by new cells. The clinical consequence is corneal edema and blurred vision. Currently, the only effective treatment is corneal transplantation, but there is a global lack of donor tissue and surgical specialists. An estimated 13 million people world-wide need a cornea transplantation, yet only 185,000 corneas are transplanted per year. New treatments less dependent on donor tissue are therefore needed. These could be regenerative therapy, cell therapy or tissue engineering. It is envisaged to induce regeneration by surgical or medical stimulation in vivo, and to grow ECs in vitro for cell injection therapy or transplantation on a tissue engineered carrier membrane. To develop these new treatment strategies, it is pivotal to increase our understanding of the endogenous endothelial regeneration and to establish which cell type is optimal for cell therapy.

Methods

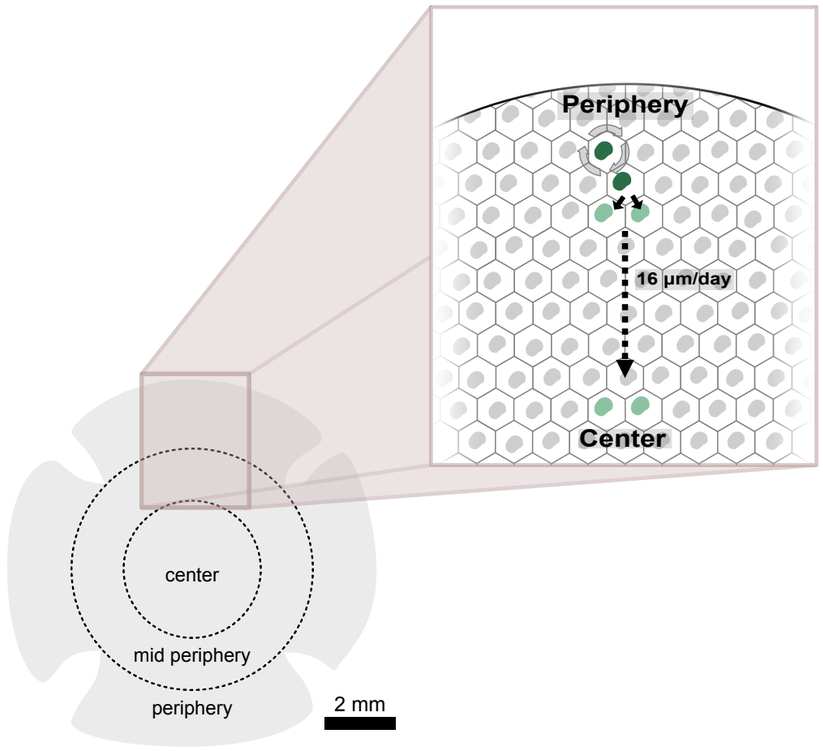
We studied EC proliferation in young rabbits in vivo to identify regenerative patterns and subpopulations of cells to be utilized in future cell therapy. The ECs were labeled with the thymidine analogue 5-ethynyl-2′-deoxyuridine (EdU) in a pulse-chase design to search for label-retaining cells (LRC), proposed to be progenitor cells. EdU was injected intraperitoneally (IP) or intracamerally (IC) after endothelial wounding by transcorneal freezing. The corneas were flat-mounted and evaluated by confocal fluorescence microscopy. Digital image analysis in Fiji (ImageJ) software was used to quantify Ki67-expressing and EdU-labeled ECs.

Results

- Proliferating ECs are located exclusively in the peripheral zone under normal conditions with a higher density in the superior region.
- IC-injection, but not IP-injection, of EdU can be used to label and track proliferating ECs.
- An endothelial wound is healed by local EC proliferation and migration, but ECs in the peripheral zone, distant from the wound, exhibit stem cell-like behavior by adjusting their proliferative activity and being recruited towards the wound. The centrally located ECs are not recruited to a distant wound
- The LRCs are located in the peripheral endothelium and in/at the transition zone (TZ) to the trabecular meshwork, but never in the center or midperiphery of intact corneas.

Conclusion

In this thesis, we conclude that the peripheral endothelium is important for homeostasis and regeneration of the endothelial monolayer. The proliferative activity is regulated dynamically and region-specific, and the regulatory pathways might be utilized to pharmacologically stimulate controlled EC regeneration. Our findings support the hypothesis that progenitor cells are located in the peripheral endothelium or the TZ. Further characterization of the proliferative ECs in the peripheral endothelium is required and could reveal whether these cells might be the optimal cell type for cell therapy or tissue engineering.



Endothelial homeostasis and regeneration. Proposed model of endothelial homeostasis and regeneration: The peripherally located EdU label- retaining endothelial cells (green) are progenitor cells undergoing asymmetrical division and the daughter cells migrate towards the central cornea. *Illustration by Correll & Moschowits.*

CONTACT

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