Understanding the complexity of the corneal endothelium for regenerative medicine

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Impact statement

Introduction

It has been estimated that at least 12.7 million people worldwide require a corneal transplantation but have no access to donor tissue.[1] This estimation is based on 2012–2013 data and probably undervalues the current figures, as it does not include individuals that require keratoplasty but are not yet registered for transplantation and does not account for the future projection of corneal-related blindness.

The loss of vision associated to endothelial dysfunction has a major effect on the quality of life and causes difficulty in mobility, increased risk of falls and fractures, higher risk of depression and anxiety, and a greater likelihood to entering nursing homes, among other impacts.[2] Furthermore, vision impairment poses a major financial burden associated with a loss of productivity.[2] Developing novel therapeutic approaches is therefore crucial to provide swift and safe treatment to those in need. This thesis aims to assist the development of novel cell and regenerative medicine therapies, with the ultimate mission of granting better eyesight. In this section, we aim to provide insight into the scientific, economic, and societal implications related to the findings presented in this thesis.

Scientific impact

The research in this thesis has generated novel knowledge and crucial reference datasets that can impact the development of novel therapies (Chapters 4, 5, and 6), which have been made publicly available in the Gene Expression Omnibus (GSE186433) and DataVerseNL (doi:10.34894/X7ZSDZ).

Firstly, the human cornea was traditionally considered to be a tissue composed of three main cell types, namely epithelial cells, stromal keratocytes, and endothelial cells, a view that does not account for the true cellular heterogeneity. The results reported in this thesis portray the human cornea as a highly heterogeneous tissue at the single cell level, with each corneal layer comprising several cell populations

(Chapter 4). Secondly, our findings revealed the heterogeneity of primary cultured corneal endothelial cells (Chapter 5), providing insights into the alterations that occur through their primary expansion and identifying markers to enrich for clinical-grade cells.

Overall, the single cell transcriptomic (scRNAseq) datasets presented in Chapters 4 and 5 identified subpopulations with different roles in the maintenance of corneal homeostasis. These readily available and accessible datasets can provide a reference baseline to impact future research. The information gained in Chapter 4 can be used for better disease profiling when comparing these data to scRNAseq studies on specific corneal diseases. This could allow the identification of disease-specific genetic mechanisms in the aim to improve therapies. Secondly, the identification of novel cell-specific markers described in Chapter 4 and 5 can play a crucial role in the selection of cell types that are suitable for therapy, potentially improving therapeutic outcomes of cell-based therapies. These markers can also be used to improve stem cell differentiation to specific corneal cell types by targeting the enrichment of the specific markers. Besides the cellular markers highlighted in Chapters 4 and 5, the transcriptomic pathway-level information provided in Chapter 5 provides pivotal background to understand the alterations that occur in CECs upon their *in vitro* culture. These findings open the possibility to improve the current primary culture protocols by targeting specific pathways with small molecules, which could enable sourcing more cells from a single donor or to expand CECs from older donors.

In Chapter 6 we used an interesting approach to gain insights on the proliferation capacity of CECs. By isolating and comparing endothelial tissue of species with non-proliferative CECs, namely sheep and human, with rabbit proliferative CECs at the transcriptomic level, we were able to highlight pathways that could drive the proliferation potential (and thereby regenerative potential) of human CECs. These findings open novel paths for the improvement of regenerative therapies for treating

corneal endothelial dysfunction by specifically targeting the highlighted pathways, but also provide the scientific community with reference datasets of corneal endothelial samples of human, sheep, and rabbit, which are readily usable in other studies. Besides generating a central reference dataset, we have developed an automated pipeline for a cross-species comparison, which has been made publicly available in GitHub (Chapter 6). This pipeline is not specific to corneal endothelial research and can be applied to any tissue and cross-species comparison, enabling a fast and reproducible data analysis in other fields and species comparison.

Impact on clinical translation

Besides the generation of scientific knowledge associated to this thesis, the findings reported in Chapter 6 on the specific markers to select or assess the quality of primary cultured corneal endothelial cells have opened the possibility to file for intellectual property protection. The patentability of our findings has two major repercussions. Firstly, it opens the option to license the markers for the assessment of the therapeutic potential of corneal endothelial cells, which might have a direct financial impact in case of licensing out the technology to a third party. Secondly and most importantly, it opens the possibility for the development and commercialization of a corneal endothelial cell–based therapy without some of the barriers currently faced. Namely, markers associated with the therapeutic quality of primary expanded corneal endothelial cells such as CD166 or CD44 have already been patent-registered in the European Union, and can only be used in the therapeutic setting with a license. As the success of a cell-based therapy heavily relies on the quality assessment of the generated cells, the existing patents might limit the development and application of a corneal endothelial cell–based therapy if there is no licensing agreement. The discovery and patent protection of the markers reported in Chapter 6 opens an alternative possibility for assessing the quality of the corneal endothelial cells intended as a cell-based therapy.

Societal and ethical impact

Corneal endothelial regenerative medicine aims to treat those without access to a donor cornea. But there is a major worldwide imbalance in corneal-related blindness and access to donor corneas in different areas of the world. Most European and North American countries can meet the donor tissue demand, and in some specific cases such as in the United States of America or the Netherlands, can even export surplus corneas to other regions in need. It is in fact in African, Asian and some Middle Eastern countries where donor tissue shortage is a major burden, and patients lack access to treatment in the short-term.

It is essential to understand the worldwide imbalance when developing regenerative medicine approaches for treating corneal endothelial dysfunction. Corneal endothelial cell therapies will probably be more expensive than corneal transplantation. We can use Holoclar as a reference, an autologous transplantation of corneal epithelial stem cells to treat unilateral limbal stem cell deficiency, which has a price of EUR 105,000 per eye in Europe.[3] Taking this in mind, cell therapies to treat corneal endothelial dysfunction are only conceivable in countries with a self-sufficient donor system, with the exception of Japan or Singapore, countries that suffer from donor cornea shortage but have the financial means and facilities for a cell transplantation program. The work reported in this thesis can potentially help to reduce the costs of a cell therapy. Specifically, improvements to the primary cell culture protocols based on our findings could increase the cell pool derived from a single donor, reducing the therapeutic cost. Furthermore, the cost effectiveness of corneal endothelial cell–based therapies can be dramatically reduced by using cell scaffolds, as reported in a recent study.[4]

From the perspective of a global and communal attitude, the use of a corneal endothelial cell therapy in a self-sufficient country could generate a surplus of donor corneas, which in turn could be exported to countries without the infrastructure to

develop and implement a cell therapy. However, there is the need to justify the development and use of a cell-based therapy when there is a treatment and donor tissue readily available for those in need. For the approval of a corneal endothelial cell–based therapy, which is considered an advanced therapeutic medicinal product in the European Union, the therapy must either provide a very strong rationale for a return on investment to elicit commercial interest or provide an improvement compared to the current lamellar therapy; therefore, a corneal endothelial cell–based therapy should be measured against the outcomes of the current DMEK. Considering this, primary cultured corneal endothelial cells could be engineered in a "super-DMEK" graft with a high cell density (≥3,000 cells/mm^2), potentially increasing graft survival. Finally, logistic solutions to transport bioengineered endothelial grafts and frozen cells could also allow countries lacking the necessary infrastructure to benefit from a cell-based therapy.

In the short term, developing and applying a corneal endothelial cell–based therapy in the countries with major cornea shortages is not feasible. For this reason, we might consider the use of other cost-effective therapeutic modalities, such as the Descemet's stripping only combined with the use of Rho associated protein kinase inhibitors or the use of the revolutionary endothelial keratoprosthesis with the capacity to regulate corneal deturgescence, such as EndoArt. Their relatively low cost and the minimal infrastructure needed could allow their implementation, reducing the need for transplantation.

In our view, regenerative therapies should be combined with the international development of efficient eye bank infrastructures and clinical facilities, as well as with promoting organ donation. The present global shortage of donor corneas, expected to increase as the world population ages, can only be tackled with an international effort and a communal attitude.

References

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