Biobanking of Human Retinas: The Next Big Leap for Eye Banks?

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Abstract

Retinal degenerative diseases are one of the main causes of severe vision impairment and regenerative medicine is attracting much attention as a potential therapy. Although highly desirable, the reactivation and proliferation of endogenous stem cells in vivo is not sufficient to generate enough cells to restore visual function after retinal injury. Thus, the replacement of exogenously derived normal donor cells is a promising solution. The challenge is to develop therapies with sufficient amounts of cells being harvested or expanded from donor tissues. Eye banks could overcome this issue by harvesting endogenous adult retinal stem cells from different donors.

Significance

Retinal degenerative diseases are one of the main causes of severe vision impairment and regenerative medicine is attracting much attention as a potential therapy. Although highly desirable, the reactivation and proliferation of endogenous stem cells in vivo is not sufficient to generate enough cells to restore visual function after retinal injury. Thus, the replacement of exogenously derived normal donor cells is a promising solution. The challenge is to develop therapies with sufficient amounts of cells being harvested or expanded from donor tissues. Eye banks could overcome this issue by harvesting endogenous adult retinal stem cells from different donors.

Regenerative Medicine for Retinal Degenerative Diseases Has Started

Retinal degenerative diseases (RDDs) caused by inherited monogenic defects (Stargardt’s disease, retinitis pigmentosa [RP]) or acquired multifactorial retinal diseases (age-related macular degeneration [AMD], glaucoma, and diabetic retinopathy) are one of the main clinical causes of often incurable, progressive, and severe vision impairment in the developed world [1]. Thus, extensive research effort is put into the development of new therapeutic options, of which regenerative medicine-based therapies are currently attracting much attention. However, although the induction of adult endogenous retinal SCs to regenerate lost neurons would be highly desirable, to date, the reactivation and proliferation of retinal SC in vivo is not sufficient to generate enough cells to restore visual function after widespread retinal injury or disease in the adult human eye [2, 3]. Thus, the replacement of exogenously derived normal functional donor cells is an alternative alternative and a promising solution. A number of tissue sources are under consideration, including SCs derived from nonocular tissue (e.g., bone marrow-derived mesenchymal stem cells [BM-MSCs], human embryonic stem cells [hESCs], induced pluripotent stem [iPS] cells), as well as retinal SCs derived from fetal (progenitor retinal SCs) or adult retinas. Adult retinal SCs were identified in specific retinal regions (e.g., the ciliary epithelium, retinal pigmented epithelium [RPE], iris, and Müller glia cells [MGCs]) [4]. Moreover, based on the recent clinical trial results using hESCs, fetal cells, or BM-MSCs for treating different RDDs, restoration of visual function by SC replacement strategies could be a real possibility [4]. Furthermore, whereas the first preliminary clinical trial using IPS cell-derived RPE cells to treat patients with advanced exudative AMD has started as well [5, 6], there are no reported clinical trials that use cultivated adult donor retinal SC-derived RPE or photoreceptor (PR) replacement therapies [4, 7]. A lot of recent research studies on adult retinal SCs have been performed on animal models or in vitro cultured cells, probably because of the limited availability of fresh human posterior eye tissues (vitreous, retina, and choroid) [8]. To address this, in our previous reports we showed that eye banks with large numbers of globes collected yearly could represent a biorepository option to retrieve potential retinal SC and progenitor cells from different parts of the retina and could be a breakthrough in the future delivery of ex vivo prepared customized (histocompatible) retinal tissue on scaffolds for transplantation purposes. In this Perspective, we will consider how the biorepositories could influence the future strategies for retinal stem cell therapies.

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However, because the degradation process is a critical step, short postmortem time (retinal tissues have to be as fresh as possible) is crucial for the proper isolation and preservation of suitable tissue material (e.g., in the case of RNA extraction) for further research activities. In addition, the use of freshly obtained donor retinal tissues would also be desirable in the case of future cell-based therapy applications because prolonged postmortem time and inadequate storage conditions might reduce the percentage of functional stem/progenitor cells and affect their proliferative/colony-forming potential by inducing the activation of differentiation and apoptotic pathways. Thus, successfully validated retrieval of adult donor retinal SC could be a promising and challenging alternative to current retinal stem cell therapies based on pluripotent SC (iPS cells or embryonic/fetal stem cells), with less ethical and tumorigenic concerns. In this Perspective paper, we will consider how the biorepositories could influence the future strategies for adult retinal SC-based therapies. To that end, we also attempt to delineate a possible future production of validated and safe retinal donor grafts on different scaffolds or donor cell suspensions, which could potentially enable a breakthrough in retinal transplantation surgery for severe RDDs.

**ARE EYE BANKS READY FOR THE NEW CHALLENGES OF REGENERATIVE MEDICINE FOR RETINAL DISORDERS?**

Each year, eye banks with a high number of collected eye globes are provided with precious posterior eye tissues such as retina, choroid, and optic nerve. In the past decade, eye globes represented approximately 15%-30% of the total eye tissue specimens collected in the Veneto Eye Bank Foundation (Venice, Italy), which corresponds to approximately 1,100 eye globes collected in the year 2014 (Fig. 1). Of these globes, 60% were suitable for cornea collection. Importantly, the remaining 40% of eye globes could still be used to harvest valuable retinal and choroidal tissues. As reported in our previous publications [8, 9], when correct and precise excision techniques are used, all the posterior eye tissues can be excised and prepared for further distribution to scientists and ophthalmologists. Moreover, eye banks could provide additional valuable information, such as gender, age, cause of death, and other epidemiological data that are available as part of the donor selection procedures [8, 9]. This can further help in the correlation studies and improve the quality assurance. Thus, biorepositories of human posterior eye tissue could provide sufficient amount of human retinal tissue for further population-based genetic analysis, enabling improved investigation of multifactorial RDDs that afflict large segments of the population. Moreover, identifying DNA sequence variation in retinal population studies could lead to genetic variants associated with higher susceptibility to some major RDDs or could be linked to some diseased phenotypes. Retinal transcriptome analysis could further provide additional information on retinal gene regulation, which is crucial to understanding normal retinal functioning (e.g., PR biology) [10]. This insight could also help to better understand some pathophysiological processes in monogenic inherited diseases with known tremendous genetic heterogeneity, such as RP [11]. Despite the impact that biorepositories of posterior eye tissues might have on the development of retinal genomic, proteomic, or epigenetic studies [10], a major breakthrough could originate from the possibility to harvest and biobank adult retinal SC and prepare validated retinal donor tissue for transplantation (Fig. 2), as discussed in the following sections.

**ADULT RETINAL STEM CELLS: RATIONALE AND PROGRESS**

In the last decade, great progress has been made in the identification and isolation of human progenitor/stem cells for retinal regeneration purposes [4]. Currently, adult human retinal SCs have been identified in different parts of the posterior eye tissue [12]. A population of retinal SCs has been assumed to reside in the ciliary marginal zone of human eyes, even if there is no consensus regarding the differential potential of these isolated cells [13, 14] based on previous in vitro studies [15–17]. Secondly, human iris pigmented epithelial (IPE) cells have also been identified to possess some retinal progenitor cell-like properties, which additionally express neurotrophic factors [18] known to slow the degenerative process in a paracrine model of action [1]. Abe et al. [19] already reported promising clinical results from the transplantation of autologous IPE cells to replace defective or diseased RPE cells in patients with AMD. More recent studies using human MGCs showed that, in vitro conditions, they have the capacity to produce various types of retinal neurons including postmitotic photoreceptors and retinal ganglion cell precursors [12] when stimulated to proliferate following retinal injury [20]. Promisingly, Giannelli et al. [21] isolated and expanded a homogenous population of human adult MGCs from surgical samples and postmortem retinal tissues that could self-renew for a relatively extensive period providing a number of cells sufficient for several applications. Surprisingly, more recent reports on RPE tissue derived from human adult donors showed, for the first time, that RPE cells could be successfully coaxed into a multipotent state, with donor age having no influence on the expansion of these cells in vitro [22]. These cells were able to undergo several rounds of divisions and could be passaged for at least six to eight times when cultured under adherent conditions, prior to differentiation into multiple lineages, including central nervous system and mesoderm-associated lineages [22]. Moreover, recent reports [23, 24] proposed that fully mature photoreceptors can also integrate within the normal retina but exhibited poorer survival as if the donor cells were at the correct stage of development at the time of transplantation even into the mature recipient retina [4].

Thus, based on recent reports, there is increasing evidence demonstrating that adult donor-derived retinal SCs could be a promising alternative for regenerative SC-based therapies and could perhaps in the future overcome some of the main challenges from the currently running trials based on hESC-, BM-MSC-, and iPS-derived SCs [25]. To point out some of the current issues, hESC treatment necessitates harvesting donor cell material from developing human embryos, which besides being immunogenic raises ethical and legal concerns [4]. On the other hand, although iPS can be derived from the patient’s own cells (such as fibroblasts, the most common source), they have a long differentiation time window. This is associated with laborious and costly culturing procedures with low efficiency in generation of desired retinal cells [4]. Furthermore, both types of pluripotent cells are endowed with a tumorigenic potential based on greater genomic instability [26]. Additionally, although autologous bone marrow-derived stem cells are likely to be less immunogenic after transplantation as hESCs, they are usually only available in very limited amount and could further secrete proangiogenic factors, which are avoided in exudative AMD [1, 25].

As discussed above, many issues remain unsolved, and eye banks could contribute to the solution. With the help of biorepositories, some of the major challenges regarding adult retinal SCs could be overcome. A major challenge is to generate a sufficient number of transplantable cells [27] and, in parallel, to achieve high and consistent levels of integration [28]. Eye
banks with large numbers of harvested posterior eye tissues could potentially overcome this issue not only by harvesting SCs from different donors, but also by optimizing the transplantation protocols. Additionally, because of the research results that showed less immunogenic potential of cultured cells compared with freshly isolated cells [4], eye banks could address also this important issue with the development of culturing procedures to obtain less immunogenic retinal explant tissue. Although it is not yet known how stable the newly generated cells are over time, eye banks could provide good manufacturing practice (GMP)-compliant, reliable, validated, pretested, and robust differentiation protocols for the generation and cryopreservation of desired retinal tissue before further clinical application.

**Figure 1.** The number of harvested eye tissue specimens from 2004 to 2014 in the Veneto Eye Bank Foundation (Venice, Italy).

**TRANSLATION POTENTIAL OF BIOBANKED RETINAL TISSUES: THE CLINICAL FRONTIER**

Currently, retinal transplantation strategies have largely fallen into two main approaches: transplantation of different retinal cell sheets (whole retinal sheet [29] or preprepared cell monolayers [30, 31]) or the transplantation of cell suspensions intravitreally (10,000,000 cells [0.1 ml] [32]) or subretinally (approximately 1,500 to 10,000 cells [19, 33, 34]). The results of previous studies have already demonstrated that in addition to the correct developmental stage of donor cells, their interactions with the recipient retinal environment are essential in determining transplantation outcome [27, 35]. Therefore, migration and survival of donor cells in the recipient retina are crucial to have a therapeutic benefit. Appropriate delivery can help to place these cells into the right position [27, 35]. Thus, different types of RDDs (e.g., AMD or glaucoma) will most likely need different surgical approaches. The subretinal application of donor cells bypasses the mature inner limiting membrane and has the advantage of being immediately adjacent to the outer nuclear layer (where RPE cells and PR cells reside). However, the intravitreal application of these cells is less invasive, being readily accessible to normal surgical procedures and near the ganglion cell layer [25].

In agreement with this, in past surgical attempts to treat RDD, different types of cells as well as different surgical approaches have been used to replace the diseased cells, with mixed visual outcomes [7]. In AMD, diseased macular RPEs were replaced with rotated pedicle flaps of peripheral RPE-choroid, autologous free RPE-choroid grafts, sheets of fetal RPE, and cell suspensions of peripheral RPE [25] or IPE [19]. The early clinical trial results using allogenic RPE or IPE cell suspensions were disappointing because of cell rejection or poor integration of donor cells [7, 36]. On the contrary, the results of visual acuity were improved in patients with AMD after autologous translocation of RPE cells, Bruch’s membrane, and choroid [31]. Moreover, the results of a phase II clinical trial of treating patients with RP and AMD with transplanting fetal retinal sheets with its RPE were very encouraging, with graft survival and reported improvement of visual acuity [7, 29]. Considering this, methods using cell suspensions are not likely to lead to the development of derived cells into desired cell monolayers [25], which is necessary to assure proper cell orientation and organization [37]. This suggests that not only proper cell organization is critical for their function, but cells can also...
undergo apoptosis [38]. Thus, transplantation of whole retinal or monolayered cell sheets seems to be superior to suspension cell technique, even if the results of further trials are awaited to elucidate this issue better. In addition, for the transplantation of monolayers of PR progenitor cells, it has been reported that the survival was increased when cocultured with embryonically derived RPE [39], suggesting that dual replacement could be a promising strategy [25].

From these observations, it is reasonable to conclude that an enormous potential resides in the future development and preparation of different retinal cell suspensions or sheets for transplantation purposes. Moreover, as the first clinical studies also demonstrated, activation of the host immune system was a major barrier to successful transplantation [4, 19, 36]. Interestingly, cultured cells appeared to be less immunogenic, compared with freshly dissociated cells [4]. Thus, eye banks with large numbers of donors could generate human leukocyte antigen-haplotype-matched retinal SC banks that may avoid this issue.

Looking at the future, eye banks could further support new interdisciplinary studies and facilitate development of various retinal tissue sheets (e.g., RPE monolayers, dual cell sheets PR/RPE) on various substrates (lens capsule, collagen type I [30], on thermally responsive polymer poly-[N-isopropylacrylamide]-grafted culture plates [40]). This could further improve and enhance the delivery of fragile donor cells into the recipient retina in vitreoretinal surgical procedures.

**Future Promises of Retinal Biobanking**

In recent years, various types of stem cells have been transplanted into the eye to treat degenerative retinal diseases with some promising initial results of phase I or II clinical trials. However, some major issues must be overcome before it will become a routine clinical practice. The challenge for the future would be to develop ethically acceptable retinal SC therapy without immunological rejection and with sufficient amounts of cells being harvested or expanded from donor tissues. Thus, eye banks with large numbers of globes collected yearly could potentially overcome this issue not only by harvesting adult retinal SC from different donors, but also by optimizing the transplantation protocols to provide GMP compliant, reliable, validated, pretested, and robust differentiation protocols for the generation and cryopreservation of desired retinal tissue before further clinical application.

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**Figure 2.** Sources of adult retinal stem cells from donor eye globes for harvesting and biobanking. (A): Schematic cross-section of a human eye demonstrating the main sources of adult retinal stem cells, which include the ciliary epithelium, iris, RPE, and Müller glia cells. (B, C): With correct and precise cell harvesting techniques, these retinal stem cell populations can be isolated (B) and cultured and expanded in vitro (C) for further distribution. (D): Schematic presentation of possible future application of eye bank-derived, validated and safe retinal donor grafts on different scaffolds (e.g., monolayer sheets of RPE) or donor cell suspensions, which could potentially enable a breakthrough in retinal transplantation surgery. Abbreviation: RPE, retinal pigmented epithelium.
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AUTHOR CONTRIBUTIONS

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DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

The authors indicated no potential conflicts of interest.

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