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Development of Better Treatments for Retinal Disease Using Stem Cell Therapies

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Abstract

The retina is a complex, light sensitive tissue layer on the inner surface of the eye, which functions to translate light stimuli into nerve impulses which travel to the brain via the optic nerve. Retinal diseases such as glaucoma, macular degeneration and retinitis pigmentosa can lead to significant vision problems and even blindness. Although there are some existing treatments for retinal disease, current treatments are invasive, need to be regularly repeated and do not have the capability of regenerating the damaged retinal architecture. The demand for more effective treatments for retinal disease is therefore high. Stem cell therapies and tissue engineering techniques for ocular disease have advanced significantly over recent years with promising results. However, challenges still remain with aspects such as; the delivery and integration of regenerative materials to the eye, overcoming the possibility of immune rejection and the guidance of neural growth to establish functional connections. This review describes the main approaches developed for retinal repair using regenerative medicine to date, including stimulating possible endogenous repair processes and cell replacement therapies. Subsequently, we report some of the newer and emerging strategies for retinal tissue engineering using biomaterials, which attempt to address the current challenges associated with retinal cell replacement therapy.

Keywords

Stem cell therapies, Retinal tissue engineering, Scaffold materials, Macular degeneration, Glaucoma, Retinitis pigmentosa

Figure 1: Optical image of a cross section of pig retinal tissue stained with hematoxylin and eosin, showing the multiple layered structure.

Introduction

Retina is a vital tissue determining human vision. It lines the back of the eye and converts focused light from the lens into neural signals, and then sends these signals on to the brain for visual recognition [1]. The tissue is made up of several neuronal layers consisting of at least eight different cell types, as shown in figure 1. Degenerative diseases of the retina include glaucoma, macular degeneration, diabetic retinopathy and retinitis pigmentosa. According to the World Health Organisation, glaucoma and diabetic retinopathy account for approximately 17% of world blindness. Furthermore, age-related macular degeneration is ranked the third leading cause of world blindness, after cataract and glaucoma [2]. Macular degeneration and retinitis pigmentosa are typically characterised by the degeneration of the photorec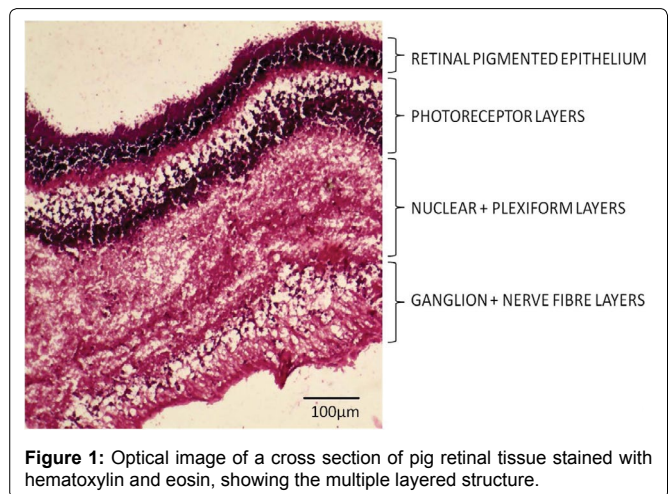eptors and retinal pigmented epithelium (RPE), a monolayer of cells which aligns a basement membrane, known as the Bruch's membrane, situated between the neural retina and the choroid [3]. Glaucoma is characterised by the degeneration of retinal ganglion cells (RGCs). These conditions can lead to significant vision problems and blindness if left untreated [4].

Existing treatments for glaucoma include a surgical procedure named trabeculectomy, for the treatment of advanced glaucoma which can no longer be controlled with medical therapy. This procedure involves surgically making an incision in the conjunctiva and sclera so that fluid can egress into the sub-conjunctiva of the eye and thereby reduce intraocular pressure (IOP). However, the control of IOP after trabeculectomy deteriorates over time, furthermore surgery is an invasive procedure and can lead to inflammation [5] and infection. Alternatives to trabeculectomy such as a tube shunt procedure can still lead to complications such as bleeding inside the eye, infection and a buildup of fluid behind the retina [6]. Other retinal diseases such as macular degeneration or diabetic maculopathy can be treated by intravitreal injections containing ocular drug treatments to target the retina, which can be effective in restoring some level of vision. However, the injections are invasive, require repeated treatments and can lead to significant adverse events such as endophthalmitis, a potentially blinding infection [7]. There are currently no commercially available treatments for retinitis pigmentosa.

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There is therefore need for more effective treatments for retinal diseases. Stem cell based therapies and tissue engineering for retinal disease has advanced significantly over recent years with promising results. However, challenges still remain with aspects such as; the delivery and integration of regenerative materials to the eye, overcoming the possibility of immune rejection and the guidance of axonal growth to establish useful connections [8]. This review describes the main approaches developed for retinal repair using regenerative medicine to date, including possible endogenous repair processes and cell replacement therapies. Subsequently, we report some of the newer and emerging strategies for retinal tissue engineering using biomaterials, which attempt to address the current challenges associated with retinal cell replacement therapy.

Endogenous Repair Processes

One approach to improve the treatment of retinal disease is the possibility of inducing endogenous repair processes to regenerate lost retinal neurons and damaged RPE. Studying the regenerative capability of organisms which already naturally possess the ability of retinal regeneration can give researchers an insight into the possibility of inducing endogenous regeneration in the human retina [9]. Potential sources of endogenous retinal regeneration include the RPE and Müller glia [10].

RPE cells

RPE cells of various species have demonstrated their differentiation potential into photoreceptor, amacrine, glial and ganglion cells [11-13]. Remarkably, it is known that certain species of amphibian, such as the newt, are capable of endogenous regeneration of the entire retina via RPE cells [14]. In an attempt to enhance the endogenous repair potential of mammalian RPE, some groups have investigated the regenerative potential of the mouse retina via RPE. One study found that intravenous administration of a low dose of sodium iodate in mice enhances RPE cell proliferation and migration by selectively injuring the RPE and subsequently triggering a sequence of pathophysiological effects which support endogenous regeneration of retinal tissue following injury [15]. However, further studies have also shown higher doses of sodium iodate to be toxic to RPE cells and retina [16]. Therefore, further experiments to investigate the correct dosage of sodium iodate would be encouraged to ensure its safety as a potential therapeutic. The existence of RPE stem cells in the human retina was reported recently in 2012 [17]. The regenerative potential of human RPE is still relatively unknown; however the possibility of RPE replacement for retinal repair in humans appears to be promising. This will be discussed further in the review.

Müller glia

Another potential source of endogenous regeneration is the Müller glia. It is known that organisms such as the Zebrafish are capable of endogenous retinal regeneration via the müller glia, as their müller glia de-differentiates and develops into proliferating neurogenic progenitor cells after injury [18]. Some *in vitro* studies have found that Müller glia from the primate retina can also develop into progenitor-like cells, but the capability to regenerate retinal neurons remains unclear [19]. Retinal regeneration via Müller glia-derived progenitor cells (MGPCs) requires five cellular actions, driven by signals emitted from the damaged or dying neurons. The first action is de-differentiation, where cells acquire progenitor phenotype and cease to function as glia. The second action is proliferation, which is required to produce a greater quantity of cells, including progeny to differentiate into new neurons and replace the Müller glia. However, it is believed that much of the proliferation which takes place in the mammalian retina is part of scar formation, rather than neuronal replacement [20]. The third action is the migration of dividing progenitor cells, in order to replace neurons within the various retinal layers. Following migration is the action of neural differentiation, to allow Müller glia derived cells to entirely differentiate into retinal neurons. The final action then involves integration into the retinal circuitry, where newly-generated neurons create synaptic connections

in order to contribute in retinal function. Currently, this integration process also appears to be lacking in the bird and mammalian retina [21]. Initial studies in the human retina include Jayaram *et al*. (2014), who found that human MGPCs can differentiate into photoreceptors when cultured on basement membrane protein in the presence of taurine, fibroblast growth factor 2, insulin-like growth factor 1 and retinoic acid. Furthermore, they found that MGPCs had the potential to recover retinal function in a disease model of retinitis pigmentosa [22]. However, difficulty to culture primary human Müller glial cells means that current research is limited.

Cell Replacement Therapies

A more widely considered approach to improve the treatment of retinal disease is the possibility of retinal repair using cell replacement therapies. Therapies include embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), mesenchymal stem cells (MSCs), adipose stem cells (ASCs) and retinal progenitor cells (RPCs), as well as RPE replacement. Many groups have successfully transplanted these cells into animal models and some therapies are even commencing into Phase I/II clinical trials.

Embryonic stem cells

ESCs are capable of self-renewal indefinitely when provided with the correct culture conditions, whilst still maintaining pluripotency [23]. Various research groups have found the potential to guide ESCs toward lineages such as RPE cells and photoreceptors [24], as well as RGCs [25], for transplantation into the retina. Some research groups have even found transplanted ESC derived photoreceptors to restore some level of visual function in *Crx*-deficient mice [26]. However, challenges with ESC derived cell transplantation include the ability to obtain stable cell sources that integrate safely into the diseased retina. There is concern that the plasticity of ESCs may be a problem, raising the risk of inappropriate progeny such as tumours [27]. A second challenge is the ability to develop a realistic up-scaling strategy in order to generate the high amount of cells required for transplantation into patients [28]. A third hurdle is the possibility of immuno rejection. The extensive remodelling that occurs following retinal degeneration can result in upregulation of inflammatory proteins and alteration of the blood-retinal barrier, which is likely to increase the chance of immuno rejection. Therefore, a better understanding of the immune status of a degenerated retinal environment is required [29]. A fourth major hurdle for the transplantation of stem cell derived retinal cells, such as photoreceptors and RGCs, is that very few cells manage to integrate successfully into the retina [30]. This has also been found for photoreceptors and RGCs derived from other types of stem cell.

Induced pluripotent stem cells

iPSCs are derived from reprogrammed peripheral cells (e.g. fibroblasts and lymphoblasts) into a pluripotent state. These cells can be directly generated from adult cells and therefore carry less ethical concerns than ESCs [31]. Most cell types in the retina (including RPE and photoreceptors) have been differentiated successfully from iPSCs [32]. Retinal cells derived from patient-specific iPSCs could be used for autologous transplantation, which has a reduced risk of immune rejection as it uses the patient's own cells. However, personalised cell therapy is time-consuming and expensive; furthermore iPSCs may require gene correction before transplantation. Human leukocyte antigen (HLA) matched allogeneic iPSCs, may also provide an alternative cell therapy in certain cases [33]. Information of current clinical trials using iPSCs can be found in table 1. Although ongoing clinical trials appear promising, previous trials such as an age-related macular degeneration treatment developed by the Masayo Takahashi group in Japan recently had to be halted due to a risk of tumour development [34]. Therefore, iPSC safety as a cell therapy is unknown until current and ongoing clinical trials have ran successfully and released their results.

Mesenchymal stem cells

Mesenchymal stem cells (MSCs) are multipotent stem cells

Table 1: A summary of cell replacement therapies currently in development for retinal repair, including the main challenges and clinical trial information (adapted from http://clinicaltrials.gov).

Cell Therapy	Main Challenges	Clinical Trial Examples
Embryonic stem cells (ESCs)	> Obtaining safe, stable and effective cell sources > Finding a realistic up-scaling strategy > Chance of immunorejection	> Advanced Dry Macular Degeneration treatment (California, US) [52] > Age related Macular Degeneration treatment (Israel) [53] > Stargardt Macular Dystrophy treatment (US) [54] > Advanced Dry Age related Macular Degeneration treatment (US) [55] + long term follow up study [56] > Advanced Dry Age related Macular Degeneration treatment (Korea) [57]
Induced pluripotent stem cells (iPSCs)	> Potential time + cost of autologous cell therapy > May require gene correction before autologous transplantation	> Investigation of genetic retinal diseases (Minnesota, US) [58] > Investigation of retinal degenerative diseases (Maryland, US) [59] > iPSCs derived from retinoblastoma patients (Tennessee, US) [60] > Age related Macular Degeneration treatment (UK) [61]
Mesenchymal stem cells (MSCs)	> Large scale differentiation of MSCs into functional cells makes cell replacement therapy problematic	> Stem cell ophthalmology treatment study (Florida, US) [62]
Adipose stem cells (ASCs)	> Risk of transplanted cells migrating towards non-targeted regions > Potential costs of autologous cell therapy	> Glaucomatous neurodegeneration treatment (Russia) [63]
Retinal progenitor cells (RPCs)	> Ability to integrate into retinal circuitry > Chance of immunorejection	> Retinitis pigmentosa treatment (UK + US) [46,47] > Retinitis pigmentosa treatment (California, US) [64]
Retinal pigmented epithelium (RPE) transplantation	> High chance of immunorejection > Survival + function of transplanted RPE difficult on aged and deteriorated Bruch's membrane	> Acute Wet Macular Degeneration treatment (London, UK) [65] > Age related Macular Degeneration treatment (Austria) [66]

derived from mesenchymal tissues such as bone marrow, adipose tissue, umbilical cord and placenta [35]. MSCs have been found to differentiate into photoreceptor-like cells, as well as RGC-like cells, making cell replacement therapy a future possibility using these cells. MSCs have also been found to provide neuroprotection for degenerating retinal cells via expression of a variety of neurotrophins which are beneficial for retinal cell survival [36]. Whilst retinal cell replacement therapy using MSCs remains problematic at the present time, some studies using MSCs for their neuroprotective mechanism are promising. For example, one study which involved the injection MSCs intravitreally into a mouse model of acute retinal injury, found that transplanted cells survived for at least 3 months and significantly protected damaged retinal cells long term [37].

Adipose stem cells

ASCs are mesenchymal stem cells which, like other stem cells, have the capacity to differentiate into multiple cell types. However, these cells are derived from the more easily accessible adipose tissue, rather than bone marrow [38]. The administration of ASCs into animal models of retinal degeneration is currently being investigated for the potential treatment of diseases such as diabetic retinopathy [39], glaucoma [40] and macular degeneration [41]. As autologous transplantation is also possible with ASCs, the risk of immune rejection is reduced. A transplantation study involving injection of human ASCs into a rat vitreous cavity found that cells survived and integrated with the eye relatively successfully. However, there was concern that some cells appeared to migrate across the blood-retina barrier and towards non-targeted regions, which could be considered a risk for transplantation to patients [42]. Therefore, more experiments to fully understand ASC cell mechanisms during transplantation will be required before an optimised treatment can be developed.

Retinal progenitor cells

RPCs are typically more predisposed towards a cell fate in comparison to stem cells because they are obtained from donated foetal tissue, rather than embryonic tissue [43]. Some groups have successfully transplanted this cell type into animal models of retinal degeneration [44] and some have even shown evidence of improved visual acuity in rats, due to photoreceptor preservation via transplanted RPCs [45]. Companies such as 'ReNeuron' are currently commencing this therapy into Phase I/II clinical trials as a potential treatment for patients with retinitis pigmentosa [46,47]. If RPCs can be successfully transplanted into patients, one of the most likely challenges for this treatment will be whether cells are able to integrate into the complex human retinal circuitry. Subsequently, if evidence for photoreceptor preservation is observed, the ultimate challenge will then be whether the photoreceptors can function well enough to improve visual acuity. Furthermore, like ESCs donor tissue is used to obtain RPCs, meaning there could be a chance of immuno rejection.

RPE replacement

Another possible cell replacement therapy is replacement of the RPE. The ability to generate large amounts of functional RPE from ESCs and iPSCs makes this cell type an ideal candidate for transplantation [48]. However, RPE transplantation in suspension and RPE/choroid patch translocation has previously faced several challenges. Allogeneic RPE transplants have shown eventual rejection and/or a lack of functional recovery, plus autologous RPE transplants have also shown problems [49]. The aging process and degenerative disease causes the RPE to accumulate lipofuscin, which diminishes cellular structures and causes a slowing of metabolic capacity. The Bruch's membrane also accumulates debris, making nutrient transport less efficient and the survival/function of transplanted RPE challenging on such an altered substrate [50]. The future potential of each cell therapy will be unknown until current clinical study results are revealed. One notable clinical study was undertaken in 2012, where ESC derived RPE was transplanted into patients with stargardt's macular dystrophy and Macular degeneration. Results found transplanted RPE to have no apparent rejection after 4 months. Therefore, with further development this therapy could overcome the challenge of immune

rejection and potentially be used for photoreceptor and central visual rescue [51]. A summary of all cell replacement therapies discussed, including current clinical trial information, can be found in table 1.

Retinal Tissue Engineering Approaches

Whilst cell replacement therapies appear promising, low cell survival and limited integration remain major challenges for this method of retinal repair. Furthermore it can be difficult to retain injected cells at specifically targeted regions of the retina, such as underneath the fovea [67]. Current tissue engineering approaches attempt to overcome these hurdles by supporting cell survival, delivery and integration. These include the development of 2 or 3-dimensional biomaterial scaffolds, as well as development of improved retinal disease models for the testing of neuroregenerative materials.

Scaffolds for retinal progenitor cell grafting

Many biomaterial scaffolds are currently in development for the grafting of RPCs. For example, Neeley *et al.* (2008) developed a microfabricated thin, porous poly (glycerol-sebacate) scaffold for RPC grafting. The reported scaffold possesses improved mechanical properties which are more closely similar to the retina in comparison to other scaffolds. Results indicate that RPCs adhered strongly to this scaffold and expressed a combination of immature and mature markers, suggesting a tendency towards cell differentiation [68]. In continuation of this work, the same research group has since transplanted the RPC grafted scaffold into mice. Transplantation proved relatively successful with long term RPC survival and expression of mature markers in the retinal tissue [69]. More recently, Yao *et al.* (2015) developed a biodegradable, thin-film polycaprolactone scaffold. Results indicate that the nano-topographies of the scaffold can interact with RPCs and have the potential to direct these cells towards photoreceptor cell fate before transplantation. The therefore scaffold provides an improved biodegradable platform to guide RPC differentiation and allowed for better organisation and delivery of RPCs to retinal tissue [70]. Although polycaprolactone is known to have improved biodegradability and good ability to drive hRPC differentiation into photoreceptors, poor adhesive qualities limit its use. Therefore, another research group has made a surface modification to a polycaprolactone scaffold by incorporating vitronectin-mimicking oligopeptide, forming a hybrid. Results showed improved cell adhesion of human RPCs and, despite no evidence of functional rescue, the RPCs showed improved migration, integration and survival when transplanted into a degenerated mouse retina on the hybrid scaffold [71].

Scaffolds for RPE transplantation

Many biomaterial scaffolds are also in development for the improvement of RPE transplantation. For example, Liu *et al.* (2014) developed a supporting scaffold on which RPE would be pre-cultured and supported to mature into a functional monolayer. The scaffold was tested *in vitro* using human foetal RPE cells and transplanted subretinally into rabbits. Results found that RPE culture was enhanced with the scaffold and showed improved subretinal biocompatibility with scaffolds of 200 nm fibre topography. Therefore, the use of biomaterial scaffolds can be considered a future direction for improving cell-based therapies to treat retinal disease [72]. However, challenges continue to remain in ensuring that scaffolds do not cause further damage to the retina, whilst still allowing for sufficient interaction between the retina and other cell layers. Therefore the surface topography, thickness, mechanical properties and degradation characteristics must be considered in scaffold design to ensure that they are suitable and beneficial for the treatment of ocular disease.

Biomaterials for potential RGC repair

At the moment, few biomaterial scaffolds have been developed for the treatment of RGC degenerative diseases such as glaucoma and optic nerve stroke. In diseases such as glaucoma RGCs are permanently damaged, leading to cell death and interruption of retinal signalling to the brain [73]. Due to their highly delicate and disparate axonal projection patterns, RGC replacement therapy for diseases such as glaucoma is highly challenging. Many more experiments to fully understand the complex *in vivo* circuitry and how replacement cells could integrate are required before a potential RGC replacement therapy can be developed [74]. Over the next few years however, we may see the development of biomaterial scaffolds and peripheral nerve bridges which attempt to repair injured endogenous ganglion cell axons, rather than replace them. Research into smart biodegradable implants aiding axonal regeneration following spinal cord injury has already been reported, which aim to stimulate repair by 'bridging the gap' between damaged axon sites. This approach has significant potential to be used in the visual system and appears to be more promising than RGC replacement therapy at the present stage [75].

Development of in *vitro* or *ex vivo* retinal tissue models

Another tissue engineering strategy which will be hugely beneficial for improving the therapeutic potential of cell replacement therapies is the development of improved *in vitro* or *ex vivo* retinal tissue models, for the testing of neuroregenerative materials such as cell replacement therapies or drug intervention. Multicellular retinal tissue models with preserved cellular interactions are preferable in comparison to *in vivo* study using live animal models, as there are less ethical implications and the tissue environment can be better controlled and manipulated [76]. Organotypic slice culture involves the *in vitro* growth of complex tissues, such as the retina, whilst still preserving a large amount of *in vivo* function and physiology [77]. Many groups have successfully cultured rat retina using this method [78,79].

As the neuronal structure of the porcine retina is significantly similar to that of the human retina [80], it is preferable to explore organotypic explant culture of the porcine retina. One notable organotypic retinal tissue system developed recently preserves all layers of full thickness porcine retinal tissue for approximately 7 days. As the photoreceptor layers faces upwards, oxygen supply to the photoreceptors and RPE layer is increased and therefore improves the viability of cultured tissue [81]. However, the filter paper substrate used in this model appears to be very basic and is likely to dehydrate the retinal tissue. Another model currently in development attempts to address this issue further by culturing full thickness porcine retinal tissue on a more biocompatible substrate; collagen hydrogel. The collagen hydrogel mimics a vitreous-like substrate which better supports the integrity of full thickness retinal tissue explants with minimal distortion, as well as improving tissue viability for up to 2 weeks [82]. Figure 2 is an optical coherence tomography image showing a pig retinal explant cultured over 4 days on collagen hydrogel
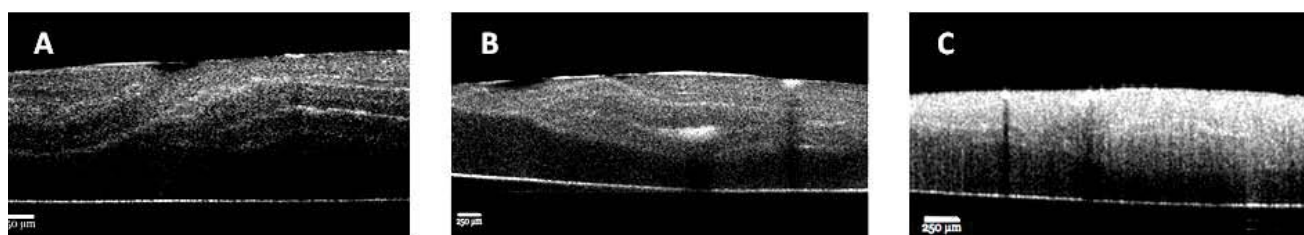

Figure 2: Optical coherence tomography images of a pig retinal explant cultured for 4 days. The layered structure was well resolved. The RPE layer is on the top of the sample.

with delineated layered structure. With further development, it can be anticipated that the testing of potential cell replacement therapies and drug interventions on improved retinal tissue models such as this one will advance the progress of these cells into clinical trials and therapeutic applications significantly.

Conclusions + Future Perspectives

Evidently, the possibility of endogenous regeneration, as well as cell replacement therapies using ESCs, iPSCs, MSCs, ASCs, RPCs and RPE replacement are likely to become major implements in the future progress of regenerative medicine. To date, the main challenges of cell replacement therapy include; the delivery and integration of regenerative materials to the eye, overcoming the possibility of immune rejection and the guidance of neural growth to establish functional connections. The development of tissue-engineered constructs, such as scaffolds and smart biodegradable implants, may help to overcome some of these challenges by improving the delivery, integration and survival of transplanted cells. Furthermore, the testing of stem cell derivatives on improved retinal models which better mimic *in vivo* physiology and function will be hugely beneficial for exploring the potential therapeutic application of these cells. Assuming that enough funding will remain in this research field, a combination of the strategies discussed may revolutionise the way in which we treat retinal disease in the future.

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