

Towards stem cell-based therapies for Retinal Neurodegenerative Diseases

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Key words. retina • stem cell • neuroprotection • transplantation • ganglion cell • photoreceptor • retinal pigment epithelium • glaucoma • AMD • retinopathy

ABSTRACT

Loss of sight due to irreversible retinal neurodegeneration imposes a significant disease burden on both patients and society. Glaucoma and age-related macular degeneration are the commonest neurodegenerative blinding diseases in the developed world, and both are becoming increasingly prevalent as populations age. Our heavy reliance on our sense of sight means that visual loss often severely restricts day-to-day life, making it difficult to function without additional support. Visual impairment also limits employment possibilities, adding to the economic burden. Current therapies for many degenerative retinopathies are limited in their efficacy, often treating the effects of disease rather than the underlying causes. Consequently, the development of novel adjunctive neuroprotective and

neuroregenerative treatments are important goals. Evidence from animal models suggests that stem cells could be useful as part of novel new treatment strategies for eye disease. The accessibility of the eye and extensive repertoire of available surgical techniques may facilitate the translation of stem cell-based therapies, for example via transplantation, to the retina more rapidly than to other parts of the central nervous system. This concise review will examine how cell therapies are being applied experimentally for neuroregenerative and neuroprotective treatment of currently incurable degenerative retinal diseases. Furthermore, recent progress towards clinical translation of such therapies will be highlighted.

INTRODUCTION

Visual impairment imposes a substantial disease burden on society. It is associated with both significant economic impact and reduction in quality of life. Visual impairment raises serious social challenges for both patients and their families, interfering with day-to-day life. Importantly for adults, visual impairment can limit employment possibilities. These limitations are difficult to quantify precisely, but they clearly impact on the quality of life of visually impaired people and their families. This has been recognized by

the WHO in a global initiative, "VISION 2020: The Right to Sight", which aims to eliminate many avoidable causes of visual impairment by the year 2020.

While some causes of visual impairment, for example cataract, are reversible and readily treated, others, such as glaucoma and macular degeneration, are both common and often irreversible. Furthermore, many of these pathologies are associated with increased age and, therefore, are becoming increasingly prevalent in aging populations [1, 2]. Many of the most common irreversible blinding

Author contributions Summary: N.B.: Conception and design, collection and/or assembly of data, manuscript writing, final approval of manuscript; K.M.: Manuscript writing, final approval of manuscript

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pathologies involve neuronal loss from the retina, which is the light-sensing tissue of the eye (Figure 1). The retina is a part of the central nervous system (CNS) and, like the brain, it is unable to regenerate neurons lost to disease. Therefore, stem cell therapies are attracting attention as possible future treatments for currently untreatable and irreversible retinal pathologies.

It is worth highlighting the advantages of the retina, and eye in general, as a target for cell-based clinical therapies. While the retina constitutes a part of the CNS, it is a highly accessible tissue compared to the brain. Ocular surgical techniques are very well developed, with intraocular injection, implantation of prosthetic lenses and other devices, retinal surgery and transplantation of non-neuronal ocular tissues all part of routine clinical care. The eye is also relatively small, reducing the number of cells required for cell-based therapies, and isolated, reducing the risk of systemic side effects. The eye has a degree of immune privilege that may help to reduce the rejection of certain transplanted cells. In addition, the available tools for measuring ocular structure and function are arguably more highly evolved than for any other part of the body. Thus, it is not surprising that stem cell treatments for some ocular diseases are in, or approaching, clinical trial.

This concise review will briefly outline retinal neurodegenerative pathologies and provide examples of how stem cells are being applied experimentally in an effort to develop novel therapies for currently intractable causes of blindness (Figure 1). It will also highlight recent progress towards clinical translation of such therapies.

Brief overview of common retinal neurodegenerative diseases

Retinal neurodegenerative diseases can be broadly divided into those that affect the outer retina and those that affect the inner retina. Outer retinal pathologies often result in the death of photoreceptor neurons (Figure 1), which are the primary sensory neurons responsible for detecting light. Photoreceptors synapse with bipolar cells, which in turn pass

the visual signal to retinal ganglion cells (RGCs). RGCs are the projection neurons of the retina and their axons form the optic nerve, connecting the retina to visual pathways in the brain. Neurodegenerative diseases of the inner retina can affect both bipolar and RGCs, the loss of which disrupts the flow of information through the visual pathway. Several other cell types contribute to retinal function, including two classes of interneurons (horizontal and amacrine cells), radial glial Müller cells (functionally similar to astrocytes in the brain) and the retinal pigment epithelium (RPE; an epithelial layer between the retina and choroid). RPE dysfunction can result in secondary photoreceptor degeneration as this tissue plays a key role in maintaining photoreceptor homeostasis. A population of astrocytic glia also resides within the retinal nerve fibre layer, however the Müller cells appear primarily responsible for supporting retinal homeostasis and function.

A very common outer retinal pathology that causes photoreceptor loss is age-related macular degeneration (AMD). It has been estimated that AMD alone accounts for about 9% of global blindness [3]. This is a group of diseases where photoreceptor death occurs secondary to RPE dysfunction. Features of AMD include RPE atrophy ('dry' AMD) and choroidal neovascularization ('wet' AMD). Another important outer retinal condition is retinitis pigmentosa (RP), a group of genetic disorders, affecting either photoreceptors or RPE, which cause blindness via progressive photoreceptor degeneration.

Glaucoma refers to a group of conditions that together comprise the most common inner retinal neurodegenerative disease – reportedly affecting 60.5 million people in 2010, predicted to rise to 79.6 million by 2020 [1]. In glaucoma, it is primarily RGCs that die, causing optic nerve degeneration and impairing the retinal connection to the brain. Common risk factors for the development of glaucoma are increased age and elevated intraocular pressure. Glaucoma is often asymptomatic until late in disease and significant visual field loss often occurs prior to diagnosis. The only clinically proven therapy for glaucoma is

reduction of intraocular pressure, which usually slows further disease progression but often fails to halt it completely. Another common cause of inner retinal neurodegeneration is ischaemic retinopathy, a group of diseases in which the retina vasculature is damaged leading to deprivation of oxygen and nutrients, and sometimes pathological neovascularization. Diabetic retinopathy, retinopathy of prematurity, and vascular occlusions are all relatively common causes of ischaemic retinopathy.

Most of these conditions are characterised by neuronal loss and are possible candidates for stem cell therapy. There are two broad approaches to developing such therapies: neuroprotection and cell replacement.

Stem cell therapies for retinal neuroprotection

It is now apparent that many types of stem cells possess inherent neuroprotective properties when transplanted into the injured CNS. Stem cells, in particular somatic (neural tissue-derived as opposed to pluripotent cell-derived, or ‘neuralized’ stem cells) neural stem cells and mesenchymal stem/stromal cells (MSCs), are reported to elicit neuroprotective effects via the inherent secretion of high levels of neurotrophic factors [4] and/or inflammatory modulators [5]. In addition, cell transplantation has been used as a vehicle for selective neurotrophic factor delivery, using a variety of cells including stem cells, genetically modified to hyper secrete neurotrophins [6-8]. Within the CNS, neuroprotective stem cell therapies are likely to be translated to clinical treatments more rapidly than neuroreplacement therapies given they require only that transplanted cells survive *in vivo* and continue to provide support to the host neurons without significant adverse effects.

More recently, such experimental therapies have been explored in animal models of retinal disease. The inherent secretion of neurotrophic factors by grafted cells has generated interest in the glaucoma field as impaired neurotrophin support is strongly implicated in RGC death. Recently, we examined the ability of

neurotrophin-secreting cells to protect RGCs from death in a rat glaucoma model [9, 10]. Initially, we discovered that intravitreal transplantation of somatic neural stem cells prevented the death of about half the RGCs normally killed by an ocular hypertensive injury [9]. Interestingly, this neuroprotective effect was achieved despite vitreal graft placement and no retinal integration of transplanted cells. This suggested secreted neurotrophins were mediating this neuroprotective effect. Subsequently, we repeated these experiments to determine whether a similar effect could be induced using MSCs, a stem cell type renowned for its neuroprotective qualities. MSCs can be isolated and cultured from a variety of tissues, including the bone marrow, making them an attractive source for autologous transplantation. MSCs secrete a battery of neurotrophins and anti-inflammatory cytokines, and are reported to protect neurons from death after transplantation into many different models of neurodegenerative disease. Furthermore, MSCs are reportedly able to ‘home’ or preferentially migrate to sites of neural injury after systemic delivery in order to elicit their protective effects. Encouragingly, we demonstrated that MSCs offer robust protection to RGCs in a rat glaucoma model after local intravitreal delivery [10]. This neuroprotection occurred in the absence of significant contact between engrafted cells and the retina, suggesting a secreted neurotrophin mechanism. Intraocular transplantation has also been reported to protect RGCs [11, 12] and photoreceptors [13, 14] in other models of retinal degeneration. In contrast to observations from other CNS pathologies [15], we did not detect migration of intravenously delivered MSCs into the injured retina and systemic delivery was not neuroprotective.

Promisingly, an implantable cell-encapsulation device (NT-501) has been engineered and is currently undergoing clinical trials for the chronic delivery of ciliary neurotrophic factor (CNTF) in AMD and RP patients [16]. CNTF is an important survival factor for photoreceptors and encapsulated cell-based delivery of CNTF has demonstrated significant photoreceptor protection in a dog model of RP [17]. The

implantable, removable, semi-permeable, polymeric device is loaded with a cell line genetically modified to secrete high levels of CNTF. This device allows diffusion of CNTF while sequestering the implanted cells away from host inflammatory attack. It was originally tested in rabbits to determine longevity and CNTF release profiles [18]. This study demonstrated that viable CNTF-secreting cells remained within the device for up to 1 year *in vivo*. A subsequent phase I clinical trial demonstrated the device was safe [19], and ongoing phase III (RP) and phase II (AMD) trials are now assessing efficacy [16]. This development is interesting to researchers searching for a safe method to deliver neuroprotective cells, for example MSCs in glaucoma, to the eye for long-term treatment. Ideally, such a method would be clinically safe, reversible if complications are encountered, long-lasting, replaceable if needed, and would provide an environment within which the secretory cells remain functional *in vivo*. To date, the NT-501 device is the first to meet these criteria, and its adaptation offers the possibility of rapid clinical translation for experimental treatments based on cell delivery of neurotrophic factors in other common retinal diseases. Clinical neuroprotective cell-mediated drug delivery for AMD, and other degenerative retinopathies, is also being developed by EyeCyte Inc (USA).

Another cell-based therapy approaching clinical translation for retinal degeneration is subretinal transplantation of RPE for the treatment of AMD. Imminent clinical trials for such therapy are being planned by several groups worldwide, including Prof Pete Coffey (UCL) in conjunction with Pfizer Regenerative Medicine in the UK [20], Cell Cure Neurosciences Ltd in Israel, and Advanced Cell Technology in the USA. This treatment aims to replace dysfunctional RPE with functional RPE generated from embryonic stem (ES) cells to preserve photoreceptors and visual function. Similar therapies have proven efficacious in animal models of photoreceptor degeneration, including the Royal College of Surgeons (RCS) rat where the ability of RPE cells to phagocytose photoreceptor outer segments is impaired. Subretinal transplantation

of healthy RPE cells, either freshly isolated or generated from ES cells, into the RCS rat has demonstrated photoreceptor protection with preservation of neuronal connectivity and visual function [21-24]. It is hypothesized that this effect is mediated through both the provision of neurotrophic factors and renewed RPE phagocytosis of photoreceptor outer segments. Furthermore, as both macular translocation (where the retina is detached and rotated to place the macula over healthy RPE) and RPE patch transplantation (where a patch of healthy RPE cells from the peripheral retina is placed under the macula) have both demonstrated limited clinical success in some patients [25, 26], it is anticipated that replacement of dysfunctional RPE using an ES cell source could improve vision in carefully selected AMD patients in the near future provided technical barriers to successful graft integration (for example damage to Bruch's membrane) are overcome. Such experimental approaches include engrafting RPE cells as a monolayer attached to a supporting membrane scaffold [27], or modifying Bruch's membrane to enhance attachment [28].

Stem cell-based therapies are also being explored for ischaemic retinopathies. This topic was recently reviewed in depth by Stitt *et al* [29], where the authors hypothesize that reparative intra-retinal angiogenesis and/or vascular stem cell-mediated repair could be used as a treatment for this group of diseases. As mentioned above, ischaemic retinopathies, including common diseases like diabetic retinopathy and retinal vein occlusion, involve vasodegeneration leading to hypoxia, which can trigger tissue release of growth factors and cytokines [30]. This, in turn, causes blood vessel leakage and neovascularization, which can impact negatively on retinal function. Current clinical treatments include intravitreal injection of corticosteroids or VEGF-antibodies, and laser photocoagulation, which alleviate vascular leakage and macular oedema but can cause unwanted side effects and do not address the underlying pathology. The underlying vasodegeneration involves loss of endothelial cells, pericytes and smooth muscle cells, eventually culminating in vascular occlusion and hypoxia [30]. As

discussed in detail by Stitt *et al* [29], endothelial progenitor cells have demonstrated capacity for vascular repair and regeneration, and, as such, their application to therapeutic angiogenesis is being explored for ischaemic diseases [31, 32]. This approach is currently being developed by EyeCyte Inc (USA) for clinical application.

Stem cell therapies for retinal neuroregeneration

As with other degenerative CNS diseases, the use of stem cells for tissue regeneration in retinal pathologies is also under intense investigation. However, the therapeutic replacement of neurons in the mature retina is extremely complicated as newly engrafted cells must not only differentiate into the appropriate neuronal cell type, but also regenerate appropriate connections within a highly ordered, hard-wired, spatially-organised neural network. Given the complexity of this task it is likely that neuroprotective stem cell therapies will reach the ophthalmological clinic more quickly than neuroregenerative therapies. However, neuroprotective treatments can only hope to halt disease progression, whereas neuroregenerative applications may be able to reverse functional loss and actually improve visual impairment.

Neuronal replacement for both inner and outer retinopathies is being explored experimentally, primarily via transplantation of various stem/progenitor cells. Many cell types have been used in these experiments, most commonly ES cells and somatic neural stem cells. *In vivo* experiments have revealed that the mature retina presents an inhibitory environment to the integration of engrafted cells [33] – typically more so than observed in the brain. This barrier to integration appears to be retinal in origin, restricted to the mature retina, and not dependent upon transplanted cell type. However, it has been observed that retinal injury, modulation of the inflammatory/inhibitory retinal environment or manipulation of cells prior to transplantation can enhance the retinal migration of engrafted cells [34-40]. In particular, it appears that reactive Müller cells form a major barrier to the migration of transplanted cells into the

retina, and blockade of Müller cell reactivity can significantly enhance integration of engrafted cells [35, 41].

Although numerous studies have now demonstrated that stem/progenitor cells can be engrafted into the injured mature retina there remains a relative lack of convincing evidence that these cells fully differentiate into *functional*, integrated retinal neurons. It is often reported that engrafted cells exhibit immature retinal neuronal markers after integration but fail to progress to mature marker expression, despite migrating into the retina and even extending neurite-like processes. It has been hypothesized that this lack of functional integration may be due to the absence of appropriate differentiation signals from the mature retina or a deficiency of the engrafted cells. In a comprehensive study, MacLaren *et al* [42], expanding on earlier demonstrations of improved retinal function after stem cell integration [43], reported that the developmental age of donor cells can be critical for the successful integration of transplanted cells, and their functional differentiation into photoreceptors, in the adult or degenerating retina. In this study it was discovered that donor cells isolated from the developing retina at the peak of photoreceptor genesis are much more successful at integrating with the established circuitry than those from other sources. Furthermore, the authors reported synaptic integration of engrafted cells, along with neuronal replacement and improved visual function in a retinal degenerative model, for the first time. The total number of cells that migrated into the retina was still small, but the same research team has subsequently found that pharmacologically reducing Müller cell reactivity can enhance outer retinal integration [41]. Related research has supported these results [44-46] and this outcome has also been demonstrated in the inner retina [35]. More recently, the important influence of cell dissociation and culture methods on transplant integration has also been illuminated [46]. With a view towards clinical translation, it has been reported that immature retinal cells generated from human ES cells can integrate into the retina, after subretinal transplantation, where

they exhibit differentiated photoreceptor features and improved functional light responses in a model of photoreceptor degeneration [47]. Further development of methods to enhance photoreceptor differentiation from stem cells and evidence for functional recovery [48], combined with the possibility of using an abundant ES cell source, is gradually propelling stem cell-mediated replacement of photoreceptors toward future clinical treatment. However, several important hurdles to clinical translation, including investigating key safety issues (for example potential tumorigenicity) and determining optimal cell source for particular therapies (discussed elsewhere, require addressing before such therapies could be made available to patients.

In contrast, successful transplantation-based replacement of RGCs is lagging behind that achieved for photoreceptors. This is perhaps unsurprising given the complexity of replacing RGCs in the mature retina, which, unlike monosynaptic photoreceptors, would need to establish connections with both afferent targets in the retina and efferent targets in the brain, concomitant with projecting a long axon through the length of the optic nerve. Moreover, newly integrated cells would need to broadly conserve the retinotopic map. To date, almost none of these requirements have been adequately addressed in the mature mammalian retina. We have previously demonstrated very limited integration of transplanted cells into the inner retina following experimental glaucoma [34]. Moreover, while other researchers report better retinal migration of engrafted stem cells, coincident with expression of some RGC protein markers and extension of neurites (perhaps due to different cell sources [36, 39, 49]), evidence for synaptic integration and functional improvement remains elusive. However, incremental advances in the fields of RGC-directed differentiation of stem cells (including embryonic stem [ES] and induced pluripotent stem [iPS] cells), retinal transplant integration and optic nerve regeneration continue to be made, hinting that eventually successful protocols will converge to achieve RGC replacement in disease.

Endogenous replacement of retinal neurons is also under investigation. Unlike some evolutionarily older animals, such as fish and birds, the mature mammalian retina does not exhibit any reparative capacity. However, within the last decade several groups have demonstrated that proliferative cells, which display some of the hallmarks associated with neural stem cells, can be isolated from the mature retina (rodent and human [50-53]). Two possible sources have been identified for these retinal stem cells: the ciliary body, and the Müller glia. Both cell types have been shown to differentiate *in vitro*, where they can be induced to express protein markers of various mature retinal neurons, although the neurogenic capability of proliferative ciliary margin cells has been questioned [54, 55]. More recently, it has been demonstrated that mild excitotoxicity can trigger *in vivo* proliferation of a Müller cell subpopulation [56, 57]. These newly born cells exhibited some potential neurogenic capacity and survived in the retina for up to 3-4 weeks. Furthermore, it has subsequently been reported that Notch and Wnt signalling can stimulate *in vivo* Müller cell proliferation and regeneration of photoreceptors [58]. While modest to date, and lacking evidence for the functional integration of newly generated cells, these tantalizing results suggest the exciting possibility of modulating endogenous retinal stem cell behaviour for future neural repair/replacement.

CONCLUSION

As with other neurodegenerative diseases, retinal pathologies desperately need effective neuroprotective and neuroregenerative therapies. However, as ophthalmic surgical interventions are well developed, and as the eye is a relatively isolated structure where treatments can be directly visualised and functional improvement easily measured, the retina may possess an advantage in the race to translate experimental stem cell-based therapies to the clinic. It is clear that stem cell transplantation can be neuroprotective in a variety of models of common retinopathies, and the recent development of an encapsulated cell delivery device presents the very real possibility of moving such therapies into the

clinic as an approved, effective treatment soon. In addition, transplantation of ES-derived RPE for the treatment of AMD is now approaching clinical testing. Retinal neuroregeneration is also a topic of intense investigation as a means to reverse blindness. To date, stem cell transplantation in animal models has been most effective for the replacement of photoreceptors, although this therapeutic approach is also being explored for inner retinal pathologies. Finally, the discovery of a population of proliferative cells in the mammalian retina has raised the possibility of harnessing endogenous retinal stem cells to elicit retinal repair. Together, these recent advances provide significant hope that novel adjunctive stem cell therapies may soon be developed to prevent blindness in progressive

retinopathies that are currently inadequately treated.

ACKNOWLEDGMENTS

NDB and KRM are supported by grants from Fight for Sight (UK) and the Glaucoma Research Foundation. KRM is supported by the Jukes Glaucoma Research Fund and the Cambridge NIHR Biomedical Research Centre.

Disclose of Potential Conflicts of Interest

The authors indicate no potential conflicts of interest.

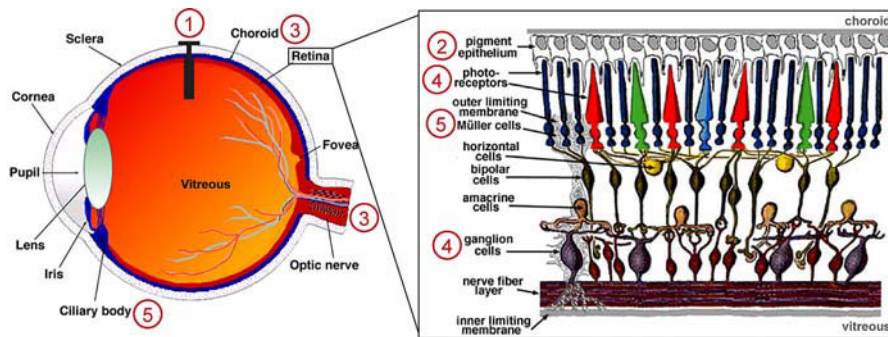
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Figure 1. Schematic of the human eye and retinal anatomy highlighting experimental stem cell therapies under investigation. The neurosensory retinal tissue lines the posterior segment of the eye. Light passes through the cornea, lens, vitreous and retina before it is absorbed by photoreceptor neurons. Photoreceptors synapse with bipolar cells, which in turn pass the visual signal to retinal ganglion cells, whose axons form the optic nerve and connect the retina to visual pathways in the brain. Horizontal and amacrine cells are retinal interneurons that modulate intra-retinal signalling. Müller cells, a type of radial glial cell, and pigment epithelial cells provide key physiological support to retinal neurons. The possibility of using stem cells for neuroprotection and/or neuroregeneration is being explored for several common neurodegenerative retinopathies, as summarized in this diagram. Abbreviations: AMD = age-related macular degeneration; RP = retinitis pigmentosa; ES = embryonic stem cells; RPE = retinal pigment epithelium; RGC = retinal ganglion cell; * = treatment currently in, or approaching, clinical trial. *Diagrams courtesy of Webvision, an online resource from the John Morgan Eye Center, University of Utah, UT, USA.*



- ① Implantable cell-encapsulation device for chronic intraocular delivery of secreted neurotrophins in AMD & RP*
- ② Transplantation of ES-derived RPE for recovery of RPE function & photoreceptor protection in AMD*
- ③ Vascular stem cell repair and therapeutic angiogenesis in ischaemic retinopathies
- ④ Transplantation of stem/progenitor cells for photoreceptor replacement in outer retinal disease, & RGCs in inner retinal disease
- ⑤ Modulation of retinal stem cells, either Müller cell or ciliary body origin, to elicit endogenous retinal repair/regeneration