Writer: Maya Chaddah
Editor: Lisa Willemse
Reviewer: Derek van der Kooy

Originally published by the Stem Cell Network September 2012

Diseases of the Eye

Research is a dynamic enterprise that generates a wealth of knowledge. It provides a forum for debating ideas and working them into evidence-based theories. The clinical trial setting puts these theories to the test and may lead to evidence-based medicines that can alleviate symptoms or cure disease. But the process of taking research from bench to bedside is a lengthy one, and demands not only vision but also years of hard work and dedication on the part of scientists, physicians and patients. This document presents basic information about diseases of the eye and frames the context for the discussion that follows about the future application of stem cells to treat these conditions. Readers may also wish to peruse additional web resources or speak with their physicians for more information.

About diseases of the eye

Diseases and conditions that cause visual impairment or blindness have the potential to drastically reduce quality of life for individuals and increase the economic burden to society. Vision loss can result from injuries or from diseases that affect many different parts of the eye, including the cornea, retina and optic nerve. Some of the diseases can be traced to a genetic defect while others stem from complications arising from illnesses, such as diabetes. The most common eye diseases, notably cataracts, glaucoma and age-related macular degeneration, are all associated with aging.

Anatomy of the eye

Before embarking on how stem cells may be able to help diseases of the eye, it is worth taking a brief look at the anatomy of the eye, and paying special attention to the retina.

The eye is essentially a hollow ball. There are three main layers that lie against each other to form the eyeball: the outer fibrous layer, the middle vascular layer and the inner neural layer. The eyeball is protected by the conjunctiva, a thin layer that covers the inner surface of the eyelid and outer surface of the eye. The outer layer of the eyeball consists of the sclera and cornea. The sclera, or white of the eye, is made of a tough tissue that connects with the cornea, which is the transparent bulge covering the coloured iris and black pupil at the centre of the eye. The border between the sclera and the cornea is called the limbus. The middle layer of the eyeball, or choroid, houses the blood vessels, lymphatic vessels, iris, and ciliary body. This layer regulates the amount of light entering the eye, maintains the aqueous humour and controls the shape of the lens, located just behind the iris. The lens focuses visual images on the retina, which is the inner neural layer of the eyeball. The retina is the crucial light sensing part of the eye that conveys visual information to the brain. Beginning as the thin ring around the iris, the retina lines the inner curvature of the eyeball, and exits the eye as the optic nerve.

Three fluid-filled chambers stabilize the eye and give it its shape. The anterior chamber (front) is the space between the cornea and the iris, and the posterior chamber (back) is between the iris and the lens. These chambers contain a watery liquid called the aqueous humour, produced by the ciliary body. The vitreus is largest chamber in the eye and it is filled with a glassy gel-like substance that fills the space between the lens and the back of the eye.

Writer: Maya Chaddah
Editor: Lisa Willemse
Reviewer: Derek van der Kooy

Originally published by the Stem Cell Network September 2012

More about the retina

The outer layer of the retina consists of the retinal pigmented epithelium (RPE) which absorbs light. The inner layer contains neurons and support cells that process visual images, and blood vessels that supply the eye with nutrients. Multiple layers of neurons in the retina relay light information from one to the other. Just underneath the RPE lies the first layer of neurons, millions of photoreceptors called rods and cones that sense light intensity or colour, respectively. The rods and cones are not evenly distributed throughout the retina. Instead, the rods are concentrated around the periphery while the cones are mostly at the back, concentrated in a small region called the macula where rods are totally absent. The central portion of the macula is called the fovea, housing the highest concentration of cones and providing us with the sharpest sense of sight. The rods and cones in the retina transmit visual signals to neurons called bipolar cells that in turn pass the information to retinal ganglion cells whose nerve endings merge to become the optic nerve. Support cells called horizontal cells help facilitate the communications between photoreceptors and bipolar cells while amacrine cells control communications between the bipolar cells and the retinal ganglion cells. Support cells called Muller glia and astrocytes are also present in the retina.

When we look at an object, light travels through the cornea and the pupil and is focused by the lens through the vitreus onto the back of the retina. The retina translates the light information to the brain, the last link in the neural network, which processes the information into images that allow us to visualize the world around us. Damage to any of the layers of the retina can have a profound effect on vision.

Neurodegenerative diseases of the retina

Neurodegenerative diseases of the retina progressively affect the visual network that transmits signals to the brain, ultimately leading to visual impairment or blindness. Diseases of the retina either affect the outer retina or inner retina. Examples of the former are age-related macular degeneration (AMD) and retinitis pigmentosum. AMD is a group of diseases where the photoreceptors can no longer function as a result of damage to the RPE, the outermost layer of the retina. In children, the most common form of macular degeneration is called Stargardt's macular dystrophy. Retinitis pigmentosum is also a disease of the outer retina and it affects the photoreceptor neurons, situated just underneath the pigmented epithelium. Examples of diseases that affect the inner retina are glaucoma, where the death of RGCs compromises the optic nerve, and ischemic retinopathy where damage to blood vessels results from pre-existing conditions such as diabetes, prematurity, or occlusions (blockages the blood vessels).

Causes, Symptoms and Treatment

Some eye diseases, such as Stargardt's disease and retinitis pigmentosa (RP) can be traced to a genetic defect, while others - most notably cataracts, glaucoma, and AMD - are associated with aging. For some of these conditions, such as cataracts or corneal scarring, the prognosis is good because surgery is available to replace the cloudy lens or cornea. In other conditions, such as glaucoma and AMD, the prognosis is poor. In all cases, early diagnosis is critical for trying to prevent or slow the onslaught of irreversible damage.

Glaucoma

Glaucoma is the name given to a group of different diseases that cause damage to the optic nerve and the field of vision. Over 60 million people are estimated to have glaucoma, and it is the leading cause of blindness in African Americans. VISION 2020, a World Health Organization global initiative, estimates that 4.5 million people are blind as a result of glaucoma, and this number is expected to rise with the aging population.

Glaucoma is insidious and its diagnosis often occurs only after much vision has already been lost. Irreversible damage leading to blindness occurs after the optic nerve and field of vision have slowly but progressively been damaged. Glaucoma is generally painless, but a minority of patients may experience a red and painful eye. Risk factors for acquiring this disease are increased age, usually over 40, and elevated pressure in the eye. The latter is not always the case as some people with the disease may have normal eye pressure. A positive family history and certain ethnic groups also predispose to higher disease risk.

Writer: Maya Chaddah
Editor: Lisa Willemse
Reviewer: Derek van der Kooy

Originally published by the Stem Cell Network September 2012

The main treatments for glaucoma aim to reduce the high pressure within the eye: topical drops containing blood pressure lowering drugs; laser treatment to improve the flow of fluids out of the eye; surgical implantation of a filter (trabeculectomy) or valve to release fluids from the eye. Although reducing the pressure in the eye can be beneficial, it cannot stem the tide of disease progression.

Age-related macular degeneration (AMD)

AMD is the leading cause of blindness in the industrialized world and accounts for approximately nine per cent of all blindness globally, affecting around three million people. With the burgeoning population, these statistics are expected to double by the year 2020. Risk factors for this disease include age, smoking, family history of AMD, and high blood pressure, cholesterol, fat intake and body mass index. Individuals of European ancestry who carry a variation in the gene that codes for complement factor H, a regulator of the immune system, are also at higher risk of disease.

In AMD defects in the retinal pigment epithelial cells result in damage to the photoreceptors, the first neurons in the retinal network that supports vision. The failure of photoreceptors (especially the cones in the macula) leads to progressive loss of central vision and sometimes scarring in the back of the eye. If abnormal, new blood vessels start to grow in the retina and leak fluid, this condition leads to an advanced, rarer form of the disease known as “wet” AMD, which accounts for 10 per cent of cases. The more common form of the disease accounting for 90 per cent of cases is called to ‘dry’ AMD because the RPE layer atrophies or ‘dries out’. Dry AMD is now the leading cause of blindness in people over 60.

There is no cure for AMD. For some patients, treatments such as antioxidants and zinc show modest effects in terms of slowing disease progression. In selected patients, injections of anti-vascular endothelial growth factor or surgical manipulation of the macula may improve vision, but the outcome of these treatments is not always beneficial. New, broadly applicable therapies to treat this disease are lacking. The possibility of protecting, replacing or repairing the RPE layer in the retina through stem cell therapies is becoming a more tangible option for filling this therapeutic void, especially since the RPE layer is the only part of the retina that does not have to make synaptic connections with other neurons.

Corneal scarring

Because the cornea lacks blood vessels and lymphatic vessels, it cannot easily heal upon damage due to mechanical injury or diseases that cause scarring and vision loss. However, the ability of the eye to withstand grafts without rejection combined with the anatomical characteristics of the cornea has led to the success of corneal transplants which have been perfected to the point where they are now routine surgeries that successfully restore damaged vision. One of the most common reasons for having a corneal transplant is blindness caused by infection with herpes simplex virus. The waiting list for corneal transplants is very high, with approximately 500,000 pending cases per year in the US. The ability to keep up with this demand is a growing concern since the procedure is limited by the availability of healthy corneas that come from recent cadavers.

Can Stem Cells Help?

Strategies for using stem cells to treat eye diseases take advantage of the properties of stem cells and/or their products to protect neurons (neuroprotection) and regenerate damaged cells within the eye. The eyes themselves are particularly well suited to transplant therapies because they are inherently protected from normal inflammatory immune responses that counter disease. This protected status is referred to as ‘immune privilege’, displayed only by the eye and the brain. The special anti-inflammatory environment in the eye is created in part by a physical blood-tissue barrier that prevents the influx of inflammatory immune cells, but also by active immunological mechanisms that sway the immune response in these sites from inflammatory to less harmful.

The stem cells best suited for neuroprotective or transplant strategies may come from the eye or from elsewhere in the body. In the eye itself, many different types of stem cells have been identified, each of which performs a particular function. Limbal stem cells, also called corneal stem cells, support the cornea and protect the eye from wear and tear by refreshing the cells on the surface of the eye. Conjunctival stem cells play a role in continually bathing the eye in

Writer: Maya Chaddah
Editor: Lisa Willemse
Reviewer: Derek van der Kooy

Originally published by the Stem Cell Network September 2012

tears and mucous. Both limbal and conjunctival stem cells can be grafted onto existing tissue to repair damage. Retinal stem cells are located at the retinal ciliary margin and able to make photoreceptors.

Stem cells that originate outside the eye also show promise for repairing eye damage and restoring vision loss. They include adult stem cells (bone marrow stem cells, mesenchymal stem cells, neural stem cells) induced pluripotent stem cells and embryonic stem cells. The content below highlights some of the advances towards using stem cells for treating various diseases of the eye.

Neuroprotective strategies

Many preclinical studies in animals have confirmed that both neural stem cells originating in the brain and mesenchymal stem cells from bone marrow secrete factors that protect injured tissues and also modulate the inflammatory response of the immune system. Stem cells can be genetically manipulated into super secreting factories that churn out high concentrations of these beneficial factors, an approach perhaps well suited for treating diseases like glaucoma, where the death of retinal ganglion cells may be linked with an eye environment that lacks protective factors. Pre-clinical studies in rats have corroborated that both neural stem cells and mesenchymal stem cells can provide neuroprotection to retinal ganglion cells that would otherwise have been damaged due to high pressure within the eye.

Cell transplantation strategies

There is currently no therapy for curing neurodegenerative eye disease so the idea of using stem cells to replace damaged cells holds great appeal. In the case of corneal transplants, advances have come relatively quickly. Research has shown that limbal epithelial cells and human embryonic stem cells can generate new corneal tissue that can then be grafted into place. The retina, on the other hand, is not so hospitable a site as the cornea for transplants and tends to actively inhibit the integration of newly transplanted cells. Researchers have managed to get around the issue and can now transplant various types of stem cells into mouse retinas, but it seems that the environmental cues that stimulate differentiation of the transplanted stem cells into the needed cell types are lacking. Further study is needed to detail the signals within the eye that can differentiate transplanted stem cells and facilitate intrinsic repair of damaged tissue.

As a logical next step, researchers turned to directly differentiating the needed cells from stem cells in the laboratory prior to transplanting them into the eye. This approach was tested in 2006 when retinal pigment epithelium derived from human embryonic stem cells was transplanted subretinally (between the RPE layer and photoreceptors) in a mouse model of macular degeneration. Improved vision in the mice proved that the transplanted cells were able to rescue damaged photoreceptors. Although human embryonic stem cells can be readily grown into retinal pigment epithelium, it is crucial that they be tested for purity and screened to make sure that the transplanted cells are free from undifferentiated cells that could cause tumours. Scientists are striving to make these safeguards possible and are excited about the possibility of using human embryonic stem cells as an inexhaustible source of RPE that could heal neurodegenerative diseases of the retina. Moving forward, researchers are tweaking protocols and adding molecules that guide differentiation more precisely in an effort to improve the amount of RPE cells that human embryonic stem cells can produce.

Researchers are also trying to use stem cells to replace damaged retinal ganglion cells in diseases such as glaucoma. In this case, the big challenge is to get the transplanted cells to integrate and to make synaptic connections with both the bipolar cells that bring the visual signal from photoreceptors and the optic nerve carrying the signals to the brain. In rat models of glaucoma, stem cell progenitors obtained from a variety of sources have thus far not proven able to meet these requirements.

A number of different techniques are now available to reprogram adult somatic cells into induced pluripotent stem cells (iPSCs). These cells are similar to embryonic stem cells in their capacity to generate many different mature cell types. In the past few years, researchers have been able to make human photoreceptors and RPE from iPSCs and are steadily working out the details for how to use them in a clinical setting. One advantage of iPSCs is that they are patient-specific and thus should be able to minimize graft rejection. For patients, being able to lower the level of immunosuppressant drugs would always improve quality of life. In addition, some experiments have shown that having normal immune regulatory mechanisms active in the eye may actually help grafts to be accepted long-term. To avoid

the transplanted cells succumbing to the same fate as the original healthy cells, it is thought likely that iPSCs made from patients would first have to be repaired via gene therapy before being transplanted.

Canadian contributions

In the developing mouse embryo, once retinal stem cells have created their differentiated progeny they apparently become dormant. Unlike the blood and some organs, retinal stem cells responsible for early development of sight do not regenerate differentiated retinal cell types like photoreceptors in the adult. Thus, any damage to the retina in adulthood had long been considered irreversible. When retinal stem cells were first isolated in the mouse in 2000 by Canadian researcher Vince Tropepe, it kindled hope that perhaps retinal damage long considered irreversible might in fact be reversed.

Four short years after retinal stem cells were first isolated in the adult mouse eye, a team in Derek van der Kooy's lab at the University of Toronto identified human retinal stem cells that were present in donor eyes from the young to the old. The seminal experiments demonstrating that human retinal stem cells might someday be co-opted for the purpose of treatment were done first in mice and then in chicks. In newborn mice eyes, the transplanted retinal stem cells could integrate and differentiate into cells of the retina, especially the photoreceptors found in the outer layer. In embryonic chick eyes, the transplanted stem cells could also form retinal ganglion and horizontal cells.

In collaboration with Molly Shoichet's lab, also at the University of Toronto, van der Kooy has continued to investigate both how retinal stem cells develop and their therapeutic potential in animal models of human retinal disease. In mature animals, the best location to transplant stem cells is subretinally, or just between the RPE and photoreceptor layers. However, integration and survival of the transplanted cells is an ongoing challenge. To address this issue the Toronto researchers have developed an injectable biodegradable gel matrix (known as HAMC) seeded with retinal stem cell progenitors. This medium is a superior way of delivering retinal stem cells into mice and can give rise to more continuous integration of the transplanted cells into the RPE layer of the eye. This new delivery procedure is minimally invasive and could conceivable be used in a clinical setting to help regenerate large tracts of RPE that are destroyed in diseases such as wet AMD.

Many Canadian researchers are also investigating the signals that control the differentiation of retinal stem cells into the various cells that populate the retina. Understanding these signals will help researchers to manipulate stem cells towards different fates and to create more enriched sources of particular types of retinal cells for transplantation. Valerie Wallace, acting head of the Foundation Fighting Blindness, has identified Wnt and Sonic hedgehog as signals that affect the fate of retinal progenitors. Wallace's lab continues to perfect in vitro strategies for growing and differentiating stem cells with the long-term view of using the progeny for retinal transplantation in humans.

One issue with retinal stem cells is that they don't tend to make a high percentage of photoreceptors when cultured in a dish. In collaboration with scientists from Japan and the USA, Canadian researchers at the Universities of Toronto and Dalhousie have hit on a way to genetically modify human retinal stem cell precursors so that they are able to churn out a higher percentage of photoreceptors. Transplanted into mice, the genetically modified cells integrate and differentiate better than unmodified retinal stem cells and can ignite functional repair of damaged retinal tissue. These preliminary results bode well for being able to use the method of gene modulation for creating future photoreceptor-based transplant therapies.

Clinical studies

The clinical trials underway are the culmination of extensive preclinical studies whose results have paved the way for the opportunity to test the regenerative or neuroprotective capacity of stem cells in human subjects.

Embryonic stem cells

One of the most prominent cell transplant studies, spearheaded by The London Project in the UK and sponsored by Advanced Cell Technology, was reported recently in a 2012 issue of The Lancet. This report has stirred great excitement in the scientific community because it is the first published description of human embryonic stem cell-derived cells being transplanted into humans. During the trial, 50,000 human RPE cells, generated from embryonic stem

cells, quality controlled and tested for safety in mice, were transplanted subretinally into two patients; one with dry age-macular degeneration and the other with Stargardt's macular dystrophy. The aim was to test whether the RPE transplants were safe and well tolerated. Most importantly, would the cells form tumours or be rejected by the immune system? Since the transplants were not autologous, or from the patients themselves, two immunosuppressant drugs were administered one week before the surgery and continued for six weeks after the surgery, after which time one of the drugs was discontinued.

Four months after the transplant, the grafts had not been rejected nor was their any evidence of abnormal growth or inflammation. The transplanted cells appeared to attach to Bruch's membrane, the layer between the retinal pigmented epithelial cells and the choroid, and this is a key requirement for further integration of the cells into the damaged RPE layer. Neither of the patients lost any vision during the trial and both showed a degree of improved vision as measured by increased recognition of hand motions and letters on a visual acuity chart. The patient with macular degeneration improved from being able to read 21 letters to 28 letters by week 6 after the transplant. The graphic artist with Stargardt's was able to read 5 letters after 5 months and also reported improved colour and contrast vision.

Although these results are very encouraging, the researchers admit that they are unsure whether the transplanted cells may yet be rejected or whether the visual gains are a result of the transplanted cells, from the immunosuppressant therapy or even as a result of a placebo effect. They will continue to monitor the patients, and also work on optimizing the stage of RPE cells created in the laboratory for transplantation. The long-term goal of future trials would be to treat patients with earlier stage disease so that the chance of photoreceptor rescue and visual gains could be maximized.

Autologous limbal stem cells

Autologous limbal stem cells are also being investigated for their ability to regenerate corneal tissue in people whose eyes have been badly burned. Provided that one of the eyes is undamaged, a sample of resident limbal stem cells can be harvested, grown in the laboratory and transplanted back into the patient's burned eye. An Italian trial tested this approach in 112 patients and published their findings in 2010. In 75 per cent of patients, the autologous limbal stem cell grafts were able to regenerate functional, renewing corneal epithelium and restore normal vision. The before and after pictures were remarkable, showing the cloudy corneas scarred by acid burns and the clear transparent corneas that regenerated after the transplant. The effects were long-lasting and patients were followed for up to 10 years in some cases, providing much hope for using this treatment to regenerate damaged corneas.

Retinal cells

Pointing the way for future stem cells studies are the results from a study sponsored by Neurotech, the company that created the NT-501 implantable device. Loaded in the device are genetically modified cells, derived from human retinal cells, that produce ciliary neurotrophic factor (CNTF). This factor can promote the survival of photoreceptors and retinal ganglion cells. The device is implanted into the back of the eye where it releases a continuous source of CNTF. A big advantage of this method is that graft rejection is minimized because the genetically modified cells are trapped in the device and therefore do not come into contact with the immune system. Following the initial safety checks in a phase I trial, Neurotech has since completed a phase II trial testing the device in patients with dry age-related macular degeneration as well as phase II and III trials in patients with retinosa pigmentosum. In all three trials, the device was well tolerated and appeared to slow the rate of vision loss. A small phase II trial on patients with retinitis pigmentosum is currently underway testing for cone photoreceptor preservation and whether the device precipitates any adverse effects, rejection or shifting from the site of implantation. This therapy holds much promise for many other retinal neurodegenerative diseases as well, and there are also ongoing phase I trials testing NT-501 in patients with glaucoma and ischemic optic neuropathy. The company has also created another model, NT-503, loaded with cells that produce a protein that blocks new blood vessel formation. They plan to start a phase I trial testing the device in patients with wet age-related macular degeneration. Although the genetically modified cells in the devices are not stem cells, the method itself points to a potentially safe way of delivering stem cells, such as mesenchymal stem cells, that could make neuroprotective factors to treat diseases such as glaucoma or AMD.

The content reviewed in this document is but a smattering of the preclinical and clinical trials currently underway to assess the ability of stem cells to treat diseases of the eye. The advances to date on both fronts are quite incredible. In

Writer: Maya Chaddah
Editor: Lisa Willemse
Reviewer: Derek van der Kooy

combination they provide the basis for a realistic future where stem cell therapies will be a viable option for restoring damaged vision.

Web Resources

- CNIB: http://www.cnib.ca/
- The Foundation Fighting Blindness (Canada): http://www.ffb.ca/
- Foundation Fighting Blindness: http://www.blindness.org/
- The London Project (UK): http://www.thelondonproject.org/
- AMD Alliance International: http://www.amdalliance.org/
- National Eye Institute: https://www.nei.nih.gov/
- Vision Action Plan [.PDF file]: http://www.who.int/blindness/Vision2020_report.pdf
- Vision 2020: http://www.iapb.org/vision-2020
- EuroStemCell: http://www.eurostemcell.org/factsheet/eye-and-stem-cells-path-treating-blindness

Selected Reading List

- Ballios et al. A hydrogel-based stem cell delivery system to treat retinal neurodegenerative disease. Biomaterials. 2010; 31: 2555-2564.]
- Bull N, & Martin, K. Concise Review: Toward Stem Cell-Based Therapy for Retinal Neurodegenerative Diseases. Stem Cells 2011;29:1170-1175.
- Emerich DF, Thanos CG. NT-501: An opthalamic implant of polymer encapsulated ciliary neurotrophic factor-producing cells. Curr Mol Ther 2008; 10:506-515.
- Harrison's Principles of Internal Medicine. 18th Edn. /editors. Dan L. Longo et al. The McGraw-Hill Companies, Inc. 2012.]
- Rama P. et al. Limbal stem cell therapy and long-term corneal regeneration. New England Journal of Medicine. 2010;36(2):147-155.
- Schwartz S et al. Embryonic stem cell trials for macular degeneration: a preliminary report. The Lancet 2012;379:713-720.
- Tibbetts et al. Stem cell therapy for retinal disease. Current Opinion in Opthalmology. 2012;23(3):226-234.
- Timoyouki Inoie et al. Maximizing Functional Photoreceptor Differentiation from Adult Human Retinal Stem Cells. Stem Cells. 2010;28(3):489-500.
- Tropepe et al. Retinal stem cells in the adult mammalian eye. Science. 2000 Mar 17;287(5460):2032-6.
- Wallace V. Concise Review: Making a Retina – From the Building Blocks to Clinical Applications. Stem Cells 2011;29:412-417.
- Wing, YY et al. Progenitors for the Corneal Endothelium and Trabecular Meshwork: A Potential Source for Personalized Stem Cell Therapy in Corneal Endothelial Diseases and Glaucoma. J Biomed Biotechn. 2011; 2011:412743.