Preclinical Research of Mesenchymal Stem Cell-Based Therapy for Ocular Diseases

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Abstract

Mesenchymal stem cells (MSC) are specific cell types that enable tissue renewal within the body and are activated during the regeneration processes in response to injury. These cells are present in all human and animal tissues. In medical and biological research, MSCs are isolated and purified from the donor organism and cultured in vitro before use in the treatment of a variety of diseases and conditions associated with tissue damage and cell loss, including ocular lesions. This approach is called cell-based therapy or regenerative medicine. Despite the many existing therapeutic strategies in the area of cell therapy in relation to curing ocular diseases in recent years, advances and new regenerative therapy methods have also been developed and consolidated, giving us a new perspective.

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Therefore, it is necessary to implement standardization and comparison of cell therapy results. Experimentation using animal models has played a central role in biomedical research. The safety and efficacy of new drugs are usually tested in animal models of human diseases prior to entering human clinical trials. Nevertheless, the pathophysiological mechanisms of eye diseases are complex and multifactorial; hence it is crucial that experimental animal models with clinical relevance provide adequate information and sufficiently replicate the eye diseases being assessed and demonstrate the effects of MSC therapy.

Keywords

Experimental · Eye · Humans · MSCs · Ocular · Regenerative

Abbreviations

ADMSC	Adipose-derived mesenchymal stem cells
AMD	Age-related macular degeneration
BDNF	Brain-derived neurotrophic factor
BMSC	Bone marrow stromal cells
CNV	Choroidal neovascularization
DR	Diabetic retinopathy
ES	Embryonic stem cells
GCL	Ganglion cell layer
GFP	Green fluorescent protein
PVPC	Multipotent perivascular progenitor cells derived from human
hESC	Embryonic stem cells
hRPC	Human retinal progenitor cells
LEC	Lens epithelial cells
LHON	Leber's hereditary optic neuropathy
MSC	Mesenchymal stem cells
NF1	Neurofibromatosis type 1
POAG	Primary open-angle glaucoma
RCS	Retinal dystrophy of Royal College of Surgeons rats
RGC	Retinal ganglion cells
RP	Retinitis pigmentosa
RPE	Retinal pigment epithelial cells
SC	Stem cells
UMSC	Umbilical cord mesenchymal stem cells

1 Introduction

Dysfunction of the visual system can significantly reduce the quality of human life, as the brain receives more than 80% of the incoming information via the eyes. According to the World Health Organization (WHO), in 2020, there were more than 1.3 billion people with visual impairments; 217 million people within this group had

poor vision, and 36 million were blind. The leading causes of visual impairment are uncorrected refractive errors, cataracts, age-related macular degeneration, glaucoma, diabetic retinopathy, and corneal opacity (Lancet Global Health 2019).

Despite the availability of various medications and therapies available for use in ophthalmic practice presently, not all ocular diseases are treatable. Therefore, stem cell (SC) therapy has recently become more widespread. Many publications indicate its effectiveness in treating intractable eye diseases for both humans and animals (Zakirova et al. 2015, 2019).

The role of experimental animal models for obtaining information about the effectiveness and safety of cell therapy, including SC therapy, has significantly increased. Most studies are currently conducted on rodents, and on large animals, such as rabbits, dogs, pigs, sheep, goats, and non-human primates (Harding et al. 2013).

There is presently a wide range of potential SC-based drugs that can be used for medical purposes. Currently, embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs) somatic stem cells, as well as differentiated cells derived from SC, mesenchymal stem cells (MSCs) from adipose tissue (ADMSCs), MSCs from bone marrow (BM-MSCs), neural stem cells, endogenous retinal stem cells, and stem cells derived from ciliary, retinal pigment epithelium, and umbilical cords (UMSCs) are used for therapy (Harding et al. 2013).

Currently, preclinical animal models are used to develop methods for treating eye surface diseases, glaucoma, retinal diseases, diabetic retinopathy, and age-related macular degeneration. Many experimental animal models imitate eye diseases, but none of them reflect the incredible complexity that the human disease possesses. Therefore, to replicate or emulate different aspects of human pathology, several experimental models of the same disease are usually required. Some of the important and commonly used experimental animal models used for testing cellular technologies in ophthalmology are summarized in Table 1.

2 Models of Corneal Diseases

The cornea is a piece of transparent tissue in the anterior aspect of the eye. It is a protective physical and biological barrier against the external environment and provides a refractive force for the concentration of light on the retina (Lewis et al. 2010).

The cornea comprises three main cell types: stratified surface epithelium, stromal keratocytes, and the innermost single-layered endothelial cells, which are neuroepithelial in nature (Sagizade et al. 2017). Surface epithelial cells are essential for corneal transparency and visual acuity. Corneal epithelial cells are continuously enhanced by a population of SC or progenitor cells, which are located in the corneal limb. However, severe corneal damage caused by chemical or mechanical exposure, and immune or hereditary diseases, can lead to corneal inflammation, ulceration, neovascularization, and deficiency of limbal SC. All of these situations can result in loss of vision.

Table 1 Animal models used for testing cellular technologies in ophthalmology

Eye tissue	Animal	Model	Cell type	References
Cornea	Mouse	The transgenic model. Lumican UMSC knockout mice.	UMSC	Liu et al. (2010)
		Transgenic mice model with mucopolysaccharidosis type VII	UMSC	Coulson-Thomas et al. (2013)
		Mechanical damage	BMSC	Shukla et al. (2019)
		Laser induction of damage	ADMSC	Zeppieri et al. (2017)
		Thermal damage	BMSC	Lan et al. (2012)
	Rat	Chemical burns	BMSC	Ma et al. (2006)
		Chemical burn with alkali	MSC	Faruk et al. (2017)
			BMSC	Li et al. (2015)
			MSC	Yao et al. (2012)
		Chemical damage by 100% ethanol	MSC	Oh et al. 2008
			BMSC, corneal epithelial cells	Oh et al. (2009)
		Surgical injury	ADMSC c PAX6	Joe and Gregory-Evans (2010)
		The dry eye syndrome caused by benzalkonium chloride	MSC	Beyazyıldız et al. (2014)
	Rabbit	Chemical alkali burn	MSC	Ye et al. (2006)
			BMSC	Gu et al. (2009)
		Surgical injury	ADMSC	Galindo et al. (2017)
			BMSC	Then et al. (2017)
		Model of bullous keratopathy	Primary corneal endothelial cells	Peh et al. (2017)
	Dog	Spontaneous model of dry keratoconjunctivitis	MSC	Sgrignoli et al. (2019)
		Spontaneous corneal wounds and ulcers	MSC	Falcão et al. (2019)
Lens	Rat	Induced by sodium selenite-	Wharton jelly MSCs	Maleki (2015)
		Model of diabetes 2 type	ADMSC	Yu et al. (2019)
	Rabbit, Macaque	Surgical method	LEC	Haotian et al. (2016)
Retina		Laser induction	BMSC, RPE	Hou et al. (2010)
	Rat	Diabetes model induced by streptozotocin	hESC-PVPC BMSC	Kim et al. (2016)

(continued)

Table 1 (continued)

Eye tissue	Animal	Model	Cell type	References
	Rat	RCS rats with dystrophy	hRPC hRPC/hBMSC RPE RPESC-RPE	Semo et al. (2016), Zhao et al. (2017), McGill et al. (2017), Davis et al. (2017)
	Rat	Model of retinal degeneration by sodium iodate injection	hESC	Park et al. (2011)
	Mouse	Transgenic model, Rd1	ESC и IPSC Precursors of rods	Assawachanaont et al. (2014), Barnea-Cramer et al. (2016), Singh et al. (2013)
	Rat	Optic nerve injury	MSC MSC from cord blood	Mesentier-Louro et al. (2014), Zwart et al. (2009)
	Rat	NMDA model	ESC	Aoki et al. (2008), Divya et al. (2017)
	Cat	NMDA model	MSC from Muller's glia	Becker et al. (2016)

Corneal transplantation is currently a reasonably effective method for managing corneal damage. However, the main problem with this treatment is the rejection of the transplants following this therapeutic technique. The use of allogeneic or autologous limbal cell transplantation is also limited due to the significant risk of systemic immunosuppression. Autologous transplantation can also lead to damage in the contralateral normal eye. Therefore, the search for a safe and adequate source of cells to create a bioengineered corneal epithelium is still ongoing, and one which is under active research at the present.

Numerous experimental studies have attempted to use mesenchymal stem cells (MSCs) as a bioengineered corneal epithelium. Research has also confirmed that epithelial-like corneal cells can be formed from mesenchymal and embryonic stem cells (ESCs) extracted from bone marrow. The numerous potential advantages of adipose tissue-derived MSCs (ADMSC), including easy availability, low immunogenicity, high pluripotency, lack of ethical controversy, and reduced risk of infection, have made them a particularly attractive cell source for bioengineered corneal epithelium (Sun et al. 2018).

MSCs can treat both congenital corneal diseases and various types of injuries and damages (Joe and Gregory-Evans 2010). Additionally, there are many animal models designed to reproduce inherited diseases and injuries affecting the cornea. Therefore, the therapeutic efficacy of MSCs has been tested on various animals (mice, rats, rabbits, dogs, and primates).

In addition to different species, different model types have been used, including transgenic animals, experimental animals with natural spontaneous corneal diseases, and induced models.

Liu and colleagues used knockout mice as model animals for the Lumican gene (Liu et al. 2010). The mice received UMMC transplantations to facilitate studying their potential use as a therapy for congenital corneal diseases caused by genetic mutations. As a result, it was found that transplantation into the UMMC corneal stroma increases the thickness of the stroma and reduces corneal opacity. The turbidity of the corneal stroma was determined by measuring the total pixel intensity of light scattering through a three-dimensional volume, while the thickness of the corneal stroma was calculated by measuring the axial distance from the anterior to posterior stroma (Liu et al. 2010).

Coulson-Thomas and Caterson used genetically modified MPS VII mice with type VII mucopolysaccharidosis as a model to study the therapeutic properties of mesenchymal stem cells (Coulson-Thomas and Caterson 2013). Their study was aimed to establish cell therapy effectiveness in mucopolysaraidosis VII (Sly syndrome). The researchers used UMSCs, which were transplanted into the corneal stroma. A significant number of the UMSCs delivered to the cornea survived the transplant process and remained in the cornea throughout the treatment period. The data obtained showed that human UMSCS transplanted into the murine cornea with MPS VII prevented the accumulation of glycosaminoglycans in the corneal stroma and keratocytes. There was also a lack of corneal opacity, which showed that UMSCs could prevent the disease progression (Coulson-Thomas et al. 2013).

Shukla and Mittal (2019) used a mechanical approach to damage the cornea. The corneal epithelium and anterior stroma, which account for approximately one-third of the total thickness of the cornea, were mechanically removed using an Algerbrush brush. The study was conducted to determine the effectiveness of BMSCs on damaged cornea and conjunctiva within the eye. The cells were injected subconjunctivally, intravenously, and intraperitoneally for an hour after the injury had been induced. Studies have shown that subconjunctival or intravenous administration of BMSCs has higher therapeutic efficacy compared to local or intraperitoneal administration following corneal damage. The authors noted a decrease in corneal opacity, tissue fibrosis, and the expression of pro-inflammatory cytokines (Shukla et al. 2019).

In a laser-induced model of corneal damage in mice, ADMSC administration had significantly smaller defects of corneal epithelial (Zeppieri et al. 2017). In a thermal moxibustion murine model of corneal damage, the ability of MSCs to penetrate into damaged tissue and promote repair of corneal was investigated (Lan et al. 2012). BMSCs were administered intravenously following injury, and the outcomes were evaluated using epifluorescence microscopy whereas the epithelial regeneration was evaluated via corneal fluorescein staining. Studies have shown that the systemic administration of MSCs affected the damaged cornea and showed their long-term survival. The researchers also noted that after the cornea had been damaged, the introduction of BMSCs resulted in a significant and rapid regeneration of the corneal epithelium (Lan et al. 2012).

The effectiveness of human BMSCs in treating chemical burns in the corneal epithelium of rats has also been investigated. BMSCs were cultured on the human amniotic membrane, and 7 days after a chemical burn had been administered,

amniotic membranes with BMSCs were transplanted onto rodent corneas. The anatomical effects and vision of these rodents were measured once a week with a slit lamp and optokinetic head tracking response, respectively. The resulting data showed that BMSCs successfully repaired damaged corneal surfaces in rats (Ma et al. 2006).

Faruk et al. (2017) induced corneal damage using an alkali solution in rats. Studies have shown that systemic injection of autologous MSCs leads to regeneration of the corneal epithelium and inhibits neovascularization in the induced chemical burn of the cornea. The authors showed that MSCs had a therapeutic effect and inhibited early inflammatory responses in local corneal cells (Faruk et al. 2017). This method of corneal damage has also been used in rats. A model of corneal burn, using an alkali, was created by placing a 3 mm diameter piece of NaOH-soaked filter paper onto the right eyes of rats. Immediately after the injury, and 3 days later, the rats were given a subconjunctival injection containing a 2×10^6 MSCs suspension. Studies have shown that subconjunctival injection of MSCs reduces inflammation of the locally burned cornea by inhibiting the infiltration of inflammatory cells and the production of pro-inflammatory cytokines (Yao et al. 2012).

The therapeutic effects of MSCs have also been tested in a model where 100% ethanol was applied to the eye's surface. Afterward, a reservoir with a hollow tube pre-filled with MSCs and culture medium was placed on the cornea and left in situ for 2 h. The reservoir was then removed and the eyes sutured. In this study, the authors demonstrated that MSCs reduced corneal inflammation and neovascularization and this was associated with IL-10 and TGF-beta1, IL-6, and TSP-1 upregulation, and IL-2, IFN-gamma, and MMP-2 downregulation, as well as decreased tissue infiltration by CD4 + cells (Oh et al. 2008). The same researchers also applied the same rat model of 100% ethanol chemical damage where BMSCs and human corneal epithelial cells were directly injected into the cornea. This technique resulted in upregulated expression of IL-6, VEGF, TGFbeta1, MMP-2, and thrombospondin-1, and suppression of MMP-9 secretion by the damaged epithelial cells (Oh et al. 2009).

One study investigated surgical corneal damage with ADMSC with PAX6 therapy. The research revealed that PAX6 induces differentiation of ADMSCs into epithelial-like corneal cells in vitro. ADMSCS reprogrammed with PAX6 also repaired damaged corneal surfaces in vivo (Joe and Gregory-Evans 2010).

The therapeutic efficacy of systemic administration of BMSCs after applying an alkaline burn to the experimental rats was investigated. Efficacy was assessed by corneal reepithelization, corneal opacification, and neovascularization. In the publication, the authors concluded that systemically transplanted BMSCs can take root in the damaged cornea, promoting wound healing through differentiation, proliferation, and synergy with hematopoietic stem cells (Li et al. 2014).

The therapeutic efficacy of topically applied MSCs for dry eye syndrome has been extensively reported (Hirayama 2018; Villatoro et al. 2017). The effectiveness of topical application of MSCs was assessed to treat dry eye syndrome (keratoconjunctivitis sicca; KCS) caused by benzalkonium chloride (BAC) in an experimental rat model. Eye drops containing ADMSC were applied topically every day for a

week. The effectiveness of the treatment was evaluated using the Schirmer test, evaluation of the time of destruction, assessment of the eye surface, the index of corneal inflammation, and histological and electron microscopic analysis. Studies have shown that topical application of ADMSC can be a safe and effective treatment for dry eye syndrome (Beyazyıldız et al. 2014).

Mesenchymal stem cells (MSCs) are also known to promote the engraftment of cell and organ transplants due to their immunotherapeutic and immunomodulatory characteristics (Beeken et al. 2021). Indeed subconjunctival injection of MSCs to rats was effective in increasing the survival rate of corneal allografts. This effect was due to the inhibition of inflammatory and immune responses (Jia et al. 2018).

The possibility of using MSC systemic administration to accelerate the healing of corneal wounds after the alkaline burn of rabbits has been investigated. The clinical outcomes were evaluated using corneal reepithelization, corneal opacification, neovascularization, and immunohistochemical studies. The experiments conducted showed that systemically transplanted MSCs could engraft the damaged cornea, stimulating wound healing by differentiation, proliferation, and synergy with hematopoietic stem cells (Ye et al. 2006).

Gu and Xing (2009) studied the efficacy of MSCs in rabbits concerning whether BMSCs can differentiate into corneal epithelial cells. Corneal damage in rabbits was caused by contact with 1 N NaOH-soaked filter paper for 30 s. Twenty-eight days after corneal injury, fibrin gels were transplanted onto the rabbit cornea. The corneas of the rabbits were observed each day for follow-up with a slit-lamp microscope system (SL-1600; Nidek Co., Ltd., Aichi, Japan) to assess reepithelization, neovascularization, and transparency. The data showed that following BMSC transplantation, the damaged surface of the rabbit cornea was successfully reconstructed, and some of the transplanted cells directly participated in the healing process of the damaged corneal epithelium (Gu et al. 2009).

The effects of human ADMSCs on surgically damaged cornea have also been investigated. Studies have shown that ADMSCs transplanted to the surface of the eye migrated to the inflamed tissues, reduced inflammation, restrained the development of neovascularization and corneal opacification, and partially restored the phenotypes of the limbal and corneal epithelium (Galindo et al. 2017). Molecular profiling revealed partial restoration of corneal epithelial cell markers CK3 and E-Cadherin and the limbal epithelial cell markers CK15 and p63, that was lost during experimental corneal failure. The same research group has reported that subconjunctival injection of MSCs is less invasive and allows high-dose administration of MSCs besides being more efficacious (Galindo et al. 2021).

Evaluation of autologous BMSCs for the treatment of corneal stroma defects in rabbits showed interesting preliminary results. Animals were subjected to deep lamellar corneal dissections, and clinical outcomes were assessed via corneal reepithelization, corneal opacity, corneal thickness, and histology. Studies have shown that the use of BMSCs did not achieve complete transparency in the cornea. It has been suggested that remodeling the corneal stroma to fully restore the original optical qualities requires a longer time ranging from several months to several years.

The authors noted that locally transplanted BMSCs could be a valuable source of corneal stroma regeneration (Then et al. 2017).

The beneficial therapeutic effect of isolated primary human corneal endothelial cell transplantation on a preclinical model of bullous keratopathy in rabbits has shown promising results. Primary corneal endothelial cell transplantation gradually reduces the thickness of the cornea during the first 2 weeks. It completely restores it to a thickness of approximately 400 microns by the third week of transplantation. In contrast, the cornea of control rabbits remained significantly thicker, more than 1000 microns ($p < 0.05$) throughout the study. This study data showed that the use of primary human corneal endothelial cells opens up excellent prospects in the clinic (Peh et al. 2017).

In addition to experimental animal models, it has been highlighted that veterinary patients, such as dogs, are increasingly recognized as critical translational models of human diseases. For example, the etiopathogenesis of canine diseases is similar to that of humans (Brown 2016). Therefore studies of keratoconjunctivitis in dogs can help to develop therapeutic approaches which may also benefit people. Researchers have evaluated the intralacrimal transplantation of allogeneic MSCs in dogs with mild, moderate, and severe dry conjunctivitis. The data retrieved showed that allogeneic transplantation of MSCs on dogs was safe as no side-effects were observed with allogenic MSCs. The authors also showed improvements in the condition of the treated eyes. Experiments have shown that the use of MSCs is effective and safe for experimental dogs and, therefore, this type of treatment may be used in clinics after additional tests (Bittencourt et al. 2016).

In another canine study, this time in corneal wounds, the therapeutic efficacy of MSCs was tested in dogs. Previously, the experimental animals had been diagnosed with deep corneal ulcers, and for the duration of the experiment, the animals did not receive any immunomodulatory drugs. The researchers noted that all dog owners had signed written consent before this experimental procedure was started. Moreover, all owners were fully informed that the safety, complications, and effectiveness of cell implantation in corneal ulcers were unknown and not very well-established. The effectiveness of the treatment was evaluated using ophthalmological examination on three occasions, once prior to MSC therapy, then on days seven and 14 following their respective treatment. The tests included criteria such as clinical signs and the size and depth of the ulcers. MSCs were administered sub-conjunctively at a dose of three million cells. Studies have shown that the effectiveness of treatment was 84.6%, that is, the healing was observed in 22 of the 26 experimental dogs (Falcão et al. 2019).

Keratoconjunctivitis, or dry eye syndrome, is mainly an immuno-mediated degenerative disease directly affecting patients' vision and quality of life; it is one of the leading causes of ocular diseases in both dogs and humans. Dogs are excellent animal models for facilitating the understanding of this disease. One trial used 22 animals that were already unwell to study the effectiveness of MSCs. MSCs were administered locally and the treatment effectiveness was evaluated based on clinical signs, ophthalmological tests, histology, and immunohistochemistry. The work showed that the introduction of MSCs improved the condition of the cornea in

experimental dogs, and relapses were only observed in seven of the 22 treated dogs (Sgrignoli et al. 2019).

3 Models of Lens Diseases

The structure of the human lens is reflected in Fig. 1.

A cataract is a pathological condition associated with the opacity of the eye lens which causes varying degrees of visual impairment up to complete loss of vision. A cataract is one of the problems that affect the clarity of vision and can be caused by a number of factors, including aging, eye injuries, inflammation, and other circumstances. According to the latest estimate, cataract occurrence is responsible for 51% of the world's blindness, affecting around 20 million people. An effective way to treat a cataract is surgery; the natural lens of the eye is removed and replaced with an artificial lens (Snellingen et al. 2002).

Maleki (2015) induced lens damage in rats and rabbits via administration of sodium selenite. They conducted research to identify the therapeutic properties of SCs derived from Wharton's jelly by introducing SCs into the lens via surgical manipulation. The effectiveness of this treatment was evaluated by determining the expression of βB1 and βB3-crystallin genes and by evaluating the lens' morphological ultrastructure by electron microscopy. Studies have shown that the SCs obtained from Wharton's jelly, differentiated into the lens fibers and then restored lens structure within the capsule. The authors suggested that this method of cataract treatment could be applied in clinical practice following additional safety parameter studies (Maleki 2015).

To evaluate the complications associated with the development of type 2 diabetes pathologies, the effectiveness of ADMSC was investigated. This work illustrated the

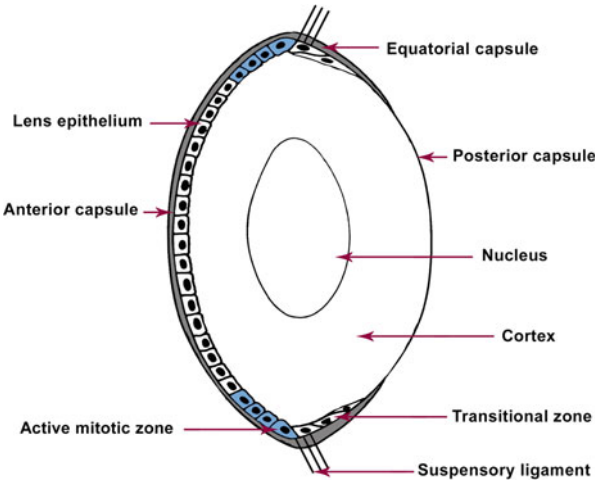

Fig. 1 Diagram showing the structure of the human lens

positive effects on various body systems in the experimental rats, including the lens of the eye. In the group of animals that received ADMSC intravenously, lens opacification was not observed, results which contrasted to those observed in the control group (Yu et al. 2019).

A new surgical method for the treatment of cataracts, which consisted of preserving endogenous lens progenitor cells (LEC), was developed as it was thought that the cells might provide functional regeneration of the lens in rabbit and macaque models. Although this method is conceptually different from modern practice, since it preserves the endogenous LEC and their natural environment as much as possible, it also restores the functionality of the lens (Haotian et al. 2016).

4 Retinal Models

Retinal diseases are one of the leading causes of blindness in the modern world. Previous research has shown that many retinal diseases observed in animals are similar to those seen in people. This work has primarily led to an improved understanding of the pathogenesis of retinal diseases and has also provided the means to test possible treatments, including cell therapy.

In the diseases of the external retina, such as age-related macular degeneration (AMD) and diabetic retinopathy (DR), two of the most common causes of blindness in the developed countries, the main body of in vivo models are those showing abnormal retinal angiogenesis or damage to the retinal pigment epithelium (RPE) (Liu et al. 2017).

One model with induced choroid neovascularization (CNV) caused by a laser was used to study the therapeutic effect of bone marrow-derived cells. The intravenously administered MSCs penetrated the damaged area and differentiated into several cell types and participated in the development of neovascularization without stagnation in other organs, thereby suppressing the growth of choroid neovascularization (Hou et al. 2010). Li and colleagues also used laser photocoagulation to induce CNV to investigate whether subretinal transplantation of RPE expressing Fbln5 could suppress CNV in vivo. One week after laser-induced CNV, RPE cells were transplanted into the subretinal space, with the pZlen-Fbln5-IRES-GFP vector placed in the right eye and the pZlen-IRES-GFP vector in the left eye. CNV was then evaluated using fundus photography, fundus fluorescence angiography, and hematoxylin and eosin staining. CNV appeared 1 week after photocoagulation and reached a peak of activity after 3 weeks. The transplanted RPE cells survived for at least 4 weeks and migrated to the retina. Subretinal transplantation of RPE cells, with Fbln5 expression, resulted in a significant reduction in the total lesion area. The researchers concluded that subretinal transplantation of RPE cells with Fbln5 expression inhibited laser-induced CNV in rats and therefore, represented a promising therapy for this condition (Li et al. 2015).

Neovascularization has been reproduced in a diabetes model induced by the administration of STZ in rodents. It was hypothesized that multipotent perivascular progenitor cells derived from human embryonic stem cells (hESC-PVPC) would

improve the damaged retinal vasculature. Indeed a single intravitreal injection into Brown Norway diabetic rats caused the localization of cells in distinct perivascular areas of the retinal vasculature and stabilized the rupture of the hemato-retinal barrier. This study shows the therapeutic potential of hESC-PVPCs in diabetic retinopathy by imitating the role of pericytes in vascular stabilization. The researchers highlighted that this study represents a simple method for creating perivascular progenitor cells from human embryonic stem cells. These cells have common functional characteristics with pericytes, which are irreparably lost at the onset of diabetic retinopathy. Animal studies have shown that replenishing damaged pericytes with perivascular progenitor cells can restore the integrity of retinal vessels and prevent loss of vitreous fluid. These data provide promising and compelling evidence that perivascular progenitor cells could be used as a novel therapeutic agent to treat patients with diabetic retinopathy (Kim et al. 2016).

The effectiveness of BMSCs in diabetic retinopathy was studied in a streptozotocin (STZ) model in male albino Wistar rats. After 3 months of induced diabetes, the right eye was intravitreally injected with green protein-labeled guidelines and the left eye was injected with a balanced saline solution. The introduction of BMSCs increased retinal gliosis in the diabetic group compared to the control group of animals. Immunofluorescence analysis revealed that BMSCs mainly integrated into the inner retina. Overall, this study showed that intravitreal administration of BMSCs improved visual function (Çerman et al. 2016). Attention is currently being paid to models with photoreceptor damage from retinitis pigmentosa and various hereditary disorders of central vision. It should be noted that these animal models are a target for the transplantation of early photoreceptors or RPE cells because RPE cells are responsible for protecting the function of photoreceptors.

The efficacy and safety of human retinal progenitor cells (hRPCs) in experimental RCS rats with dystrophy have also been researched. The hRPCs have been tested to maintain visual function and evaluation was undertaken using optokinetic head tracking and retinal structure studies. The safety of the NPHR was evaluated by subretinal cell transplantation into rats and wild-type mice of the bodily lower-III stages with analysis at 3, 6, and 9 months after transplantation, respectively. Studies have shown that the optimal dose of hRPCs for preserving visual function and retinal structure in dystrophic rats was between 50,000 and 100,000 cells. The human retinal progenitor cells integrated and survived in the retinas of dystrophic and wild-type rats up to 6 months after transplantation. No signs of tumors were detected. Therefore human retinal progenitor cells appear to be safe and effective in this preclinical model. It has been shown that they can be used in the early stages of retinal degeneration and the areas of intact retina without the risk of adverse effects on visual function (Semo et al. 2016).

Qu, Linghui et al. (2017) studied the effects of both hRPCs and BMSCs in a different disorder, retinitis pigmentosa (RP). The researchers have noted that both hRPCs and human BMSCs (hBMSCs) are widely and practically used for transplantation. In this study, the possibility of using combined transplantation of the above cells was tested. The researchers transplanted hRPCs and hBMSCs into the subretinal space of RCS rats. Studies have shown that combined hRPCs/hBMSCs

transplants supported electroretinogram results much better than single transplants. The thickness of the outer nuclear layer also showed the best results during combined transplantation. It was also recorded that the cells in the combination treatment migrated better than single transplantation. Photoreceptor differentiation of cells in the retina rats treated with combined cell transplantation also showed a higher ratio than those treated with a single type cell transplant. Finally, microglial activation and Muller cell gliosis were more effectively suppressed following a combined transplantation protocol, indicating better immunomodulatory and anti-glial effects. The researchers stated that the combination of hRPC and hBMSC transplantation was a more effective strategy for treating SC-based retinal degenerative diseases (Qu et al. 2017).

Substitution therapy in degenerative diseases such as age-related macular degeneration has also been investigated. A modified trans-scleral injection method has been developed that uses specific angles and needle depth to successfully and consistently deliver retinal pigment epithelial cells into the rat's subretinal space and prevent excessive retinal damage. Efficacy was demonstrated in RCS rats sustaining for 2 months (Zhao et al. 2017).

The efficacy of RPE cells obtained under xenon-free conditions from clinical and xenon-free human embryonic stem cells (OpRegen) after transplantation into the subretinal space of the RCS rats has also been characterized. The rats received a single subretinal injection of xeno-free RPE cells, whereas a physiological saline solution and non-operated eyes served as a control. Optomotor behavior tracking was used to evaluate functional effectiveness and recovery of photoreceptors, and survival of the transplanted cells was assessed using histology and immunohistochemistry. The subsequent studies showed that the outer nuclear layer (ONL) was significantly thicker in the eyes treated with the cells than in the controls. Transplanted RPE cells were identified in the subretinal space, and integration into the RPE monolayer was also revealed. It was proven that the OpRegen RPE cells survived and restored visual function and preserved rod and cone photoreceptors for an extended time period (McGill et al. 2017).

During subretinal transplantation of RPE-derived stem cells (RPESCs) and RPE cells (RPESCs-RPE), the preserved vision was observed in a model of RPE cell dysfunction in RCS rats. The researchers noted that the stage of differentiation that the RPESCs-RPE had acquired before transplantation significantly affected the effectiveness of vision restoration. While the cells at all tested stages of differentiation protected photoreceptor layer morphology, the intermediate stage of RPESCs-RPE differentiation, obtained after 4 weeks of culture, was more consistent in protecting vision than the subsequent generation that differentiated during culture weeks 2 or 8 (Davis et al. 2017).

Park and colleagues used an experimental rat model of retinal degeneration developed by an intravenous injection of sodium iodate. Human embryonic stem cells (hESCs) as a therapeutic agent for treating degenerative retinal diseases and proposed to differentiate the cells into retinal pigment epithelium (RPE) using particular culture conditions. The putative RPE cells (10^5 cells/5 μl) were transplanted into the subretinal space. The animals were killed at either 1, 2, or 3 weeks

after transplantation for immunohistochemistry to check transplanted cell survival and their fate. The putative RPE cells derived from hESCs had the morphological characteristics of human RPE cells. The implanted RPE cells survived in the subretinal space for up to 4 weeks post-transplantation, and the expression of RPE markers was confirmed via immunohistochemistry. Thus, the researchers showed the potential of using hESC-derived RPE cells for cellular therapy of retinal degenerative disease (Park et al. 2011).

In another model of retinal degeneration, Rd1 mice were used to replicate rapid progressive retinitis pigmentosa with end-stage retinal degeneration. Development of a structured outer nuclear layer with internal and external segments during the transplantation and integration of three-dimensional retinal tissue derived from mouse embryonic stem cells or induced pluripotent stem cells were investigated. The resulting retinal sheets were transplanted into the subretinal space of mice aged 6–8 weeks and sacrificed from 2 weeks to 6 months after transplantation. The transplanted retinal sheets obtained from both mESCs and iPSCs survived and matured in the highly degenerated retinas of the experimental animals. This study is a "proof-of-concept" for retinal transplantation in late retinal degenerative diseases (Assawachananont et al. 2014).

The therapeutic potential of photoreceptor precursors derived from human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs) have been studied to confirm their potential in retinal degeneration. ESCs and iPSCs were cultured in four stages under certain conditions, resulting in a somewhat homogeneous population of photoreceptor-like progenitors. After transplantation into the experimental mice model of end-stage retinal degeneration, these cells differentiated into photoreceptors and formed a cell layer associated with the host retinal neurons. Visual function in the animals of the experimental group was partially restored, as evidenced by visual behavioral tests. In addition, the degree of functional improvement was positively correlated with the number of implanted cells. The data obtained confirmed the potential of ESCs and iPSCs for photoreceptor replacement therapy aimed at photoreceptor regeneration in retinal diseases (Barnea-Cramer et al. 2016).

An experimental mouse model of severe human retinitis pigmentosa at a stage of loss native cells showed that transplanted rod precursors could transform an anatomically distinct and appropriately polarized outer nuclear layer. The three-layer organization was returned to rd1 mice that had only two retinal layers before treatment. The transplanted progenitors could integrate and develop in the degenerated retina and transformed into mature rods with light-sensitive outer segments connected to neurons. The visual function in these experimental animals was also restored after cell transplantation. Studies have shown that cell therapy can restore the light-sensitive cell layer de novo and, consequently, restore the structurally damaged visual contour (Singh 2013).

Diseases of the internal retina, such as glaucoma and various forms of optical neuropathy (ischemic, traumatic, and compression), have common characteristics of retinal ganglion cell loss (RGC). Experimental animal models that focus directly on RGC damage do not necessarily reproduce the pathogenesis of individual

conditions; however, they are widely used because of their ability to demonstrate dramatic changes in RGC function in many different animal types. Damage to the optic nerve is one of the frequently used preclinical methods to reproduce the loss of RGCs. This model was used to study RGC stimulation as a way to regenerate axons along the entire length of the visual pathway and further in the lateral geniculate nucleus, the upper mound, and other visual centers. Notably, regeneration partially restored the optomotor response, demonstrating the possibility of restoring the central vision circuit after optic nerve damage in adult mammals (de Lima et al. 2012).

The therapeutic potential of MSCs injected into the vitreous body, also on a model of optic nerve damage, has been demonstrated. Adult (three to five-month old) Lister hooded rats underwent unilateral optic nerve crushing followed by an injection of MSCs into the vitreous. Before injection, MSCs were labeled with a fluorescent dye or superparamagnetic iron oxide nanoparticles, which enabled cell tracking and their post-engraftment fate determination in vivo using magnetic resonance imaging. Retinal ganglion cell survival was evaluated at 16 and 28 days after the injury. The researchers noted that the transplanted MSCs remained in the vitreous body and were detected in the eye for several weeks. The MSCs-based therapy's success was further explained by an increase in FGF-2 expression in the retinal ganglion cell layer, and the concomitant expression of interleukin-1β. Thus, studies have shown that MSCs protect RGCs and stimulate axon regeneration following optic nerve damage (Mesentier-Louro et al. 2014).

Evaluation of human UMSCS to protect and promote the regeneration of axotomized neurons in the rat optical system "UMSK" has also been undertaken. The cells survived well up to 2 weeks post-transplantation; however, they did not significantly migrate significantly or differentiate. In the presence of UMMC grafts, it was found that the axonal processes of the host were present at the site of the lesion, and there was a stimulation of the population of endogenous neural progenitors. Four weeks after the transplant, UMMC was shown to have a neuroprotective effect, protecting a significant percentage of axotomized retinal ganglion cells. Further experiments showed that UMSCS could also promote the re-growth of axotomized RGCs (Zwart et al. 2009).

5 Model Damage to the *N*-methyl-d-aspartate Receptor (NMDA)

Glutamate excitotoxicity is a critical component of selective neuronal cell death in ischemic retinopathy and glaucoma. This process is associated with excessive stimulation of NMDA receptors in retinal ganglion cells, which leads to an intracellular influx of calcium ions, and this, in turn, leads to the activation of the apoptotic cascade. Experimental animal models using glutamate excitotoxicity focus on NMDA receptor stimulation have been developed and optimized (Casson 2006). This was used to study a constructed eye-like structure derived from ESCs consisting of retinal neural clone cells, RPEs, and lens cells to determine whether they could

differentiate into retinal ganglion cells (RGC) during retinal transplantation into the vitreous body of a damaged adult mouse (Aoki et al. 2008). Prior to this, it was shown that the cells of these eye-like structures could integrate into the developing visual bubble of chickens. ESCs were induced to differentiate into eye-like structures in vitro for 6 or 11 days. NMDA was then injected into the eyes of the mouse recipients to damage the RGC before transplantation. Cell material for transplantation was extracted from eye-like structures and transplanted into the vitreous body of both the damaged and control eyes. During the follow-up, the eyes were analyzed both qualitatively and quantitatively by immunohistochemistry 10 days or 8 weeks after transplantation. Studies showed that cells from eye-like structures derived from ESCs had integrated into the RGC layer and differentiated into neurons when transplanted into control (non-NMDA-treated) eyes; however, they rarely expressed RGC markers. Once transplanted into NMDA-treated eyes, the cells spread across the surface of the retina and covered a relatively large area of the host's RGC layer, which was damaged by NMDA. Eye cells derived from ECU often differentiated into cells expressing RGC -specific markers and formed a new layer of RGC. In addition, it was observed that a small number of these cells, originating from the ESC, expanded the axon-like processes in the direction of the optic disc. However, visually induced responses could not be recorded from the visual cortex (Aoki et al. 2008).

Transplantation of neural progenitors, derived from ECU (Es-NP) into *N*-methyl-D-aspartate (NMDA)-injected mouse models, ablated RGA in mouse models, and preclinical glaucoma (dBA/2D) models with consistently higher intraocular pressure (IOP). Visual acuity and functional integration were assessed using behavioral tests and immunohistochemistry, respectively. Studies have shown that ES-NP, GP-expressing, transplanted into mice with depleted RGA, which were injected with NMDA, differentiated into a clone of RGA. Improvement in visual acuity was observed 2 months after transplantation (Divya et al. 2017).

The effects of allogeneic Muller cat glia transplantation (fMGSC) with the ability to differentiate into cells expressing RGC markers after RGC removal using *N*-methyl-d-aspartate (NMDA) have also been investigated. In contrast to previous observations in rats, transplantation of hMGSC-derived RGCs into the vitreous body of cats formed aggregates and caused a severe inflammatory response without improving visual function. In contrast, allogeneic transplantation of feline MGSC (fMGSC)-derived RGC into the vitrectomized eye improved the threshold scotopic response (STR) of the electroretinogram (ERG). Despite the functional improvement, the cells did not attach to the retina. Also they did not form aggregates on the peripheral remains of the vitreous, suggesting that the vitreous may represent a barrier to cell attachment to the retina. This has been confirmed by observations that cell scaffolds of compressed collagen and enriched RGC preparations derived from fMGSC facilitate cell attachment. Although the cells did not migrate to the RGC layer of the optic nerve, they significantly improved the STR and photopic negative ERG response, suggesting enhanced RGC function. These results suggest that fMGSCS have a neuroprotective ability that promotes partial restoration of impaired RGC function and indicate that cell attachment to the retina may be

necessary for transplanted cells to provide retinal neuroprotection (Becker et al. 2016).

6 Models of Transient Ischemia

Transient ischemia models cause loss of RGC by occlusion or functional inhibition of the blood vessels supporting the optic nerve and retina, often followed by reperfusion of these vessels – hence named "transient" ischemia. One such model induced retinal ischemia in adult Wistar rats by increasing IOP to 130–135 mmHg for 55 min. Twenty-four hours after the induction of ischemia, BMSCs were injected into the vitreous body. Functional recovery was assessed after 7 days using electroretinography (ERG) measurements of a-wave, b-wave, P2, scotopic threshold response (STR), and oscillatory potentials (OP). Retinal damage and anti-ischemic effects were quantified by measuring apoptosis, autophagy, inflammatory markers, and the permeability of the blood-brain barrier of the retinal. BMSC distribution was qualitatively investigated using real-time fundus images. The introduction of the guiding compound into the vitreous body significantly improved the recovery of ERG a-and b-waves, OP of negative STR and P2, and also reduced apoptosis, as evidenced by a decrease in the levels of TUNEL protein and caspase-3. BMSCs significantly increased autophagy, reduced inflammatory mediators (TNF-α, IL-1β, IL-6), and retinal vascular permeability. BMSCs were preserved in the vitreous and were also observed in the ischemic retina. The results showed that intravitreal injection of PCRS protected the retina from ischemic damage in a rat model. (Mathew et al. 2017).

Li and Zhao (2014) used this model to study the effect of retinal progenitor cell (RPC) transplantation into the subretinal space (SRS) and the superior colliculus (SC) in rats (Li et al. 2014). For transplantation, cultured postnatal rat NPCs were used for 1 day, transfected with an adeno-associated virus-containing cDNA encoding enhanced green fluorescence protein (EGFP). The damage was caused by an increase in intraocular pressure to 110 mmHg for 60 min. The effectiveness of transplantation was evaluated using ERG immunohistochemistry. The transplanted cells survived for at least 8 weeks, migrated to surrounding tissues, and improved ERG responses in the rats with ECP damage. The data obtained showed that RPC transplantation in SRS and SC may be a possible method of cell replacement therapy for retinal diseases (Li et al. 2014).

7 Transgenic RGC Loss Models

Although they are more challenging to maintain and breed than traditional models, transgenic mouse models using the Cre-Lox system can provide an animal with pure RGC depletion for stem cell research. One example is the Pou4f2 knockout mouse (Brn3b). Pou4f2 is a critical gene for RGC differentiation and is expressed throughout life (Cho et al. 2012). This research group developed a mouse model in which

RGC is genetically removed in adult mice. These mice were transplanted with a GFP-labeled progenitors of the embryo's retina. These retinal cells were injected into the vitreous body of one eye of mice between the ages of 2 and 6 months. Gene expression analysis using an immune tag and the morphology of the optic nerves both visually and using histology were evaluated. The embryonic GFP-labeled RPCs were successfully introduced into the eyes of transgenic mice. Many transplanted RPCs penetrate the ganglion cell layer, and several GFP-labeled cells were found within the optic nerves. At the same time, a significant increase in optic nerve thickness was noted and more connected axons were observed in the retina following RPC injection. The results suggest a new approach to the regeneration of damaged optic nerves. They indicate that a significant number of RPCs toxin differentiate into RGC in the alien environment of the adult retina (Cho et al. 2012).

8 Model of Experimental Autoimmune Encephalopathy (EAE)

The EAE model primarily models the diseases that cause autoimmune demyelination in the central nervous system, namely multiple sclerosis (MS). Loss of RGC precedes inflammation of the optic nerve in rat models of chronic EAE, whereas, in mice, optic neuritis comes first, and loss of RGC follows. The typical induction time is 1–2 weeks. Current stem cell research on EAE models has focused on neuroprotection via neural or mesenchymal stem cells rather than directly replacing RGCs or their precursors (Pluchino et al. 2009; Lanza et al. 2009).

9 Models of Hereditary Diseases Associated with Optic Nerve Damage

Retinal degeneration resulting from hereditary diseases such as Leber's hereditary optic neuropathy (LHON) and type I neurofibromatosis (NF1) has no definitive treatment options to date. There are animal models of LHON (Yu et al. 2015). One rotenone-induced LHON model was applied to investigate the protection of visual function in stem cells. Photoreceptor progenitor cells were used, which, according to research, integrate into the ganglion cell layer (GCL). The results of the work were evaluated using magnetic resonance imaging with manganese enhancement, optokinetic responses, and ganglion cell counting. The cultured progenitor cells integrated into the GCL and positive affected maintaining retinal function (Mansergh et al. 2014).

10 Glaucoma Models

Open-angle glaucoma is the most common glaucoma subtype (Weinreb et al. 2016). It is characterized by an imbalance between the production of water from the ciliary body and its outflow through the trabecular network and uveoscleral pathways. This discrepancy can lead to increased intraocular pressure, resulting in damage to the RGC, as evidenced by pathological changes. Hao and co-authors (2013) studied the therapeutic effects of executive staff transplantation on vision loss in older rats with glaucoma caused by laser ocular hypertension. The BMSCs contributed to the survival of retinal ganglion cells in the transplanted eyes compared to control eyes. In addition, in swimming tests based on visual cues, rats with a leadership graft performed significantly better. The overall results of the study showed that BMSC transplantation therapy is effective in the treatment of older rats with glaucoma (Hao et al. 2013). An experimental model of photocoagulation of episcleral veins and limbal plexus with an argon laser has been used to study the possibility of retinal stem cell (RSC) transplantation and immunization with glatiramer acetate copolymer-1 (COP-1) copolymer. Rats were immunized with (COP-1) on the same day as glaucoma induction by photocoagulation. RSCs were cultured and transplanted intravitreally a week after laser treatment. The effectiveness of treatment was evaluated based on the expression of brain neurotrophic factor (BDNF), insulin-like growth factor I (IGF-I), and immunohistochemical studies, RT-PCR, and Western blotting were also performed. RGC survival was assessed by TUNEL staining and RGC counts. Their studies showed that the expression of BDNF neurotrophic factors in the IRF-I and RSC/COP-1 group was significantly higher than in the other groups ($P < 0.05$). In addition, the number of apoptotic RGS in the RSC/COP-1 group was markedly lower and the number of RGS in the RSC/COP-1 group was higher. Thus, the researchers concluded that the combined action of RSC and immunization with COP-1 protect RGC against apoptosis in a rat glaucoma model (Zhou et al. 2013).

Harper et al. (2011) used an experimental rat model of chronic ocular hypertension induced by laser cauterization of the trabecular network and episcleral veins in the eyes to study the ability of engineered MSCs to produce and secrete brain neurotrophic factor (BDNF) to protect retinal function and structure after intravitreal transplantation. MSCs expressing BDNF (BDNF-MSC) were transplanted intravitreally. The function of the optic nerve and retina was evaluated using computer pupillometry and ERG; quantitative assessment of optic nerve damage was performed by RGCs counts and by evaluating cross-sections of the optic nerve. After BDNF-MSC transplantation, the eyes retained significantly more retinal and optic nerve functions and showed higher RGC preservation. Interestingly the neurotrophic factors BDNF b-MSCs, transduced by lentiviruses, could persist in the eyes with chronic hypertension and provide functional and structural protection of the retina and optic nerve (Harper et al. 2011).

An ocular hypertension model for the induction of glaucoma, which was performed by cauterizing three episcleral veins (EVC) of the eyes of male Long-Evans rats, has also been utilized. One study investigated the potential of MSCs

therapy in this model and deciphered the in vitro effects of MSCs on primary cells within the human trabecular network. BMSCs were labeled with nanocrystals of quantum dots for post engraftment tracing of the cells' fate. The cells were injected in the anterior chamber of the eye for 20 days, with eye pressure monitored twice a week for 4 weeks. At the end of the experiment, the cell distribution in the anterior segment was examined by confocal microscopy on flat corneas. In addition, the effects of the BMSCs-conditioned medium on the primary cells of the trabecular network were tested in vitro. The work showed a long-lasting and rapid effect obtained following BMSCs transplantation in vivo. Injection of BMSCs into the anterior chamber of the eye in a rat model provides a neuroprotective effect in the pathophysiology of glaucoma. These results demonstrate that BMSCs represent a promising tool to treat ocular hypertension and retinal cell degeneration (Roubeix et al. 2015).

In ocular hypertension induced by an intra-chamber injection of hyaluronic acid into the anterior chamber of rats, the neuroprotective effects of BMSCs and ADMSCs, which were transplanted intravitreally, were investigated. MSCs labeled with green fluorescent protein were transplanted intravitreally 1 week after induction. At the end of the second and fourth weeks, retinal ganglion cells were visualized using the flat retinal attachment method and evaluated using immunofluorescence staining. The results of these studies showed that the number of retinal ganglion cells present was significantly higher when compared to untreated animals. The results of the immunohistochemical analysis showed that a limited number of SCS were integrated into the ganglion cell layer and the inner nuclear layer. The number of cells expressing pro-inflammatory cytokines (interferon-γ and tumor necrosis factor-α) in the group receiving MSCs decreased. On the other hand, the expression of Il-1Ra and prostaglandin E2 receptors had increased. It was concluded that intravitreal MSC transplantation had a neuroprotective effect when the experimental model was reproduced in rats (Emre et al. 2015).

11 Animal Models of Primary Angle-Closure Glaucoma (PACG)

Primary angle-closure glaucoma, in most cases, is a chronic disease with the gradual appearance of visual symptoms, which differs from open-angle glaucoma only in the degree of angle closure. Possible improvements in visual function by transplanting neural progenitors derived from ESCs were evaluated in a mouse model with glaucoma. Neural progenitors originating from ESCs (ES-NP) were transplanted in a model of preclinical glaucoma (DBA/2 J) with a persistently higher intraocular pressure (IOP). Transplantation experiments in dBA/2D mice showed no significant improvements in visual function, possibly due to the death of both host and transplanted retinal cells, which could be associated with high intraocular pressure. The results showed that strategies for controlling intraocular pressure are necessary for the enhanced survival of transplanted cells in glaucoma (Divya et al. 2017).

This chapter has given an overview of the past and present preclinical research undertaken into MSC-based therapies for ocular diseases. The potential future

research directions, complications observed to date, and exciting lines of research have been explored. Several studies have shown the efficacy and safety of MSC therapies in a number of ocular disorders and highlighted the mechanisms of action present within much of the in vitro and in vivo work conducted to date. The eye is a complex organ; replicating human ocular disorders remains a challenge and developing potential therapies is a complex and one of the most intricate and multifaceted challenges, but the one wherein progress is being made.

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