Chapter 10
Cell Delivery: Surgical Approaches

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Introduction

Retinal Degeneration and the Need of New Treatments

Retinal degenerative diseases as a group constitute one of the primary causes of permanent visual impairment, affecting millions of people worldwide. The effect of this group of conditions is debilitating with a major impact on a patient's daily life including difficulty in performing basic functions, deterioration of personal independence, and often an effect on mental health. Among the most prevalent retinal degenerative diseases

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are age-related macular degeneration (AMD) and inherited retinal dystrophies of which retinitis pigmentosa (RP) and Stargardt disease (STGD) are the commonest.

AMD represents the third leading cause of legal blindness and the most prevalent cause of permanent visual loss in the over 55 years age group worldwide [1]**.** RP constitutes the leading cause of inherited blindness estimated to affect approximately 1/4000 individuals [2], and STGD is the most common juvenile retinal degenerative disease, with a prevalence of 1/8000–10,000 young individuals. [3]

The eye has been identified as one of the most amenable organs to be targeted by the first generation of regenerative medicine techniques. It is easily accessible surgically, and there are multiple imaging modalities using only light sources which provide the ability to document structural and functional outcomes with minimal risk. Additionally, the eye and especially the vitreous and subretinal space is a relatively immune-privileged site, theoretically able to tolerate foreign antigens or non-histocompatible cells without eliciting an immune response. Hence, under normal circumstances the risk of tissue rejection after cell transplantation is reduced. Furthermore, it is a small organ, and the majority of retinal degenerative diseases initially target one type of cell (retinal pigment epithelial (RPE) cells, photoreceptors, ganglion cells, etc.), in a way that cell therapies can be focused on replacing one specific cell group by transplanting a relatively small number of cells. These advantages together with the invaluable combination of established surgical experience and current development in experimental retinal surgery have put retinal degenerative diseases at the forefront of cell-based clinical research.

In addition to the imaging and access advantages of the eye, progress in laboratory methods of differentiation and cultivation has increased the availability of various types of potentially therapeutic cells (Table 10.1). As a result, numerous clinical trials involving retinal and RPE transplantation have commenced worldwide, some of which show encouraging preliminary results, in terms of safety and possible efficacy (Tables 10.2, 10.3, and 10.4).

Therapeutic Formulations of Cell Therapies

Cell Suspension

A cell suspension consists of a liquid medium—usually balanced salt solution or other optimized aqueous medium—in which single cells or small aggregates of cells are floating. Ideally, the cells would have undergone differentiation, isolation, purification, and characterization, so that only the desired cell type is included in the suspension.

A cell delivery method in the form of suspension holds the major advantage that it requires a relatively minor surgical intervention. Cells can easily be injected in the intravitreal or subretinal space via small gauge cannulas, causing only minimal or no injury to the retina.

Currently the most common approach for implanting a cell suspension is subretinal delivery via the pars plana, i.e., the transvitreal route (Fig. 10.1c). This approach requires a standard pars plana vitrectomy and transretinal access to the

Table 10.1 Therapeutic cells: definitions and classification

Category	Definition
Stem cells (SC)	Cells in undifferentiated state, capable of infinite proliferation and able to differentiate into various cell types
Totipotent SC (a.k.a. omnipotent)	Cells capable of differentiation into both embryonic and extraembryonic cell types. Able to generate a complete, viable organism
Pluripotent SC	Cells capable of differentiation and tissue generation of any of the three embryonic germ layers, i.e., ectoderm, mesoderm, and endoderm
Multipotent SC	Cells capable of differentiation into limited cell types, able to generate tissue of a single germ layer
Oligopotent SC	Cells capable of differentiation into only a few cell types, e.g., myeloid, lymphoid SC
Unipotent SC	Cells capable of differentiation only into their own cell type, but retain ability to self-renew
Human embryonic SC (hESC)	Pluripotent SC obtained from a 5-day-old blastocyst
Induced pluripotent SC (iPSC)	Pluripotent SC obtained by adult somatic cells by dedifferentiation through genetic reprogramming
Mesenchymal SC (MSC)	Multipotent stromal cells capable of differentiation into variable cell types, i.e., chondrocytes, myocytes, adipocytes, and osteoblasts
Adipose derived SC (ASC)	Series of MSC derived from adipose tissue, capable of differentiation into endodermal, mesodermal, and ectodermal tissues
Human umbilical tissue-derived cells (hUTC)	Series of MSC derived from human umbilical cord tissue
Hunan retinal progenitor cells (hRPC)	Partially differentiated cells obtained from fetal neural retina, capable of differentiation into retinal cell, but not for infinite replication

subretinal space. Less common, but also less invasive is the intravitreal injection, which does not necessitate surgery in the operating room, but only a simple transscleral injection of the suspension into the vitreous cavity (Fig. 10.1b). Finally, a completely different method uses an "external" approach and a purpose-designed micro-catheter to deliver the cell suspension through the sclera and choroid into the subretinal space (Fig. 10.1a). A more detailed description of these methods will be given in the next section of this chapter.

Sheets/Patches

A cell sheet/patch transplant system consists of a biocompatible substrate or scaffold, seeded with the therapeutic cells in a way that they form a cellular monolayer (e.g., a RPE monolayer patch). The scaffold provides the supportive surface necessary for the cells to attach, proliferate, differentiate, and meet their structural and functional roles after transplantation. Additionally, the artificial membrane provides the required structural rigidity for the manipulations during the delivery process.

Table 10.2 Recent and current cell transplantation studies (Cell suspension approach)

Study ID	Study title	Phase	Sponsor/ collaborators	Location	Target disease	Cell source	Delivery approach	Registration date
NCT02467634	Study of HUCNS-SC Subretinal Transplantation in Subjects With GA of AMD (RADIANT)	II	StemCells, Inc.	Various retina and vitreous associates, Arizona, California, Illinois, Michigan, New York, Texas, Utah, US	Dry AMD	HuCNS-SC	Cell suspension—subretinal injection	Jun-2015
NCT02464436	Safety and Tolerability of hRPC in Retinitis Pigmentosa	I/II	ReNeuron Limited	Massachusetts Eye and Ear Infirmary, Massachusetts, US	RP	hRPC	Cell suspension—subretinal injection	May-2015
ChiCTR-ONB-15007477	Clinical study of subretinal transplantation of human bone marrow mesenchymal stromal cells with or without embryonic retinal progenitor cells in treatment of retinal pigmentosa	New Treatment Measure Clinical Study	Southwest Hospital, Shapingba District, Chongqing, China. National Basic Research Program (973 Program)	Southwest Hospital, Third Military Medical University, Shapingba District, Chongqing, China	RP	hBMMSC (Human bone marrow mesenchymal SC) with or without RPC	Cell suspension—subretinal injection	Oct-2015

ChiCTR-OCB-15007054	Clinical study of subretinal transplantation of clinical human embryonic stem cells derived retinal pigment epitheliums in treatment of dry age-related macular degeneration diseases	New Treatment Measure Clinical Study	Institute of Zoology, Chinese Academy of Sciences	Beijing Tongren Hospital, Capital Medical University, China	Dry AMD	hESC-RPE	Not Provided	Sep-2015
ChiCTR-OPC-15006757	Treatment of Dry Age-related Macular Degeneration using fetal retinal pigment epithelium	I	The First Affiliated Hospital of Nanjing Medical University	The First Affiliated Hospital of Nanjing Medical University, China	Dry AMD	Fetal RPE	Not Provided	Jul-2015
ChiCTR-OCB-15006423	Clinical study of subretinal transplantation of human embryo stem cell derived retinal pigment epitheliums in treatment of macular degeneration diseases	New Treatment Measure Clinical Study	Southwest Hospital, Shapingba District, Chongqing, China	Southwest Hospital, Shapingba District, Chongqing, China	Macular Degeneration Diseases	hESC-RPE	Not Provided	May-2015

(continued)

Table 10.2 (continued)

Study ID	Study title	Phase	Sponsor/ collaborators	Location	Target disease	Cell source	Delivery approach	Registration date
NCT02320812	Safety of a Single, Intravitreal Injection of Human Retinal Progenitor Cells (jCell) in Retinitis Pigmentosa	I/II	jCyte, Inc.	The Gavin Herbert Eye Institute, Univ California Irvine Retina-Vitreous Associates Medical Group, LA, California, US	RP	hRPC	Cell suspension—intravitreal injection	Dec-2014
NCT02286089	Safety and Efficacy Study of OpRegen for Treatment of Advanced Dry-Form Age-Related Macular Degeneration	I/II	Cell Cure Neurosciences Ltd.	Hadassah Ein Kerem University Hospital, Israel	Dry AMD	hESC-RPE	Cell suspension—subretinal injection	Nov-2014

NCT02280135	Clinical Trial of Intravitreal Injection of Autologous Bone Marrow Stem Cells in Patients With Retinitis Pigmentosa	I	Red de Terapia Cellular, Spanish NHS, Hospital Universitario Virgen de la Arrixaca, Fundacion para la Formacion e Investigacion Sanitarias de la Region de Murcia Public Health Service, Murcia, Instituto Murciano de Investigación Biosanitaria Virgen de la Arrixaca	Clinical Universitary Hospital Virgen de la Arrixaca, Murcia, Spain	RP	Autologous BMSC	Cell suspension—intravitreal injection	Oct-2014
NCT02122159	Research With Retinal Cells Derived From Stem Cells for Myopic Macular Degeneration	I/II	Ocata Therapeutics, University of California	University of California, LA, US	Myopic Macular Degeneration	hESC-RPE	Cell suspension—subretinal injection	Apr-2014

(continued)

Table 10.2 (continued)

Study ID	Study title	Phase	Sponsor/ collaborators	Location	Target disease	Cell source	Delivery approach	Registration date
NCT01920867	Bone Marrow Derived Stem Cell Ophthalmology Treatment Study (SCOTS)	Not Provided	Retina Associates of South Florida/ MD Stem Cells	Retina Associates of South Florida, Florida, US; Al Zahra Private Hospital, United Arab Emirates	Retinal Disease, Macular degeneration, Hereditary retinal dystrophy, Optic nerve disease, glaucoma	Autologous BMSC	Cell suspension—retrobulbar, subtenon, intravitreal, intraocular, subretinal and intravenous injection	Aug-2013
NCT01914913	Clinical Study to Evaluate Safety and Efficacy of BMMNC in Retinitis Pigmentosa	I/II	Chaitanya Hospital, Pune	Chaitanya Hospital, Pune, India	RP	Autologous BMMNSC (mononuclear cells)	Cell suspension—Intravitreal injection	Jul-2013
NCT02016508	Intravitreal Injection of Human Bone Marrow Derived Mesenchymal Stem Cell in Patients With Dry Age-related Macular Degeneration (AMD)	I/II	Al-Azhar University	Al-Azhar University, Egypt	Dry AMD	BMSC	Cell suspension—intravitreal injection	May-2013

NCT01674829	A Phase I/IIa, Open-Label, Single-Center, Prospective Study to Determine the Safety and Tolerability of Sub-retinal Transplantation of Human Embryonic Stem Cell Derived Retinal Pigmented Epithelial (MA09-hRPE) Cells in Patients With Advanced Dry Age-related Macular Degeneration (AMD)	I/IIa	CHABiotech CO., Ltd.	CHA Bundang Medical Center, Korea	Dry AMD	hESC-RPE	Cell-suspension—subretinal injection	Aug-2012
NCT01736059	Clinical Trial of Autologous Intravitreal Bone-marrow CD34+ Stem Cells for Retinopathy	I	University of California, Davis	University of California, Davis, California, US	Dry AMD, DR, RVO, RP, Hereditary Macular Degeneration	BMSC	Cell suspension—intravitreal injection	Jul-2012

(continued)

Table 10.2 (continued)

Study ID	Study title	Phase	Sponsor/ collaborators	Location	Target disease	Cell source	Delivery approach	Registration date
NCT01632527	Study of Human Central Nervous System Stem Cells (HuCNS-SC) in Age-Related Macular Degeneration (AMD)	I/II	StemCells, Inc.	Retina-Vitreous Associates Medical Group and Byers Eye Institute at Stanford, California; New York Eye and Ear Infirmary, New York; Retina research Institute of Texas and Retina Foundation of the Southwest, Texas, US	Dry AMD	HuCNS-SC (Human neural stem cells)	Cell suspension—subretinal injection	Jun-2012
NCT01625559	Safety and Tolerability of MA09-hRPE Cells in Patients With Stargardt Macular Dystrophy	I	CHABiotech CO., Ltd.	Republic of Korea	Stargardt Macular Dystrophy	hESC-RPE	Cell suspension—subretinal injection	Jun-2012

NCT01531348	Feasibility and Safety of Adult Human Bone Marrow-derived Mesenchymal Stem Cells by Intravitreal Injection in Patients With Retinitis Pigmentosa	I	Mahidol University, Ministry of Health, Thailand	Siriraj Hospital Mahidol University, Bangkok, Thailand	RP	BMSC	Cell suspension—intravitreal injection	Feb-2012
NCT01344993, NCT02463744 (Dry AMD) and NCT01345006 NCT02445612 NCT01469832 (Stargardt)	Safety and tolerability of sub-retinal transplantation of hESC derived RPE (MA09-hRPE) cells in patients with advanced dry age related macular degeneration (Dry AMD) and Stargardt Macular Dystrophy	I/II	Ocata Therapeutics Inc. (Formerly: Advanced Cell Technology Inc. ACT), Astellas Institute for Regenerative Medicine	Jules Stein Eye Institute, California; Bascom Palmer Eye Institute, Florida; Mass Eye and Ear, Massachusetts; Wills Eye Institute, Pennsylvania, US	Dry AMD, Stargardt Macular Dystrophy	hESC-RPE	Cell suspension—subretinal injection	Apr-2011

(continued)

Table 10.2 (continued)

Study ID	Study title	Phase	Sponsor/ collaborators	Location	Target disease	Cell source	Delivery approach	Registration date
NCT01226628	A safety study of CNTO 2476 in patients with age-related macular degeneration	I	Janssen Research & Development, LLC	Arcadia, California, Philadelphia, Pennsylvania, US	Dry AMD	hUTC	Cell suspension—subretinal injection	Oct-2010
CTRI/2010/091/00639	A Clinical Trial to Evaluate the Effect of Bone Marrow Derived Stem Cell in Diseases Like Dry Age Related Macular Degeneration and Retinitis Pigmentosa	I	Indian Council of Medical Research, India	All India Institute of Medical Sciences, Ansari Naga, India	Dry AMD, RP	BMSC (mononuclear cells)	Cell suspension—intravitreal injection	May-2010
NCT01560715 NCT01068561 NCT01518127 and NCT01518842	Intravitreal Bone Marrow-Derived Stem Cells in Patients With RP Macular Degeneration and Ischemic Retinopathy	I	University of Sao Paolo	Rubens Siqueira Research Center, Brazil	RP, Dry AMD, Ischemic Retinopathy	Autologous BMSC	Cell suspension—intravitreal injection	Feb-2010 Sep-2011 Jan-2012 Mar-2012

Table 10.3 Recent and current cell transplantation studies (Cell sheet approach)

Study ID	Study title	Phase	Sponsor/ collaborators	Location	Target disease	Cell source	Delivery approach	Registration date
NCT02590692	Study of Subretinal Implantation of Human Embryonic Stem Cell-Derived RPE Cells in Advanced Dry AMD	I/IIa	Regenerative Patch Technologies (RPT)	University of California, US	Dry AMD	hESC-RPE	Cell sheet—subretinal implantation	Oct-2015
JPRN-UMIN000011929	A Study of transplantation of autologous induced pluripotent stem cell (iPSC) derived retinal pigment epithelium cell sheet in subjects with exudative age related macular degeneration	Not Provided (Safety/ Exploratory)	RIKEN Institute	RIKEN, IBRI-Kobe Hospital, Japan	Wet AMD	iPSC-RPE	Cell sheet—subretinal implantation	Oct-2013
NCT01691261	A Study of Implantation of Human Embryonic Stem Cell Derived Retinal Pigment Epithelium In Subjects With Acute Wet Age Related Macular Degeneration And Recent Rapid Vision Decline	I	Pfizer, London Project to Cure Blindness, University College London	Moorfields Eye Hospital, University College London, UK	Wet AMD	hESC-RPE	Cell sheet—subretinal implantation	Sep-2012

Table 10.4 Recent and current transplantation studies (Device delivery approach)

Study ID	Study title	Phase	Sponsor/ collaborators	Location	Target disease	Cell source	Delivery approach	Registration date
NCT01949324	A Phase 2 Multicenter Randomized Clinical Trial of CNTF for MacTel	II	Neurotech Pharmaceuticals	Jules Stein Eye Institute, California, Bascom Palmer, Florida, Emory University, Georgia, National Eye Institute, Maryland, Massachusetts Eye and Ear Infirmary, University of Michigan, Retina Associates of Cleveland, Ohio, University of Wisconsin, US Centre for Eye Research, East Melbourne, Lions Eye Institute, Nedlands, Australia	MacTel (Type 2)	Human Cells releasing CNTF	ECT Intraocular Implant (NT-501)	Sep-2013
NCT01648452	CNTF Implants for CNGB3 Achromatopsia	I/II	National Eye Institute	National Institutes of Health Clinical Center, Maryland, US	Achromatopsia (CNGB3)	Intraocular Implant Releasing CNTF	Intraocular implantation	Jul-2012

NCT01530659	Retinal Imaging of Subjects Implanted With Ciliary Neurotrophic Factor (CNTF)-Releasing Encapsulated Cell Implant for Early-stage Retinitis Pigmentosa	II	Neurotech Pharmaceuticals, University of California	University of California, San Francisco, US	RP, Usher syndrome type 1, 2	Human Cells releasing CNTF	ECT intraocular implant (NT-501)	Jan-2012
NCT01327911	Ciliary Neurotrophic Factor (CNTF) Safety Trial in Patients With Macular Telangiectasia (Mactel)	I	Neurotech Pharmaceuticals, The Lowy Medical Research Institute Limited	Jules Stein Eye Institute, LA, California, Retina Associates of Cleveland, Ohio US	MacTel (Type 2)	Human Cells releasing CNTF	ECT Intraocular Implant (NT-501)	Jsn-2011
NTC00447954 NTC00447980 NTC00447993	A Study of Encapsulated Cell Technology (ECT) Implant for Participants With – Atrophic Macular Degeneration, – Late Stage Retinitis Pigmentosa, and – Early stage Retinitis Pigmentosa	II/III	Neurotech Pharmaceuticals	Retina-Vitreous Associates Medical Group, Beverly Hills California, Retina Group of Florida, Bascom Palmer Institute, Florida, Ophthalmic Consultants of Boston, Massachusetts, Beaumont Eye Institute, Michigan, University of Utah, US	Dry AMD Early RP Late RP	Human NTC-201 Cells Releasing Ciliary Neurotrophic Factor (CNTF)	Encapsulated Cell Technology (ECT) intraocular implant (NT-501)	Mar-2007

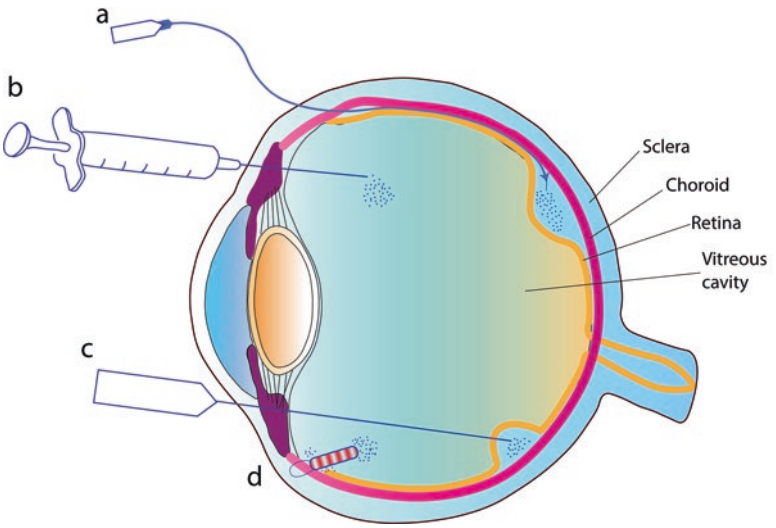

Fig. 10.1 Eye drawing illustrating the different access points and surgical approaches for therapeutic cell delivery. (**a**) Suprachoroidal approach: purpose designed microcatheter progressing through the potential space between retina and choroid, to inject the therapeutic cells into induced subretinal bleb. (**b**) Intravitreal approach: injection of the therapeutic cells directly into the vitreous cavity. (**c**) Transvitreal approach: injection of the therapeutic cells into the subretinal space via the vitreous cavity, after inducing a subretinal bleb with a small gauge cannula. (**d**) Cell device approach: intravitreal implantation and scleral fixation of therapeutic cell-loaded microdevice, which releases therapeutic factors into the vitreous cavity

In contrast to cell suspension delivery, transplantation of a cell sheet or patch requires a more complex surgical procedure. It necessitates a custom device capable of holding, protecting, and delivering the graft in a way that it sustains proper apical-basal orientation (assuming the cells are polarized) throughout its transplantation into the subretinal space. Furthermore, an adequately sized retinal incision is necessary for the sheet to be implanted. However, the benefits of this complex delivery procedure of sheet transplants are substantial, in terms of optimizing cell polarization, integration to the host tissues, and the potential size of the treated area.

Devices: Encapsulated Cell Technology (ECT)

ECT consists of a semipermeable polymer membrane capsule loaded with mammalian cells that have been genetically engineered to secrete therapeutic proteins.

Patented by Neurotech Pharmaceuticals, this novel drug delivery platform has offered an approach of overcoming the blood-retinal barrier, which—like the blood-brain barrier—restricts access of large molecules from the blood stream to the target cells. The circumvention of the blood-retina barrier is one of the major challenges for long-term sustained delivery of proteins to the retina for the treatment of a broad spectrum of eye diseases.

The semipermeable membrane of the ECT device allows the secreted protein to diffuse out and nutrients to diffuse in, but prevents access by the host immune system, thereby providing a sustainable supply of the therapeutic factor over an extended period, possibly years. In addition, the encapsulated cell implants can be retrieved from the eye at any time, providing an additional level of safety (Fig. 10.1d).

The most common therapeutic agents delivered by ECT are neurotrophic factors. These proteins can influence survival, proliferation, differentiation, and function of neurons and other cells in the nervous system and seem to hold a promising ability to retard progression of neurodegenerative disease. For the purpose of retinal neuroprotection the most studied protein is ciliary neurotrophic factor (CNTF).

It is anticipated that with further development of the ECT platform and similar approaches, future implants could become smaller and insertable in different locations, either anchored, free-floating in the vitreous cavity, or implanted subretinally, and will able to release specific proteins to replace proteins that are dysfunctional in retinal, RPE, and/or choroidal cells as a result of hereditary dystrophies.

Sites of Delivery and Current Methods of Access

It has been more than 30 years since the first description of RPE cell transplantation on to a denuded Bruch's membrane in owl monkeys, using an "open-sky" surgical technique and without attempt to reattach the retina [4]. During the last three decades, numerous transplantation techniques and cell delivery instrumentations have been introduced, a variety of which are being used currently in stem cell transplantation studies.

At present, the most broadly studied site for delivery of therapeutic cells is the subretinal space, i.e., the potential space between the neural retina and the RPE. Fewer trials are using the less complicated option of intravitreal delivery, while a very different "external" approach, which involves transscleral delivery and crossing the supra-choroidal space, has been applied for subretinal drug delivery and is now utilized for cell transplantation. Finally, future studies directed by tissue-specific treatment requirements may also focus on more accurate intraretinal and sub-RPE implantation.

Intravitreal

The intravitreal route delivers cells into the eye via injection using a small-gauge needle (Fig. 10.1b). Advantages of this method include technical simplicity and minimal invasion as it does not require a vitrectomy procedure. It can be performed in the office setting and it has been the most well studied and broadly used method for intraocular delivery of any therapeutic agent, since the advent of the anti-Vascular Endothelial Growth Factor (VEGF) injections for retinal diseases. It could be appropriate for the most prevalent diseases with high numbers of patients, such as AMD. This method,

however, also holds some significant disadvantages. First of all, it does not target the therapeutic cells directly to the degenerated tissue, and thus they have to migrate through the vitreous and retina in order to reach the outer retina or subretinal space. Transretinal migration has been shown for immune cells, RPE cells, and pigment granules {Burke:1982em} [5], while in terms of drug delivery, studies are confined to nanoparticles [6]. Another drawback of this approach is the exposure of the implanted cells in the vitreous to immune cells, such as macrophages. Transforming cells in the vitreous also have the potential to induce proliferative vitreoretinopathy and tractional retinal detachments. This potential risk has recently been accentuated by reports of severe retinal complications after intravitreal injections of experimental cell treatments [7–9].

Numerous researchers have adopted the intravitreal approach in both preclinical and clinical trials. Tracy et al. implanted bone marrow-derived mesenchymal stem cells (MSCs) from normal mice into the vitreous of mice undergoing retinal degeneration as a result of PPT1 gene mutation. The implanted cells showed survival without proliferating or invading the retina. This indicates that intravitreal implantation of MSCs is likely a safe means of long-term delivery of proteins synthesized by the implanted cells [10]. Park et al. conducted the first clinical trial in humans exploring the use of intravitreal autologous bone marrow CD34+ cells for ischemic and degenerative retinal disorders. Phase I outcomes reported feasibility and good tolerance which opened the field for further exploration [11].

Additionally, therapeutic approaches that involve factor-releasing cell devices, such as ECT for CNTF delivery, have been using the vitreous cavity as the implantation site for the device (Fig. 10.1d). The surgical procedure involves a small opening of the conjunctiva to access the sclera at the pars plana and a full thickness sclerotomy (approximately 2.5–3.0 mm) to access the vitreous cavity. The device is then inserted into the vitreous and anchored with scleral sutures. Finally, the sclerotomy is sutured and the insertion site is covered with re-apposition of the conjunctiva.

Preclinical studies using encapsulated cell-based CNTF delivery have offered evidence of photoreceptor protection in a dose-dependent manner when implanted into the eye of the *rcd1* dog with a cGMP-*PDE6b* mutation. The implants were seeded with human retinal pigment epithelium cells that had been transfected with the CNTF gene to produce CNTF protein in situ [12, 13]. Sieving et al. conducted a Phase I clinical trial of CNTF delivered by ECT in human subjects with advanced retinitis pigmentosa (RP). The planned follow-up period was 6 months in this initial study, after which the implants were surgically removed. No implant was rejected or extruded, and no severe systemic or ocular adverse events occurred. The investigators reported a trend to improved visual acuity in the study eyes [14]. Conversely, the results from a similar study by Birch et al. showed no efficacy of the CNTF against RP in the long term (60–96 months), while over the short term there were even signs of loss of visual field sensitivity of the treated eye, compared to the sham-treated eye. This loss was attributed to the active implant and was found reversible after its removal [15]. In another recent trial, patients with geographic atrophy (GA) associated with non-exudative late stage AMD received ECT implants anchored to the sclera in an anterior location in the vitreous cavity [16]. Although the trial failed to meet its primary endpoints, CNTF secretion persisted for up to 2 years [16]. More examples of trials using the intravitreal route are listed in Tables 10.2 and 10.4.

Subretinal

Both cell suspensions and cells-on-membrane sheet transplants have been targeted to this potential space, from which the new cells can interact and integrate with both the neural retina and the RPE/Bruch's/choriocapillaris complex. Due to this access, the subretinal delivery seems ideal for a large number of retinal degenerative diseases including AMD.

In most of the reported cell transplantation studies the subretinal space is accessed trans-vitreally. The procedure starts with a standard 23- or 25-gauge pars plana vitrectomy, followed by induction of a posterior neurosensory retina detachment using a stream of balanced salt solution via a small, usually 38–41-gauge cannula, in order to create a subretinal "bleb" of fluid. Subsequently, for a cell suspension implantation, another small (e.g., 38-gauge) cannula may be utilized for the subretinal injection through the same neuro-retinal puncture (Fig. 10.1c). In the case of cell sheet transplant, a larger retinotomy has to be performed in an extrafoveal location, through which the therapeutic patch is placed between the retina and the residual RPE, using a purpose-designed tool. Following inspection of the peripheral retina, a fluid-air exchange is performed, and, according to each study protocol, a tamponade agent is injected into the vitreous cavity (air, gas, or silicone oil). This approach has been tested in various therapeutic studies that have utilized stem cells as well as non-stem cell implantations.

Non-stem Cell Trials

Before the advent of stem cell-derived treatment in human trials, numerous researchers had attempted subretinal transplantation of either fetal or cadaveric tissue patches and/or suspensions, for the treatment of retinal degeneration.

Algvere et al. carried out a study of subretinal transplantation of human fetal RPE (13–20 weeks of gestational age) in patients with different forms of AMD. In one group, eyes with disciform lesions due to AMD underwent pars plana vitrectomy (PPV), excision of submacular fibrovascular membranes, and transplantation of a patch RPE transplant into the subretinal space. The patch (approximately $1.0 \times 1.5\ mm^2$) was initially sucked up into a purpose-designed glass micropipette (inner/outer diameters approx. 0.3/0.4 mm, respectively) filled with BSS and subsequently delivered into the submacular space through a retinotomy. In the second group, eyes with non-exudative AMD underwent PPV and peeling of epimacular vitreous membranes when needed, followed by the subretinal injection of a small patch-RPE transplant. The patch (0.6 mm diameter) was placed extrafoveally at the border of the GA area. In two other groups, patients with dry AMD and RPE tears respectively, were transplanted with a suspension of RPE cells through a small retinotomy, using a 20-gauge glass micropipette with a tapered tip (0.1 mm outer diameter), that had previously been flushed with sodium hyaluronate. The suspension was injected into the center of the macula. The retinotomy was small enough to self-seal and prevent the injected cells from refluxing into the vitreous cavity. In all groups the subretinal space was accessed

after inducing a neurosensory retinal detachment with a stream of fluid via a 33-gauge *Thomas* needle, so that a small bleb was created. The implantation/injection of the cells slightly enlarged the retinal bleb. The operation was completed with a fluid-air exchange and silicone oil tamponade for the first group and air-gas exchange (20% of SF6, or 12.5% of C3F8) and face-down posturing for 2-4 days for the other groups, where no subretinal tissue was removed [17]. After 24–38 months of follow-up, 12 of 16 grafts failed, and this was attributed to immune rejection. The risk of rejection seemed to be related to the integrity of the blood-retinal barrier (BRB) with both patch transplants and RPE suspensions being rejected early—within first 3 months—when placed over an exudative foveal area with compromised BRB. Nevertheless, allografts in non-exudative areas were lost more slowly—over 12–20 months—while extrafoveal transplants were retained after 30 months postoperatively.

A similar approach was reported by Kaplan et al. who describe two cases of transplantation of a sheet of human photoreceptor cells, harvested from cadaveric eyes, into two patients with retinitis pigmentosa. In this study the retinotomy was created with a myringotomy blade and extended with vertical scissors. The sheet of intact photoreceptors encased in gelatin was delivered subretinally through a pipette mounted on a specially designed delivery system. Subsequently, the subretinal bleb was partially flattened and fluid-gas exchanged was performed for pneumatic tamponade (20% SF6). Subjects did not receive any immunosuppression. There was no apparent rejection nor improvement in vision [18]. In contrast, when this group transplanted allogeneic RPE sheets into patients with exudative AMD (following choroidal new vessel excision), systemic immune suppression was required to maintain graft integrity [19].

Humayun et al. delivered a full-thickness undissociated sheet of fetal retinal tissue in the subretinal space of a patient with AMD, in addition to a microaggregate suspension of fetal retinal cells. The fetal neural retina was obtained from the optic vesicles of 14- to 16-week-old fetuses after scheduled pregnancy termination. Standard PPV and submacular surgery technique was used. A 2×2 mm^2 piece of retina was cut with microscissors and then grasped with a smooth-tip custom-built microforceps. The tip of the forceps was used to pierce the retina and, after entering the subretinal space, the tissue was released such that the outer retinal layer was facing the host RPE. Because there was an extensive disciform scar in the macula of the AMD patient, both the microaggregate suspension and the retinal sheet were transplanted in an extramacular location superior to the optic nerve head. No signs of rejection or visual improvement were shown [20].

Radtke et al. reported a case series of transplantation of fetal retinal sheets in patients with RP, and fetal retina together with its RPE in patients with advanced RP or AMD. For the delivery, a custom-made implantation instrument with a flat plastic nozzle tip at a 130-degree angle was used. The instrument maintained the orientation of the donor tissue. The loaded nozzle tip of the delivery instrument was inserted through the retinotomy into the submacular space, and the nozzle was released placing the retina/RPE sheet into the target area. The retinotomy was subsequently sealed by laser. No immunosuppression was given. Initially no signs of rejection nor improvement of vision was shown; however, in a follow-up publication, modest visual improvement was reported for 7 of the 10 patients [21, 22].

Stem Cell Trials

Transvitreal Access

In 2012 Scwarz et al. published the first description of a human stem cell-derived therapeutic trial for retinal degeneration. This was a phase I/II prospective study investigating safety in patients with advanced dry AMD or Stargardt disease. Subjects in the trial received a subretinal cell suspension of hESC-derived RPE (line MA09-hRPE). The operation followed the standard sequence: PPV, localized neuroretinal detachment, subretinal injection of the suspension in areas adjacent to GA loci, and finally air-fluid exchange. Systemic immunosuppression with Tacrolimus and mycophenolate mofetil was instituted for 12 weeks following the surgery. Schwartz et al. went on to publish their methods and the 18-month outcomes for 9 AMD patients and 9 Stargardt disease patients [23]. No serious ocular or systemic adverse events were reported. There was limited, pigmented, epiretinal membrane formation in some patients. Immune rejection was not recognized clinically. Areas of increased pigmentation at the transplantation sites were seen in 72% of subjects, while primary functional outcomes were reported to be promising. These results offered the first evidence of medium- to long-term safety, transplant survival, and possible function of pluripotent stem cell progeny in degenerative retinal disease [24]. Numerous current and recent studies have used similar methods and are listed in Table 10.2.

The subretinal space has also been used for SC-derived transplants in the form of a sheet [25, 26, 27]. Mandai et al. were the first to report the results of an induced pluripotent stem cell (iPSC)-derived RPE sheet transplantation in a patient with wet AMD. They demonstrated safety but no efficacy of their method, in terms of visual function [25]. The London Project (TLP) to Cure Blindness and University College London have commenced a Phase I study trying to reconstruct the anatomy of the subretinal space in severe wet AMD by implanting confluent, polarized hESC-derived RPE cells on an artificial basement membrane in the form of a "patch" [28]. This group uses submacular microsurgical techniques and a specially designed injector to insert the 6 × 3 mm lozenge-shaped patch into the subretinal space of patients who suffer from acute wet AMD with sudden severe vision loss due to submacular or sub-RPE hemorrhage or an RPE tear. For immunosuppression they use transient perioperative systemic steroids and, in the longer term, intraocular depot corticosteroid delivery devices. Two patients have received the patch so far, and the recently published one-year results were promising, with both patients having a significant improvement in visual acuity, reading speed, and retinal sensitivity [26]. Similar approaches utilizing cell sheet transplants are listed in Table 10.3.

Suprachoroidal Access

A completely different surgical method of accessing the subretinal space has been developed by Janssen (Titusville, NJ—division of Johnson and Johnson) in order to deliver human umbilical tissue cells (hUTCs) to patients with GA. These cells have been

evaluated in the Royal College of Surgeons rat model of retinal dystrophy and rescue degenerating photoreceptors better than other cell lines [29]. This technique utilizes a trans-scleral microcatheter-based delivery, which is advanced through the supra-choroidal space. The operation starts with a minor conjunctival dissection with surface cautery, followed by a scleral cut-down and a specialized scleral speculum insertion, 9 mm posterior to the limbus. The choroid is perforated, and a subretinal bleb is created with Healon®, under direct endoscopy (Endo Optiks, Little Silver, NJ). Subsequently, the 250-μm subretinal microcatheter (iScience Interventional, Menlo Park, CA) is inserted from the scleral opening and advanced through choroid into the subretinal space to the posterior pole (Fig. 10.1a). The tip of the catheter is illuminated and allows accurate localization to the areas of GA. The hUTC suspension is then injected by a high precision pump into the subretinal space. The catheter is carefully withdrawn, and all sclerotomies are closed with standard techniques. This surgical approach has still to be improved since some patients developed retinal tears and detachments.

Target Diseases and the Need for Specific Delivery Approaches

Retinal degenerative diseases constitute a large, heterogeneous group of inherited or acquired disorders that disturb mainly the photoreceptor and the RPE layers, the function of which constitute the most critical layers for visual function of the eye.

AMD

Age-related macular degeneration is associated with a chronic, low-grade inflammation that affects the outer layers of the central retina, starting with the degeneration of the RPE and Bruch's membrane and leading to loss of photoreceptors and subsequent Geographic Atrophy (GA). GA is expected to affect 3.8 million adults by the year 2050 [30]. Even patients with the neovascular type of the disease (wet AMD) that can be stabilized using anti-VEGF injection treatments eventually manifest dry AMD. Furthermore, although anti-VEGF treatment may delay the progression of disease, there are significant drawbacks both for the patients, regarding the duration of therapy and the risk of complications, and for the health systems, regarding the financial burden of treating constantly increasing numbers of patients.

For a surgical AMD treatment to be feasible, it has to be technically simple, with low risk of complications, applicable in an office-based ophthalmological therapeutic setting, relatively inexpensive, and suitable for large numbers of patients. Cell-based treatments, trying to replace the RPE or RPE-Bruch's complex with stem cell-derived equivalents, hold promise for the future but face many challenges in terms of delivering a viable therapeutic option on a large scale. Numerous approaches have been tried so far, with most common among them being the subretinal injection of stem cell-derived RPE cell suspensions (Table 10.2), while most recent human studies of RPE-artificial BM sheets transplanted subretinally are yet to prove their feasibility and efficacy (Table 10.3).

Inherited Retinal Disease

In addition to the epidemiological and clinical significance of AMD, the management of AMD using a cellular approach also constitutes a potential therapeutic paradigm for other disorders that affect RPE and neural retina, such as inherited retinal diseases (IRDs).

Retinitis pigmentosa (RP) is the most prevalent of the IRDs affecting approximately 1/4000 individuals [2]. It is associated with primary photoreceptor degeneration due, in most cases, to defective genes involved in their metabolism. Several studies mainly using human retinal progenitor cells or human bone marrow mesenchymal stem cells to rescue or replace the degenerating photoreceptors are now running as shown in Table 10.2. Furthermore, some subtypes of RP caused by RPE-specific genetic defects seem to primarily disturb the structure and function of this supportive epithelial layer. Dystrophies associated primarily with the RPE specific genes such as MERTK [31] and RPE65 [32] could also be potential targets for cell-based RPE therapies in the future.

Stargardt disease is a juvenile retinal dystrophy caused by a photoreceptor gene defect that is associated with increased production of toxic bisretinoids and which leads to abnormal RPE lipofuscin accumulation and secondary RPE degeneration. Classically, it presents during the first two decades of age, and it is the commonest cause of juvenile macular disease, reducing central vision in approximately 1:10000 young individuals [3]. The first cell-based therapeutic study directed at Stargardt disease attempted to replace defective RPE by subretinal injection of a stem cell-derived RPE cell suspension. The results of this trial have offered the first long-term safety evidence and also suggested potential vision and vision-related quality of life improvement.

Other IRD examples that may be treated using cell-based therapies in the future include diseases such as vitelliform dystrophy (Best disease), choroideremia, cone and/or rod dystrophies, and some forms of Leber congenital amaurosis. It is also possible that retinal disorders with breaks to Bruch's membrane and secondary RPE atrophy, such as angioid streaks and myopia, may be amenable to an artificial membrane strengthening Bruch's with RPE cell replacement to reduce the effect of the secondary atrophy.

Uveitis: "Cellular Immunotherapy"

Apart from acquired and inherited retinal degeneration, cell-derived treatments have also been directed towards modifying other disease processes such as inflammatory ocular diseases. *Cellular immunotherapy* is an approach that uses intact, fully differentiated, autologous or allogeneic mature immune cells to modulate the patient's inflammatory reaction against a specific hazard.

More specifically, cellular immunotherapy is already being studied as a treatment of CMV retinitis that typically occurs in immunocompromised patients with insufficient primary T-cell response against the virus. In this approach, partially matched donor CMV-specific cytotoxic T-cells are infused intravenously into patients with CMV retinitis who are resistant, refractory, or intolerant to conventional antiviral therapies. Primary results have demonstrated efficacy against persistent viremia or systemic infection in the stem cell transplant population [33, 34].

Discussion

Feasibility Criteria

For a surgical approach to be adopted in everyday clinical practice, it has to meet some feasibility criteria. First of all, it has to demonstrate adequate safety for both the target tissue—the retina—and the adjacent tissues. The risk of complications such as retinal hemorrhage, retinal perforation, retinal detachment, and choroidal hemorrhage has to be comparable with other already established procedures. Secondly, the cell delivery approach must secure not only the initial implantation, but also the retention of the therapeutic cells in the targeted location. Leakage of cells either into the vitreous or in the suprachoroidal space may not only compromise the treatment, but also put the patient at risk, in case of cell migration to distant organs.

Additional requirements concern the procedural complexity and efficacy. The targeted delivery has to be reproducible, with straightforward adoption by experienced surgeons. Ideally, it is compatible with commonly used surgical tools and techniques and has a duration suitable for high patient numbers. Ideally, the approach should also be adaptable to differing eye length and globe volume and expandable to be applied in a variable spectrum of cases.

Future Directions

The emerging progress in multimodal medical imaging and surgical instrumentation technology will open numerous new fields in therapeutic delivery in ophthalmology. Intraoperative OCT (optical coherence tomography) systems, already in use, and 3D surgical visualization systems are now in the process of changing the way ophthalmologists perceive eye surgery. The ability to obtain and analyze scans in real time as well as the option to superimpose simultaneous and/or previous exams onto the surgeon's view of the operational field in real time will soon provide an upgraded level of microscopic interaction between the surgeon and the target tissues.

Future developments in ophthalmological surgery, instrumentation, and robotics engineering are expected to overcome the challenge of insufficient surgical dexterity. Micro-precision devices such as surgeon extenders and teleoperated robots coupled with multimodal imaging sourced information will augment the effectiveness of eye surgeons in accessing and manipulating retinal and subretinal tissues. Targeting specific layers and microscopic structures within the retina in an accurate and safe manner may open delivery approaches that are not feasible at present [35]. In the near future, intraretinal, intra-choroidal, and intra-optic nerve cell treatments are expected to extend to currently untreatable diseases, the powerful new paradigm of cellular therapy for the treatment of an increasing number of blinding ocular degenerative diseases.

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