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Review Article

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Ophthalmic transplantology: Posterior segment of the eye – Part II

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Summary

Background: Transplants of the retina are among the new strategies being used in the treatment of genetic and degenerative macular diseases. Moreover, various cell cultures are being tested to treat retinal disorders.

Material/Methods: Literature dated from 2004 to 2011 was comprehensively examined via Medline and PubMed searches for the following terms: auto-, homo-, heterologous transplantation, retina, stem cells, cultivated cells.

Results: Tissue and cell therapy of retinal diseases are reviewed, including full-thickness retina/retinal pigment epithelium (RPE)/choroid graft; full and partial thickness RPE/choroid complex grafts; RPE/Bruch membrane complex graft; and RPE, iris pigment epithelium and stem cell grafts. Recommendations for transplants, as well as the benefits and weaknesses of specific techniques in retina transplants, are discussed.

Conclusions: Auto- and allogenic transplants of a full or partial thickness retina/RPE/ Bruch membrane/choroid complex represent an alternative treatment offered to patients with some macular diseases. Stem cell transplantation to reconstruct and regenerate the macula requires further biomolecular and animal research studies.

key words: **eye ball • transplantation • autologous • homologous • heterologous • retina • stem cells**

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Background

Genetic and degenerative retinal diseases currently present scientific and ophthalmological challenges. In the United States, approximately 6 million people have retinal diseases, in particular from retinopathies such as age-related macular degeneration (AMD), diabetic retinopathy and retinitis pigmentosa [1–3]. AMD accounts for 75% of cases of legal blindness in people over the age of 50 years in all industrialized countries [4,5]. Photodynamic therapy or intravitreal anti-vascular endothelial growth factor (VEGF) injections represent the first line of wet AMD treatment, but are of limited efficacy [4–8]. No treatment has been proposed for atrophic maculopathy [9,10]. Implantation of electronic chips to stimulate the brain, as well as transplantation of donor tissue and cells, are new therapeutic strategies currently being explored to reconstruct the photoreceptor/retinal pigment epithelium (RPE) complex and to restore central vision in different retinopathies [11–14].

An animal model of retinal degeneration demonstrated the importance of the survival and integration of transplanted tissues [15]. The human eye is an immune-privileged organ – an evolutionary phenomenon that prevents immunogenic inflammation, macular destruction and blindness [12,13,16–18]. Blood-tissue barriers that limit the ingress of blood-borne immunogenic factors; immunosuppressive properties of pigment epithelial cells; and the expression of CD95 ligand on ocular parenchymal cells, which triggers the apoptosis of effector T cells, all contribute to the immune tolerance of the eye [19,20]. Human RPE cells have the particular ability to suppress T-cell activity by direct contact (Toll-like receptors serve as the first line of defense [1–7,9,10]) and via secretion into the aqueous humor and vitreous body of soluble immunomodulatory/suppressive factors, such as cytotoxic T lymphocyte-associated antigen-2α programmed cell death 1 ligand, thrombospondin-1 and transforming growth factor [19,21–25]. These overlapping mechanisms enable acceptance of foreign tissue grafts for extended intervals – unlike conventional body sites that summarily reject such grafts – and delay symptoms of retinal rejection (ie, shrinkage, architecture loss and glial transformation accompanied by low-grade inflammation) [19,26].

Tissue Therapy in Retinal Diseases

Full-thickness retina/RPE/choroid complex allogenic grafts were performed to treat patients with retinitis pigmentosa and geographic atrophy in dry AMD [27,28]. During a 5-year study, graft samples were harvested from the enucleated eyes of human embryos after abortion in the 10th to 15th week of pregnancy [28]. An average tissue area of 3.5 mm^2 was excised directly before the transplantation. It was then placed in a special applicator (allowing gentle lifting and correct direction of the tissue) and grafted under the central retina by means of an upper nasal retinotomy. The procedure was finalized with the use of a laser around the retinotomy and performance of a perfluorocarbon-oil exchange. No intraoperative or postoperative complications were found [28]. After the retina transplantation, synaptic junction stimulation at the host/donor interface was required [29], which was performed in this group of patients by showing them various colorful moving images on video. Seventy percent of the patients (n=10) showed improved visual acuity scores 6 months after the transplantation [28]. The nutritious effect of the graft on the photoreceptors or the stimulation of Müller cells, leading to new synaptic junctions in the host/donor interface, may have contributed to better visual acuity [28]. The "new immunization" was not found in human leukocyte antigens; embryonic tissues were less immunogenic and the blood-brain barrier stopped the infiltration of the graft's antigens [28]. Five years after surgery, no graft rejection symptoms, such as macular edema or encapsulation, were found clinically or by optical coherence tomography. However, in the graft area, pigment loss occurred in 80% of patients after 3–6 months, which was not interpreted as rejection because of the improvement in visual acuity [28]. The investigators also suggested that the improvement could result not only from the graft, but also from the lensectomy and vitrectomy, which had been performed before transplantation during the same procedure [30]. The administration of neutrofins (an indirect result) rather than the replacement of the degenerated tissue could also have contributed to the good results. It is doubtful, however, whether this type of graft would be commonly accepted (because of ethical and religious dilemmas) [30].

Autotransplantation (autotranslocation) of RPE/choroid complex graft is called a full-thickness autologous patch or a patch graft (PG). Such grafts were performed in patients with wet AMD (choroidal neovascularization in AMD [CNV/AMD]) [31–35], RPE rip [36] and dry AMD (geographic atrophy in AMD) [37]. A PG consists of the RPE, Bruch membrane, and choriocapillaris, as well as medium and large choroidal vessel translocation [31,32]. The differences between PG methods [31,32] depend on the manner in which the neovascular tissue is removed and the graft is prepared, as well as on the tissue translocation technique used (see below). One method involved the removal of CNV by means of perimacular retinotomy and harvesting of the graft from the superior temporal equatorial retina [31,35]. It was first encircled with continuous laser impacts and then excised as deep as the sclera with vertical scissors. The retina was peeled from the sheet and PG was implanted under the fovea through a central retinotomy. The procedure was finalized by applying the laser around the retinotomy and performing a perfluorocarbon-oil exchange. One study [31] compared the results of this PG technique with those of full macular translocation (FMT) surgery (360°). The results after 1 year were comparable, but after 4 years the results of FMT surpassed those of PG (the deterioration of visual acuity from 0.87 to 1.38 logMAR in PG and from 0.9 to 0.69 logMAR in FMT; more extensive RPE damage and inferior fixation occurred in the PG group) [31]. Three reasons for the inferior results in the PG group have been suggested [31]: (1) Iatrogenic detachment of the retina caused by the fluid in FMT does not damage the natural, strong adhesion of the photoreceptors and RPE to the extent that peeling does; (2) in PG, the tissue is harvested from the equatorial retina and RPE, which is less specific and less sensitive than paramacular RPE, on which the retina is placed and rotated by 45° in FMT; and (3) the perfusion disorder can last for several days after PG and the translocation of the full choroid section does not prevent ischemia and does not contribute to the metabolic balance of the retina [31]. In another study [35] the investigators compared results of autologous RPE/choroid PG with an RPE cell-suspension graft. Three or more lines

in best corrected visual acuity at 24 months follow-up was found in 28.5% of patients in the RPE patch group and in 14.2% of patients in the RPE cell-suspension group; a loss of 3 or more lines occurred in 14.2% in each group (n=7 in each group) [35]. Examination by fluorescein and indocyanine green angiography showed revascularization of all PGs and normal choroidal vasculature in the area of RPE atrophy in all patients of the RPE cell-suspension group. However, part of the successfully transplanted patch grafts showed intrastructural changes on spectral-domain optical coherence tomography (ie, irregularities in the cell layers; scarring or gliosis and an absence of photoreceptors and outer nuclear layer) [35]. This may explain the limited visual gain of RPE PGs, which should theoretically be a better option than RPE suspension grafts (loose, unevenly distributed RPE cells without their own basal lamina are worse support for photoreceptors than a functional monolayer in sheet grafts) [35]. In another method for PG surgery, a temporal 180° retinotomy was performed and the mobilized retina placed on the nasal area [32]. The vast retinal area bared in this way enabled less extensive CNV removal. With special scissors, the graft was excised from the central circumference of the fundus in the superior temporal quadrant and was not encircled by the laser. In cases of hemorrhage, the nutrient vessel supporting the graft was coagulated with diathermia and/or the intraocular pressure was raised. The retina was peeled from the sheet. Perfluorocarbon injected into the subretinal area stabilized the folded retina and allowed for autotranslocation. Moreover, perfluorocarbon injected into the intrinsic surface reversed the retina and stabilized the graft. The procedure was finalized by applying a laser along the retinotomy and by performing a perfluorocarbon-oil exchange. Between 4 and 20 months after surgery, visual acuity improved in 60% of eyes, stabilized in 30% and deteriorated in 10% (n=13) [32].

The advantage of the technique is the easy access to the posterior area. This allows for delicate CNV excision and maximum preservation of residual, healthy RPE and subretinal vessels; in addition, it enables and accelerates revascularization of the graft [32]. Folding and stretching of the tissue during implantation was not required (it took place in the graft through paramacular retinotomy) [32]. The laser was not used prior to tissue resection to avoid the shrinkage and infolding of the graft's edges. It is vital for the graft to be large enough to entirely cover the area of the recipient's damaged RPE and choroid [32]. The tissue junction contributes to the nutrient bridge vessel at the host/donor interface and to early graft revascularization [32]. In indocyanine green angiography, the graft's nutrient vessels did not run radially, but in parallel as ladder rungs [32]. Moreover, proliferative vitreous retinopathy (PVR) did not occur in this group [32]. PVR occurs in 20–50% of patients after a 360° retinotomy and in 31% of patients after paramacular retinotomy; a large incision area and good perfusion of the central retina lead to PVR [38]. Lack of PVR in the above-mentioned group is ascribed to limited (180°) retinotomy in the poorly vascularized circumferential area of the retina and routine performance of phacoemulsification with intraocular lens implantation [32]. The right access allows for maximum excision of the vitreous base, which prevents shrinkage and PVR [32].

In some patients, the long-term results are not satisfactory after PG [35,39]. Extensive tissue thickness makes penetration of nutrients and reperfusion difficult, thereby leading to graft fibrosis beginning just 30–60 days after autotranslocation [39]. Animal research has shown that a partial graft, with no medium and large choroid vessels, is easily integrated with the recipient's bed. In 63% of the animals, the partial graft was found to be viable (ie, revascularization and monolayered RPE cells were present) [40].

Autologous RPE/Bruch membrane complex grafts were performed in patients with hemorrhagic CNV in wet AMD [38]. A temporal 180° retinotomy was performed with CNV removal on the external side of the retina and laser around the retinotomy with a perfluorocarbon-oil exchange. The 4 mm² graft was excised with a microvitreoretinal knife on the cauterized area from the central circumference in the superior temporal quadrant. The incision was as deep as the middle choroid. The RPE/Bruch membrane complex was separated from the choroid vessels with a special S-shaped spatula and scissors [38]. Patients showed improved Early Treatment of Diabetic Retinopathy Study visual acuity scores that averaged 28.6 to 47.7 after surgery, central fixation occurred in 30% of eyes, and the appropriate graft morphology without depigmentation was found in 82% of eyes (n=21) [38]. Visual acuity scores were reported to be comparable to the results of full-thickness grafts [34,36,37]. However, the thinness of the partial graft, without the retina and the choroids, is perceived as an advantage in this graft – it easily settles after perfluorocarbon, does not require curling, enables penetration of nutrients from the choroid to the retina, and its edges do not tend to curl, which prevents the formation of subretinal membranes [38]. According to various authors [41–44], the reported long-term results of RPE/Bruch membrane PGs are not satisfactory because the achieved overall visual gain is about 1 line of best corrected visual acuity, and proliferative vitreoretinopathy complications occur in up to 45% of eyes.

Full or partial tissue transplantation is one of the current strategies for macular pathology treatment, but most grafts fail to integrate into the recipient's bed, lose their architecture and do not function [45–47]. Moreover, allotransplants of fetal or postmortem tissue lead to rejection and the need to apply immunosuppressants and, at the same time, they raise moral and ethical concerns [30,45]. These problems are partially eliminated by the cell transplant concept [45,48].

Cell Therapy in Retinal Pathology

Autologous RPE cell grafts were performed in patients during the surgical removal of CNV in exudative AMD (CNV/AMD) [35,49] or CNV in retinal angiomatous proliferations (CNV/RAP) [49] and can be a therapeutic option for anti-VEGF non-responders or where anti-VEGF fails to improve visual acuity (RPE tear, massive subretinal hemorrhage) [35]. After pars plana vitrectomy, nasally from the optic nerve, flat detachment of the retina was created and RPE cells were mobilized in the iatrogenic bubble by means of fluid injection. The cells were aspirated, removed by centrifugation and then counted and prepared for injection of suspension. The CNV was removed by a second paramacular retinotomy, and the suspension of RPE cells was injected under the fovea. After implantation, the cells were allowed to sink in. The procedure was finalized by applying a laser to the retinotomy and performing a perfluorocarbon-oil

exchange. In a 1-year follow-up period, visual acuity improved or stabilization occurred in 80% of patients with wet AMD and in 68% of patients with RAP [49]. However, the long-term results of autologous RPE cells grafts are not promising [32,35,50]. The technique is not viable because the grafts do not contribute to functional monolayer formation and the scattered cells atrophy. Unevenly distributed, multilayered RPE cells without their own basal lamina and extracellular matrix are unable to grow well on an aged or diseased host's Bruch membrane, which is a crucial factor in the survival of transplanted RPE cells, their adherence and differentiation, and their ability to phagocytose photoreceptor outer segments [50–52]. The fovea requires much more stimuli than just new RPE cells in order to function properly [32]. A biostable synthetic membrane (ie, expanded polytetrafluoroethylene (ePTFE) polymer modified by ammonia gas-plasma treatment) was tested experimentally as a Bruch membrane prosthesis – a scaffold for RPE cell growth and differentiation [52]. Defluorination of the fluoropolymer increases surface hydrophilicity, incorporates surface polar groups and facilitates cell attachment [52]. ARPE-19 cells (a non-immortalized human RPE cell line that mimics the human situation), seeded on a modified ePTFE scaffold at a density of 1500 cells/mm^2 and cultured in the presence of retinoic acid (to promote RPE differentiation), formed a differentiated and functional monolayer capable of phagocytosing photoreceptor outer segments [52]. The scaffold serves as a carrier substrate, protected from the hostile influences of an aged and diseased Bruch membrane; facilitates cell interaction and flow of nutrients (vitamin A, glucose, fatty acid); and represents a possible treatment to repair retinal degeneration and restore vision in affected patients [50,52,53]. According to Krishna et al. [52], the biostable, biocompatible ePTFE membrane provides stable replacement of the natural Bruch membrane and long-term support for transplanted RPE cells. The membrane seems to be a better option than a synthetic biodegradable membrane or tissue-based material [50,53–57]. Despite advances in microfabrication technology, the functional results of various RPE cell transplantation techniques in humans are worse than anti-VEGF treatment and are associated with a high rate of serious complications (eg, massive hemorrhage, PVR) [35,50].

Autotransplantation of iris pigment epithelium (IPE) cells is not performed in humans because of the potential for significant damage to the iris. Non-modified IPE allotransplantation is also not performed in humans, because it does not significantly improve visual acuity [58]. However, IPE cells have become a focus of interest because *in vivo* they have been shown to express mRNA for proteins involved in the metabolism of retinol. They also phagocytize the external segments of photoreceptors and accumulate lipofuscin; thus, they significantly impact visual acuity [59]. Subretinal human IPE xenotransplantation was performed in rats (the Royal College of Surgeons) with iatrogenic macular dystrophy (genetically engineered gene change for the photoreceptors of tyrozine kinosis, leading to inhibition of phagocytosis of the external segments of photoreceptors through RPE and lipofuscin accumulation, as well as to subsequent atrophy of photoreceptors) [59,60]. A human iris (from a cadaver) was entirely excised, and the cell suspension was injected under the retina [59]. The human IPE cell xenotransplant was shown *in vivo* to protect photoreceptors without any significant adverse effects; it also produced protective neurotrophic growth agents and stimulated the regeneration of photoreceptors [59]. In future, IPE cells may turn out to be useful for transferring genetic information to photoreceptors and RPE [59]. Specific retinal cells, similar to other neurons, have limited capability for proliferating and regenerating spontaneously [48].

Stem cells, as well as engineered retina (ie, retinal aggregates that are laboratory-generated from tissue-specific progenitor stem cells [PSCs]), are new therapeutic strategies for the replacement of degenerative photoreceptors and RPE cells in patients [45,57,61–79]. The multipotent stem cells differentiate into multiple, specific cell types of 1 lineage (meso- or endo- or exo-dermy), known as PSCs [30,48]. Multipotent stem cells can be harvested from different tissues, such as embryo, neuron, bone marrow, and eye (including the ciliary body, iris, and retina) [48]. Retinal PSCs, formed from exodermy, have the ability to differentiate into photoreceptors, RPE, Müller glial cells and bipolar cells in a favorable environment that includes growth factors and/or chemical modulators [48,80,81]. At present, research on retinal PSCs has been conducted *in vitro* and with animals [45,60,78,82–84], and the source of optimum retinal PSCs has not yet been determined [48]. Embryonic human stem cells can differentiate into retinal neurons that are similar to adult retinal stem cells [80], but this manner of obtaining adult stem cells in humans has raised serious moral and ethical concerns and dilemmas [45]. Neural stem cells differentiate to photoreceptors and glia in rats, but when implanted into an animal retina, often become immature and fail to express rhodopsin [85]. However, the results of xenotransplantation of human neural progenitor cells are highly promising.

A human neural progenitor xenograft was performed in 21-day-old rats with iatrogenic macular degeneration and pigment dystrophy [60]. The xenograft sustained the visual acuity of rats for 280 days [60]. Adult human stem cells found in the retina, Müller cells or RPE are considered to be the optimum source of retinal PSCs [45,61–65]. The concept of grafting both 3-dimensional (3D) cultures and differentiated retinal PSCs seems to be promising [45,70,78,79]. 3D cultures are much better suited than monolayer cultures because the graft involves the 3D structure of the recipient's bed [45]. Retinal PSCs are cultured alone or co-cultured with RPE cells [45]. Cultured aggregates of retinal progenitors appear on approximately the 10th day of culturing. As a result of cellular bioengineering, the cultured 3D structured cells were differentiated in such a way as to create several cell lines specific for the particular tissue [45]. Photoreceptor differentiation was performed by upregulation of rhodopsin and AANAT enzyme (implicated in melatonin synthesis). This differentiation was confirmed by reverse transcription-polymerase chain reaction in reference to the following factors: rod transcription factor Nrl, Nr2e3, interstitial retinal binding protein and rhodopsin kinase. Differentiation toward other cell lineages was demonstrated by the presence of tyrosine hydroxylase in amacrine and ganglion cells, as well as by the presence of calbindin and GNB3 in cone cells [45]. The resulting 3D cultured and differentiated PSC aggregates are able to blend into the degenerated bed in the recipient and are capable of producing neurotrophic factors, leading to regeneration of the photoreceptor/ RPE complex and reconstruction of the subretinal

environment in retinitis pigmentosa and AMD in humans [45,48]. The development of microfabrication technology improved the delivery aspect of 3D aggregates for transplantation in an experimental study. A novel biodegradeable, 3D thin-film cell encapsulation scaffold served as the carrier platform for aggregates [70]. The transplantation vehicle was constructed from thin-film 2–2.5-D polycaprolactone (PCL) layers (<10 μm) thermally bonded to form the 3-D cell encapsulation scaffold (<30 μm) with varying protrusions, cavities, pores and particles [70]. The 3D scaffold was permeable and promoted the delivery of retinal progenitor cells and their appropriate retention in subretinal space [70]. In another study [78], mouse embryonic stem cells cultured with the extracellular matrix proteins added to the medium, over a period of 9 days formed 3D spherical aggregates called embryoid bodies. Subsequent invagination of its apical surface formed an optic cup-like structure with a 2-walled structure consisting of retinal pigment epithelium and neural retina, with synaptic activities. The formation of the optic cup occurred in a self-directed way (was not caused by a lens or surface ectodermal tissues). According to several studies [78,79], self-formation of fully stratified 3D neural retina tissue from stem cells opens up a new strategy for the transplantation of the artificial retinal tissue sheets, rather than simple cell grafting. This optic-cup structure can be further induced to form an entire eye structure (lens, iris, cornea, and sclera), and can serve as the cell-culture models for drug screening and characterization of the disease phenotype. Such models may be obtained with retinal PSCs, which derived from the persons with some specific mutant genotypes associated with the diseases, such as macular degeneration or retinitis pigmentosa.

Natural retinal barriers – inhibitory extracellular matrix and cell adhesion molecules (CD44, neurocan) [86], as well as the outer limiting membrane (OLM) – that form a natural barrier between the subretinal space and the outer nuclear layer, weaken cellular host-donor integration [87,88]. Degradation of these molecules and transient chemical disruption of the OLM facilitates host-donor integration in animals [87,88]. The combined transplantation of retinal PSCs with biodegradable microspheres of poly lactic-co-glycolic acid, which deliver active matrix metalloproteinase 2, stimulated the removal of the matrix inhibitory barrier and enhanced cellular host-donor integration in mice [86]. Targeted disruption of OLM junctional proteins, Crumbs homologue 1(Crb1) and zona occludens (ZO-1), as well as intravitreal injection of the glial toxin DL-alpha-aminoadipic acid 72 h prior to transplantation, induced transient chemical disruption of the OLM (maximal at 72 h, recovered by 2 weeks) and led to a significant increase in the number of transplanted photoreceptor precursors integrated within the outer nuclear layer in degenerated mouse retinas [87,88]. This study showed that targeted disruption of an undesirable physical barrier in the retina may be an effective and practical strategy for retinal repair. The usefulness of research on stem cells has also been confirmed by autologous non-myeloablative hematopoietic stem cell transplantation (HSCT) [89]. HSCT was shown to be effective in the therapy of autoimmune-related retinopathy and optic neuropathy syndrome (ARRON) that was resistant to conventional therapies (prednisone, methotrexate, cyclophosphamide, plasmapheresis, intravenous immunoglobulin) [89]. After HSCT, the clinical manifestations (visual acuity, visual field, electroretinography, and antibody activity against pig retina and pig optic nerve) of ARRON appeared to stabilize [89].

Conclusions

The techniques presented for auto- or allogenic retina transplants, both full and partial, represent the currently available surgical alternatives in the treatment of neovascular and atrophic macular diseases. However, retina transplant requires complicated vitreoretinal surgery and in some cases might be followed by delayed reperfusion of the graft.

The difficulty of retina transplantation lies in the highly specific nature of the complex and "demanding" neuronal tissue. Stem cell transplantation in retinal diseases is of increasing importance [90], but further laboratory tests in genetic engineering and animal testing are required to find an optimal source of PSCs for RPE cells and photoreceptors [45,48,60,91,92]. Stem cell transplantation also raises new ethical dilemmas that require further consideration [93].

Abbreviations

AMD – age-related macular degeneration; **ARRON –** autoimmune-related retinopathy and optic neuropathy; **CNV/AMD –** choroidal neovascularization in age-related macular degeneration; **CNV/RAP –** choroidal neovascularization in retinal angiomatous proliferations; **3D –** three-dimensional; **ePTFE –** expanded polytetrafluoroethylene; **FMT –** full macular translocation; **HSCT –** hematopoietic stem cell transplantation; **IPE –** iris pigment epithelium; **OLM –** outer limiting membrane; PG – patch graft; **PSCs –** progenitor stem cells; **PVR –** proliferative vitreous retinopathy; **RPE –** retinal pigment epithelium; **VEGF –** vascular endothelial growth factor.

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