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Regenerative Medicine and Eye Diseases

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Abstract

The chapter examines the use of stem cells in ophthalmological pathologies affecting both the anterior and posterior segments. The authors review the clinical trials that have most contributed to defining the role and potential of stem cell regenerative therapy in corneal and retinal pathology. The results described in the scientific literature are analyzed and commented, without neglecting the possible side effects related to the use of this therapy. Within the anterior segment, the greatest efforts were made to study the possible uses of limbal epithelial stem cells (LESCs). They were the first stem cells to be discovered at the level of the anterior segment and currently the only ones involved in clinical practice with satisfactory results. At this juncture there have been significant successes in the treatment of corneal stem cell deficiency and of corneal scars. The chapter later investigates the possible applications of stem cell therapy in degenerative retinal diseases, with particular reference to retinitis pigmentosa, Stargardt's disease, and age-related macular degeneration. It then describes how the use of cell therapies, in particular those that use ADSC, can contribute, through various methods, to the containment of the evolution of retinal degenerative diseases. These mechanisms cover various biological aspects and can be summarized as follows: neurotrophism, oxidation, vascular changes, apoptosis, inflammation, or immunology. The ophthalmological modalities of the cell graft and what is the ideal approach for an ophthalmological cellular surgery are later on described. Finally, the technique used by the author and the possible outcomes in the course of degenerative retinopathy are described.

Keywords: ophthalmology, stem cells, regenerative medicine, degenerative diseases

1. Introduction

Regenerative medicine has been a major topic in the last few decades. The use of stem cells has opened up new perspectives in the therapeutic approach to many different diseases. Even in the ophthalmological field, the use of stem cells has allowed an improvement in the clinical outcome of different pathologies such as limbal stem cell deficiency, corneal scarring, retinitis pigmentosa, Stargardt's disease, age-related macular degeneration, and retinal atrophy following various vascular conditions. Many of these pathologies have always been considered untreatable, leading to a progressive sight loss; the arrival of stem cell therapy has led to an entirely new clinical and therapeutic approach to these conditions. Different types of stem cells have been tested as a solution for tissue repair in the different ocular structures.

As for the anterior segment, the most used cells are limbal epithelial stem cells (LESCs), found at the level of the Vogt palisades. As regards the posterior segment, the stem cells used for the treatment of retinal degenerative diseases are embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), and mesenchymal stem cells (MSCs).

LESCs: Also known as corneal epithelial stem cells, they are located in the basal epithelial layer of the corneal limbus. They form the border between the cornea and the sclera and are implied in the regular corneal renewal. They are also implied in corneal repair activity after severe damage of corneal surface.

ESCs: The first ESCs were obtained from mouse embryos and immediately showed their ability to express neural markers and to migrate into the retina when applied intravitreally. Also, they seemed to be able to integrate into the retinal layers and act as neuroprotective factors. Clinical trials conducted in the human eyes have demonstrated that the subretinal application of these cells shows no signs of rejection, ectopic tissue development, negative proliferation, or tumor formation in a 1-year follow-up.

iPSCs: These cells are obtained from reprogramming adult somatic fibroblasts through retroviruses or lentiviruses. Compared with ESCs, they show less risk of rejection and less need for immunosuppressive therapy. However, further studies have suggested that iPSCs can stimulate oncogenes/suppress tumor suppressor genes, resulting in gene mutations and malignant transformation. The many molecular passages required for their production also seem to act as a trigger for the genetic instability shown by these cells.

MSCs: These cells are derived from many different tissues (peripheral blood, bone marrow, adipose tissue, cord blood, teeth, central nervous system, and liver). Once acquired, MSCs can be expanded in cell cultures maintaining their stemness. They can differentiate into various cells (mesodermal, ectodermal, and endodermal cells), including neuron-like cells. Since they are capable of secreting neurotrophic factors, repairing neural connections, and stimulating the formation of synapses, MSCs are also appreciated for their “structural” function. Moreover, they have shown a strong immunosuppressive action inhibiting the release of pro-inflammatory cytokines; therefore they allow both autologous and allogenic transplantation. Finally, their use does not seem to be related to tumor formation. For these reasons, researchers look at stem cells as a promising therapeutic option for degenerative retinal diseases. Nevertheless, it must be said that various ocular complications related with the use of these cells have been described (see Section 3).

2. Regenerative medicine in the anterior segment of the eye

Stem cells are unspecialized cells that have been a focal point of the field of regenerative medicine, frequently considered as the future of medicine. The first medical science branch which directly benefits from stem cells for regenerative treatment was ophthalmology. The triumph of regenerative medicine in ophthalmology can be attributed to its accessibility, ease of follow-up, and the eye being an immune-privileged organ. Two key characteristic attributes of stem cells are pluripotency, the capacity to differentiate into multiple lineages, and proliferation. These cells have the ability to replace damaged or diseased cells under certain specific circumstances. Stem cell-based therapy has now reached a state where ocular tissues damaged by disease or injury can be repaired and/or regenerated. The eye is an ideal organ for studying regenerative medicine thanks to the ease of access for the therapeutic procedure as well as its status of being an immune-privileged organ. Such therapy involves various techniques in which stem cells are injected into both

the cellular and extracellular matrix microenvironments. Corneal epithelial stem cell transplantation has been the most employed stem cell-based therapy after bone marrow transplantation. Stem cell-based treatment in ophthalmology follows two possible ways: a cell replacement therapy strategy or a strategy involving trophic factor-based guidance cues. Throughout treatment, outcomes are related to different factors like our in-depth knowledge of the disease, the source of stem cells, the plausible mechanism driving the therapeutic outcome, and the mode of treatment. Considering specifically the anterior segment, we will analyze separately stem cell pools of the conjunctiva, the cornea, the trabecular meshwork, and the lens. In addition we will make few words about the iris stem cell pool. This pool also subspecializes in three types of cells with different capacities for multiplication: putative stem cells with high reproductive potential, the generally slow-cycling activity cells, and transit amplifying cells (TAC), which have a reduced reproductive potential but rapid expansion time.

2.1 Conjunctiva

The conjunctiva, apart from being a protection against pathogenic entry, is a connective tissue provided by a high vascularization that offers channels for proper flow of nutrients and fluids. From the anatomical point of view, the conjunctiva is an unkeratinized stratified squamous epithelium, in which goblet cells are also present, that covers the exposed scleral surface (bulbar conjunctiva) and the interior part of the eyelids (tarsal conjunctiva). Conjunctival cells undertake renewal similar to the corneal epithelium, but with a still elusive source of stem cells. Conjunctival stem cells undergo a differentiation pathway that can take them to become either mucin-producing goblet cells or epithelial cells. The dividing basal cell migration starts from the bulbar conjunctiva and takes it to the corneal surface before differentiation. Conjunctival epithelial cells are negative for CK3 and CK12 but positive for CK19. As shown in clonal culture assays, the stem cells located in the fornical niche can differentiate into epithelial cells as well as goblet cells. This provides important evidence that the stem cell pool supporting conjunctival renewal is located in the fornix region. Commitment to differentiate into goblet cells occurs relatively late; in fact goblet cells are generated by stem cell-derived transient amplifying cells. The decision of a conjunctival keratinocyte to differentiate into a goblet cell appears to be dependent upon an intrinsic “cell doubling clock.” Ocular processes that affect the cornea also affect the conjunctiva; some examples are conjunctival scarring, cicatricial pemphigoid, thickening, dry eye, or mucin. In order to treat conjunctival stem cell deficiency and scarring, conjunctival autografts, oral mucous membrane grafts, nasal turbinate mucosa grafts, and amniotic membrane are often used. Conjunctival cells cultured on amniotic membrane have been used for cell transplantation in patients with limbal stem cell deficiency (LSCD). Recent patient follow-up reports have shown that transplantation of autologous conjunctival epithelial cells improved the clinical parameters of total LSCD with respect to vision acuity, impression cytology, and in vivo confocal analysis. These cells were cultivated ex vivo on amniotic membrane with the presence of epidermal growth factor, insulin, cholera toxin, and hydrocortisone to produce the corneal lineage; the cells were transplanted after 2 weeks of culture. Ultrathin polymembrane substrate has also been shown to support conjunctival epithelial cell proliferation.

2.2 Cornea

The cornea is at the outermost surface of the eye, and its fundamental characteristic is transparency, which is crucial for vision. It is a clear lens that determines the

majority part of the dioptric power of the eye (about 43D). Its normal thickness is between 520 and 540 μm and is composed of five layers which are from the outside to the inside: corneal epithelium, Bowman's layer, corneal stroma, Descemet's membrane, and corneal endothelium. Forty-five million people worldwide are bilaterally blind, and another 135 million have a severe impaired vision defect in both eyes because of loss of corneal transparency. In order to correct this kind of problems, therapies ranging from local medications to corneal transplants, and more recently to stem cell therapy, could be applied. The corneal epithelium is a squamous epithelium that has a constant renewal activity, with a vertical turnover of 7–14 days. The corneal stem cell pool is located in the limbus, at the periphery of the cornea, and these cells are called limbal epithelial stem cells (LESCs). The corneal epithelium has a renewal process which is performed by cells generated at the limbus and, migrating from there, in opposition to other squamous epithelia in which each stem cell has the role of regenerating a limited area of epithelium. In the corneal epithelium, stem cells are located at the corneal periphery in the basal layer of the limbal region, called the palisades of Vogt. These are visualized in small clusters and are strictly associated with the stromal matrix and the basal membrane, thereby assisting in cell-cell, cell extracellular matrix, and paracrine signaling communication. The corneal epithelial basal layer is composed mostly of TAC at various stages of maturity, and this could be demonstrated by their elevated expression of a specific isoform of the transcription factor p63 along with a high nuclear to cytoplasmic ratio. The positivity of ATP-binding cassette subfamily G member 2 (ABCG2) has been detected in LESCs as well as in several other cells located in the suprabasal region of the limbus, and these markers could be used to identify the LESC pool. Some reports also indicate that an RNA binding protein called Musashi-1 can be used to stain LESCs. Corneal stem cells also express some other specific markers, enolase, cytokeratin (CK)19, and vimentin, but do not express CK3, CK12, or connexin 43, which are present only in mature corneal epithelial cells. Stromal multipotent stem cells have been identified and expanded to neurospheres in cultures. Corneal stromal stem cells are located in the anterior region of the stroma adjacent to the basal side of the palisades of Vogt and were identified as a side population using the DNA-binding dye Hoechst 33342. These cells expressed genes encoding ABCG2, Bmi1, CD166, c-kit, Pax6, Six2, and Notch1 similarly as mesenchymal stem cell and corneal early development markers. Corneal stromal stem cells, when differentiated, express keratocyte markers like keratocan, ALDH3A1, CXADR, PTDGS, and PDK4. LESC deficiency, either partial or complete, is pathological and is caused by either chemical or mechanical injury or thermal burns or acquired by diseases like aniridia and Stevens-Johnson syndrome. Treatment of such conditions involves LESCs transplantation therapy. In unilateral cases of ocular disease, LESCs from the healthy eye are expanded *ex vivo* for therapeutic purposes using specific protocols which involve amniotic membrane or fibrin in the presence or absence of growth-arrested 3T3 fibroblast feeder layers. Taking in consideration non-limbal cell types, cultured oral mucosal cells and conjunctival epithelial cells have been transplanted with success to treat LSCD in humans. The peripheral cornea has been proven to contain a higher density of keratocyte precursors with high proliferative capacity. Restoration of corneal transparency, stromal thickness, and collagen fibril defects have been demonstrated as solvable through the injection of corneal stromal stem cells in mice. If it will be shown as successful, such therapy would eliminate the shortage of corneas from donors needed for transplantations. Although stem cell transplantation is performed worldwide, standardized protocols need to be established because of variability in clinical outcomes. An application example of LESCs transplant could be in patients with LSCD who are suffering from a severe loss of vision and annoying irritation, being also poor candidates for conventional

corneal transplant. Hence, new surgical strategies have been devised by transplanting LSCs from an autologous or allogeneic source. When in total LSCD only one eye is involved, the reconstruction of the damaged corneal surface can be effectively performed by the application of conjunctival limbal autograft. Although conjunctival limbal autograft has high success rates, if transplantation is carried out at the acute stage of chemical burns when inflammation remains in “active” stage, the surgical outcome is not satisfactory; this notion has been verified in a rabbit model. The potential risk to the patient’s donor eye could be reduced with the application of different techniques: the first alternative is to perform LSCs allograft, in which an allogeneic source of LSCs is derived from either living donors matched with HLA or not matching cadavers. Systemic immunosuppression with cyclosporin A or other agents is necessary because the donor tissue is allogeneic, but this solution is potentially toxic. The success rate of limbal allografts declines with time even with systemic application of cyclosporin A. Elements implicated as factors contributing to the poor prognosis for keratolimbal allografts are keratinization, severe dry eye, chronic inflammation, uncorrected lid, and lid margin abnormalities. A combined immunosuppressive regimen together with a meticulous restoration of the ocular surface defense has been shown to further improve the long-term visual outcome of keratolimbal allografts.

2.3 Trabecular meshwork

The trabecular meshwork is a tissue included between the cornea and the iris in the anterior region that has the role of draining the aqueous fluid. It is divided into three parts which have their characteristic ultrastructures: inner uveal meshwork, corneoscleral meshwork, and juxtacanalicular tissue. Intraocular pressure is determined by the correct balance between aqueous production and outflow; a malfunction in this mechanism is a possible risk factor for the development of glaucoma. Trabecular meshwork cells also are implied in the removal of debris in the circulating aqueous humor. Trabecular meshwork cellular markers are vimentin, non-muscle actin, aquaporin-1, acetylated and acetoacetylated alpha-2 adrenergic receptor, matrix GLA protein, and chitinase-3-like-1. Recently, the isolation, characterization, and specific markers of trabecular meshwork cells have been widely studied. These studies suggest that trabecular meshwork cellular population has properties similar to stem cells, expressing mesenchymal cell-associated markers such as CD73, CD90, and CD105, and they have also the ability to differentiate into adipocytes, osteocytes, and chondrocytes. Moreover, further studies showed that trabecular meshwork cells with mesenchymal phenotype are isolated as a side population or as clones expressing specific stem cell markers, not present in mature cells, such as Notch1, OCT-3/OCT-4, ABCG2, AnkG, and MUC1. These stem cells have the ability to differentiate into the trabecular meshwork lineage expressing CHI3L1, AQP1, and TIMP3 markers that underlies to a phagocytic function. Lowering the intraocular pressure is the aim of treatments for glaucoma. The idea for this came primarily from the observation that trabecular meshwork cell division increased after argon laser trabeculoplasty. Current first-line treatments are topical and oral drugs, argon laser trabeculoplasty, and some surgical approaches. Stem cells isolated from human trabecular meshwork and expanded in vitro showed evidence of the ability to home to mouse trabecular meshwork and differentiate into trabecular meshwork cells in vivo according to recent studies. The expanded trabecular meshwork stem cells expressed the stem cell markers Notch1, ABCG2, and MUC1 and were expressing also the trabecular meshwork marker protein CHI3L1. These trabecular meshwork cells were multipotent and had phagocytic properties. Some groups are working on transplanting trabecular

meshwork cells or trabecular meshwork progenitor cells combined with argon laser trabeculoplasty as a novel cell-based therapy for glaucoma.

2.4 Lens

The lens is composed of the lens capsule, epithelium, and fibers and, like the cornea, is transparent. It is hypothesized that lens-specific stem cells reside in the lens capsule, although they have not yet been identified. The most confirmed hypothesis is that this cell pool comes from the ciliary body, which is anatomically close to the lens. It has been demonstrated that lens capsule regeneration occurs in lower vertebrates from cells residing in the ciliary body. According to this fact, the probability that lens stem cells might reside in the lens capsule is high. Lens progenitor cells have been derived from human ESCs as well as from induced pluripotent stem cells (iPSCs). Lens stem cells are presumed to have a significant role in the maintenance of the lens transparency and might be implied in cataractogenesis process or other lens abnormalities.

2.5 Iris

The iris has the anatomical role of dividing the space between the cornea and lens into anterior and posterior halves. The microscopic structure consists of an anterior limiting layer that lines the anterior part of the iris stroma that contains muscles, nerves, and vessels and is posteriorly lined by a layer of pigmented and non-pigmented cells. The stroma and the vascular structure of the iris take embryological origin from the anterior region of the optic cup. Epithelial cells of the iris pigment have the ability to grow in spheres and express markers of neural stem/progenitor cells such as Msi, Nestin, and Pax6. It has been revealed by studies from the mouse iris that these cells can also differentiate both in neuronal and glial lineages and express markers such as Rho, Chx10, Otx2, and Olig2. The iris pigment epithelial cells have the potential to be used in cell-based therapy, but nevertheless not much work on validation and quality assessment has been done. Further studies are needed before iris pigment epithelial cells can be used clinically.

3. Regenerative medicine in the posterior segment of the eye

Considering the posterior segment, the main interest is focused on the retina, the target of regenerative medicine. Retinal anatomy is quite complex, and focusing to the microscopic structure, it can be divided into nine layers of nervous tissue that interfaces with the outermost layer of the pigmented epithelium. From external to internal, there are inner segment/outer segment layer, external limiting membrane, outer nuclear layer, outer plexiform layer, inner nuclear layer, inner plexiform layer, ganglion cell layer, nerve fiber layer, and inner limiting membrane.

Stem cells are immature, undifferentiated, highly proliferative cells which are capable of self-renewing and differentiating into many cell types [1]. Therefore, stem cells represent a potentially endless source of tissue renewal; that is why, in the modern era, stem cell therapy has been considered a valid approach for many different pathologies. Ophthalmologists and researchers were not slow to guess the potential applications of stem cell therapy in various degenerative retinal diseases such as retinitis pigmentosa, age-related macular degeneration, Stargardt's macular dystrophy, and other pathological conditions affecting the posterior pole of the eye, including retinal vascular occlusions [1, 2]. These pathologies are responsible for a progressive decline in visual acuity which, in the case of RP,

Stargardt's disease, and AMD, are due to a constant and irreversible loss of retinal photoreceptors and outer nuclear layers. With such premises, it is easy to imagine a therapeutic approach based on the use of stem cells to restore the lost retinal tissue. Stem cells have shown to be able to perform additional functions, such as nutritional support, apoptosis inhibition, synapse formation, immunoregulation, and neurotrophin secretion [1] and have increased even more the enthusiasm for their application in the ophthalmological field. Furthermore, the use of stem cells in the eye seems to offer numerous advantages: firstly, the amount of stem cells required is relatively low, which implies lower costs than those required for the treatment of other tissues of the human body; secondly, the surgical approach is quite easy and the transplanted cells can be easily monitored with the imaging methods currently used in clinical practice. Finally, the immune privilege of the eye allows avoiding long-term immunosuppressive treatment [1]. Several experimental studies conducted on embryonic stem cells (ESCs), induced pluripotent stem cells (IPSCs), or mesenchymal stem cells (MSCs) have demonstrated that they tend to adapt to the retinal environment and can differentiate not only into photoreceptors and RPE cells but also into Müller, amacrine, bipolar, horizontal, and glial cells [1]. Retinitis pigmentosa represented one of the first targets of stem cell therapy in the ophthalmological area: pioneering animal studies have shown that pluripotent stem cells, when placed in murine retinitis pigmentosa models, are able to survive, multiply, differentiate, organize into, and function as photoreceptor cells, developing a retina-like organizational structure [3]; using mouse models, Singh et al. have established that at a stage when all host rod cells are lost, transplanted rod precursors can lead to the re-establishment of a proper, correctly polarized outer nuclear layer, indicating that stem cells may recreate a light-sensitive cell layer *de novo* and restore structurally damaged visual circuits. It has to be said that current methods for photoreceptor derivation from human pluripotent stem cells require long periods of culture and are often inefficient. Boucherie et al. [4] reported that formation of a transient self-organized neuroepithelium from human embryonic stem cells cultured together with extracellular matrix can induce a rapid conversion into retinal progenitors, which are capable of subsequently differentiating into photoreceptor precursors in only 10 days and later acquire rod cell identity within 4 weeks.

Following such promising results, the first phase I/II clinical trials in humans were approved in the United States in 2010; hESC-derived retinal pigment epithelium cells were transplanted into the eyes of patients with Stargardt's macular dystrophy and dry age-related macular degeneration [5]. During differentiation, the stem cells "displayed typical RPE behavior and integrated into the host RPE layer forming mature quiescent monolayers" [5]; after surgery, structural exams showed that cells had attached and persisted during the study. An improvement in best corrected visual acuity was reported both in the patient affected by Stargardt's macular dystrophy and in the patient affected by dry AMD. And what is more important, in 4 months of follow-up, clinicians did not identify signs of hyperproliferation, abnormal growth, ectopic tissue development, or immune-mediated rejection [1, 5], which represent the main concern about stem cell therapy. These findings support the safety of ESC-derived stem cells [1, 6].

Since they are autologous, IPSCs (obtained from reprogramming adult somatic fibroblast cells using retroviruses or lentiviruses) seem to have an even lower risk of rejection. However, because of their abnormal genetic composition, the risk of T cell-mediated immune response or oncogenesis should not be underestimated. In 2015, in fact, a Japanese study on IPSCs that was being conducted on human retinas was interrupted because of a new genetic mutation that occurred in the IPSCs of one of the patients.

MSCs can differentiate into mesodermal, ectodermal, and endodermal cells and can be obtained from many different tissues, including cord and peripheral blood, teeth, central nervous system, liver, bone marrow, and adipose tissue [1]. Several studies have demonstrated that MSCs can easily turn into neuron-like cells and repair damaged cells through a paracrine action which results in a neuroprotective function. In rats, subretinal transplant of MSCs led to their differentiation into different retinal cell types. These results encouraged clinical trials on humans. In a study, Park et al. [2] isolated CD34+ cells from bone marrow and injected it intravitreally. They enrolled six patients affected by dry AMD, retinitis pigmentosa, or retinal vascular diseases. Follow-up included serial ophthalmic examinations, perimetry and/or microperimetry, fluorescein angiography, ERG, and OCT. After 6 months of follow-up, there was no evidence of worsening neither in BCVA nor in full-field ERG. No signs of intraocular inflammation were observed. Other studies on MSCs confirmed their safety in terms of hyperproliferation and systemic side effects. However, as reported, further MSC applications led to other sight-threatening intraocular complications such as elevated intraocular pressure, vitreous hemorrhages, tractional and rhegmatogenous retinal detachment, development of preretinal and vitreal fibrous tissue, and shallowing of the anterior chamber.

Retinal pigment epithelium replacement represents a promising evolution of stem cell therapy. The outer segments of photoreceptors have a very high metabolic demand and undergo a daily renewal; in the healthy retina, the apical processes of the RPE envelope the outer segments of rods and cones, which contain visual pigment, resulting in a diurnal outer segment recycling. Pathological conditions such as drusen deposits, accumulation of lipofuscin, or ischemic insult can result in a disruption of RPE, slowing photoreceptor metabolism and leading to cellular damage. RPE was one of the first tissues to be differentiated in vitro. Nowadays, there are many ongoing clinical trials for pluripotent stem cell-derived RPE replacement. The success of RPE replacement can be explained by various factors: for a start, RPE cell biology and phenotypes are precisely described and conserved among species [7]; the differentiation of embryonic stem cells into RPE cells follows default pathways that are well characterized; animal models of RPE dysfunction are easily available; the amount of RPE required to functionally restore affected retinas is relatively small compared with photoreceptors [7]; and the RPE layers within the retina can be easily visualized using optical coherence tomography, adaptive optics scanning laser ophthalmoscopy, and fundus image. Moreover, studies on animal models have established that sheet transplantation is much more beneficial and effective than single-cell suspension [7], making retinal patches a fascinating approach to degenerative retinal diseases. However, further studies have proven this technique unsuccessful in human models. Nevertheless, studies on retinal sheet transplantation are still ongoing.

Retinal tissue engineering is another intriguing idea for treating late-stage retinal conditions, but various technical and biological issues coming from lab-grown neuroretinal tissue design still need to be solved before it can work in clinics. The size of 3D retinal tissue derived from human pluripotent stem cells is much smaller than that required to obtain a significant clinical outcome, and the implantation of a single piece of retinal organoid may not result in an appreciable improvement in visual acuity in humans [8]. Because of their plasticity, human pluripotent stem cells make an extraordinary source for regenerative medicine. The current challenges of retinal tissue engineering include establishing reproducible protocols for the creation of retinal organoids from stem cells, producing larger pieces of retinal tissue from stem cells along with quality supporting biomaterials, improving surgical methods of delivering retinal organoids into subretinal space, and finding

biomaterials to facilitate the survival and functional integration of hPSC-derived grafts into the host's synaptic environment [8].

In conclusion, pioneering studies conducted on animal models have provided hopeful evidence for the hypothesis that stem cell therapy is a valid approach to sight-threatening degenerative retinal diseases, including retinitis pigmentosa, Stargardt's disease, dry age-related macular degeneration, and vascular occlusions. A number of phase I/II clinical trials on humans seem to have confirmed the effectiveness of this method. We now know for sure that when placed in an appropriate tissue niche stem cells not only survive and proliferate but are capable of differentiating into proper retinal cells which exhibit functional characteristics of real photoreceptors, resulting in the development of a retina-like structure [9]. Further studies are needed to put such promising experimental data into clinical practice and establish standardized procedures for the application of stem cell therapy in the ophthalmological field.

4. Cell therapy and atrophic retinal diseases: our experience

Visually impaired patients are affected by a series of different neuroretinal diseases that can target nerve cells such as ganglion cells (RCG), photoreceptors, or support cells such as retinal pigment epithelium cells (RPE). The evolution of these pathologies leads to serious impairment of vision. There are many types of retinal degenerative diseases, including glaucoma, hereditary retinal dystrophy such as retinitis pigmentosa (RP) or Stargardt's disease, age-related macular degeneration (AMD), degenerative myopia, and diabetic retinopathy (DR). In each of these pathologies, regardless of its nature, a certain sequence of molecular events gradually leads to the death of retinal cells.

These mechanisms cover various biological aspects and can be summarized as follows:

- Neurotrophic aspects
- Oxidative aspects
- Vascular alterations
- Apoptosis
- Inflammation and para-inflammation [10, 11]

The sequence can begin with oxidation, photooxidation, or photosensitivity. This is followed by the release of oxidizing substances and free radicals in the cellular environment which in turn causes lipid peroxidation, oxidation of the critical bonds in the protein chains and rupture in those of the DNA, activation of the endogenous nuclease, inhibition of the expression of the Bcl2 gene, and priming of mechanisms of cell apoptosis.

In physiological conditions, healthy retinal cells possess an arsenal of substances with protective action, including antioxidant systems (e.g., SOD) and enzymes, which serve to balance oxidants and free radicals, minimizing damage. One of the best known mechanisms to block or procrastinate apoptotic processes is the activation of the Bcl2 gene by growth factors, thus avoiding the fate of death, regardless of the triggering cause. There are cells such as Müller cells or RPE cells, capable of producing, under hypoxic conditions, angiogenic and neurotrophic

factors such as FGF and VEGF in order to counterbalance the insult, provided that it is transient [12]. In the case of cellular imbalance, for example, for genetic or inflammatory reasons, for reduction of the chorioretinal blood flow or when a large part of the cells has undergone apoptosis and death with consequent induction of a chronic para-inflammatory condition, the trigger of neuroretinal pathologies, or their progression, can occur. In our opinion, it is possible to apply a therapy aimed at reducing the impact and progression of the disease based on these mechanisms. The therapeutic aim is to slow down or prevent the death of residual retinal cells [13, 14], highlighting the possible efficacy of cell therapy on neurotrophic pathologies of the retina. Currently, in the presence of a dystrophic pathology responsible for a low vision condition, the patient can resort to visual rehabilitation using magnifying aids or filters to improve contrast. In a smaller number of centers, it is possible to benefit from therapies based on the neuro-modulation of visual signals, in order to improve not only the image on the retina but also the perception of the same at the cortical level.

However, the progressive loss of photoreceptors contributes to reducing the performance obtained with visual rehabilitation, and the social impact of the progressive loss of functional autonomy should not be underestimated. New therapeutic approaches to neuroretinal degenerations for therapy include restoring defective genes, when the disease is caused by a genetic defect, and transplanting stem cells to replace or repair defective or dead cells, regardless of the cause [15, 16]. Gene therapy is a causal therapy but is currently not clinically available, and the therapeutic results obtained experimentally are still marginal *in vivo*. For this reason, the interest of the scientific community is also addressed to stem cell-based repair strategies, consisting in the systemic or local injection of stem/progenitor cells for the treatment of multiple chronic pathologies [15, 16]. Stem cells are undifferentiated cells that have the ability to self-renew and differentiate into mature cells. On this basis, cell replacement therapy has been evaluated in recent years as a viable alternative for various pathologies. This therapy hypothesizes the generation of retinal cells from stem cells to replace damaged cells in the diseased retina. This goal can be achieved by releasing embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), and mesenchymal stem cells (MSCs) [17] in specific target positions of the eye. Stem cell therapy opens up the possibility of replacing or regenerating the cells now destroyed during the most common neurodystrophic diseases of the retina. However, ESC and iPCS have generated much controversy over ethical, immunological, and oncological issues. Instead, the use of MSC appears to be free from these concerns. The reparative therapy operated by the implanted cells aims to create better conditions for the viability of the residual cells, preventing or slowing their decline. We could therefore define cell therapy, or mediated cell therapy, or any therapeutic modality based on the use of cell grafts that aim not only at the neuroenhancement of compromised cells and the possible regeneration of some elements (such as receptors, mitochondrial components, connection fibers) but also to their integration with the above cells. It remains to be asked whether it is easier to preserve or promote the survival and function of diseased cells than to actively restore retinal cells after they have disappeared following the disease.

4.1 Mesenchymal stem cells as a therapeutic tool

MSCs are characterized by a panel of superficial cell markers proposed by the International Society for Cellular Therapy in 2006. The MSC population is defined as positive over 95% for CD105, CD73, and CD90 and negative over 95% for CD45, CD34, CD14 or CD11b, CD79 α or CD19, and HLA-DR [18]. Some molecules present on the surface of MSCs and endothelial cells, such as P-selectin and integrins,

let the MSCs themselves migrate to the lesion sites, following their intravascular administration. After joining the endothelium, the MSCs are able to cross it in a metal-dependent way. MSCs are obtainable from umbilical cord blood, peripheral blood, bone marrow, and adipose tissue. MSCs are multipotent: appropriate culture conditions associated with specific growth factors drive the differentiation of MSCs into specific cell types and can differentiate into various cell types, including osteocytes, adipocytes, vascular endothelial cells, cardiomyocytes, pancreatic beta cells, and hepatocytes. Therefore, MSCs play a key role in organogenesis, remodeling, and tissue repair. Experimental studies have also reported that MSCs have the potential to differentiate into retinal progenitor cells, photoreceptors, and retinal neuron-like cells. Furthermore, stem cells, in particular mesenchymal stem cells (MSCs), are able to perform multiple functions, such as immunoregulation, anti-apoptosis of neurons, and neurotrophin secretion, and the current opinion is that MSCs can exert neuroprotective and proregenerative effects, through the secretion of factors that act in a paracrine way. An increasing number of studies also report that MSCs are capable of giving rise to neuron-like cells. Not only are they able to differentiate into neurons for cell replacement therapy but to maintain and regulate the microenvironment through paracrine effects by modulating the plasticity of damaged host tissues [19, 20].

Of all the MSC collection sites, adipose tissue is particularly interesting and rich in stem cells derived from fat, called ADSCs [21]. These cells are able to secrete neurotrophic growth factors and promote survival, restore the release of the synaptic transmitter, integrate into existing neural and synaptic networks, and re-establish functional connections [22]. ADSCs produce bFGF, vascular endothelial growth factor (VEGF), macrophage colony stimulating factor (M-CSF), granulocyte-macrophage colony stimulating factor (GM-CSF), placental growth factor (PlGF), the transforming growth factor ($TGF\beta$), hepatocyte growth factor (HG), insulin growth factor (IGF-1), interleukin (IL) and angiogenin, ciliary neurotrophic factor (CNTF), and the brain-derived neurotrophic factor (BDNF). Another type of mesenchymal tissue is represented by adipose tissue which, just like the bone marrow, contains a large population of stem cells within its stromal compartment. Stromal adipocytes or fat stromal cells secrete a series of hormones, factors, and protein signals, called adipokines, which are associated with the role of the adipocyte in energy homeostasis. Fat cells produce the basic fibroblast growth factor (bFGF), the epidermal growth factor (EGF), the insulin-like growth factor-1 (IGF-1), the interleukin (IL), the transforming growth- β ($TGF\beta$), the pigmented epithelium-derived factor (PEDF), and adiponectin. Another type of cell of mesenchymal origin is the platelet, originating from the subdivision of megakaryocytes. Platelets, normally known for their hemostatic action, also release substances that promote tissue repair, angiogenesis, and inflammation modulation. In addition, they induce cell migration and adhesion at angiogenesis sites, as well as the differentiation of endothelial progenitors into mature endothelial cells. Platelets produce platelet-derived growth factor (PDGF), IGF-1, $TGF\beta$, VEGF, bFGF, EGF, platelet-derived angiogenesis factor (PDAF), and thrombospondin (TSP) [23].

The therapeutic potential of mesenchymal cells is based on the stabilizing effect against the retinal cells exerted by the cytokines and the growth factors released paracrinically when they are grafted. The binding of the growth factor to the specific surface receptor placed on the cytoplasmic membrane of the target cell is the initial step that triggers a cascade of events, activating particular second messengers that guarantee the signal transduction at the intracellular level. The ultimate goal is the regulation of enzyme activity or gene expression (**Figure 1**) [24, 25]. In particular, activated transcription factors, entering the nucleus and binding directly or indirectly to DNA, regulate the expression of various genes with

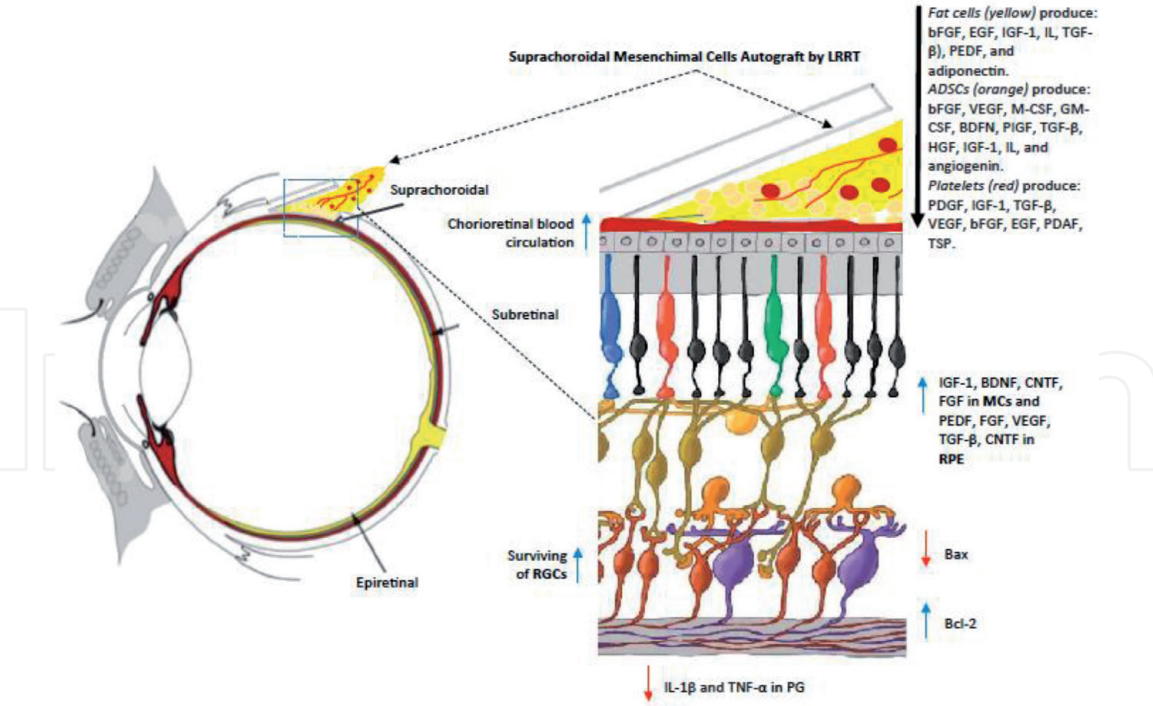


Figure 1.
The growth factors produced by mesenchymal cells implanted in the suprachoroidal space can act both directly on the retinal cells and indirectly, through the mediation of Müller (MC) and RPE cells, generating angiogenic, neurotrophic, anti-inflammatory, and antiapoptotic effects [26, 27]. Image courtesy of P. Limoli-Milan Low Vision Study Center.

different mechanisms, promoting greater synthesis of proteins including enzymes and cytokines. These end products play a key role in cell survival, as assessed by the improvement in electrical activity recorded by ERG [27]. The growth factors are essential to trigger the cell transition from G0 or resting phase to G1 or growth phase. Furthermore these molecules stimulate a wide range of cellular processes, including mitosis, cell survival, migration, and cellular differentiation.

4.2 Pathophysiological co-factoriality and cell therapy

The grafting of mesenchymal cells into the suprachoroidal space promotes a continuous paracrine increase in GF that can positively interfere with the evolution of retinal diseases in several ways.

Therapeutic activity can be classified into:

1. Hemorheological activity
2. Antioxidative activity
3. Anti-inflammatory activity
4. Antiapoptotic activity
5. Cytoprotective activity
6. Therapeutic synergy with electrical stimulation (ES)

It is worth noting that the boundaries between these categories are not necessarily defined. The hemorheological activity and its increase help to restore an

effective retinal perfusion. Photoreceptor loss that occurs in retinal diseases has been identified as the cause of microvascular dysfunction due to the release of cellular waste secondary to apoptosis. In fact, there is a correlation between the extent of the blood flow and the evolutionary stage of the atrophic pathology, in a vicious circle that leads to the final loss of other photoreceptors. Several factors such as VEGF, bFGF, angiogenin, PDAF, PlGF, PDGF, EGF, and TGF- β have been shown to promote endothelial regeneration and therefore can contribute to the reperfusion of the choriocapillaris. Furthermore, others, including TSP and PEDF, inhibit pathological neovascular processes [28, 29]. Antioxidative activity prevents oxygen-induced photoreceptor cell death. One of the underlying causes of photoreceptor deterioration, which may explain the evolution of retinal degeneration, is hyperoxia which results in a more intense oxidation process and in the formation of reactive oxygen species (ROS). Excessive generation of reactive oxygen species causes damage to membrane lipoproteins and cellular DNA, thus leading to apoptosis and the death of photoreceptors [30]. The mechanism involved in hyperoxia can be illuminated by the excessive amount of oxygen in the choroid, similar to the arterial oxygen level, which results from the deterioration and death of the photoreceptor, in addition to foveal exposure to light and the concomitant lack of anti-enzyme oxidants, such as superoxide dismutase (SOD), glutathione-peroxidase, and catalases, normally expressed in the mitochondria of the internal segments of the cone and capable of catalyzing the decomposition of hydrogen peroxide into water and oxygen molecules [31]. The concentration of bFGF within photoreceptors has been shown to increase in response to stress in order to promote retinal cell survival and prevent oxygen-induced photoreceptor cell death [32, 33]. Anti-inflammatory activity can counteract the negative effects induced by microglial activation, which occurs as soon as the apoptotic processes induced by retinal degeneration begin [34, 35]. In turn, the apoptosis and death of photoreceptors are suggested by the ignition of an inflammatory microclimate that supports the chronicity and progression of a large number of neurodegenerative diseases. In particular, RPE performs a series of essential processes for homeostasis and retinal function and constitutes the front of the immune defense of the retina: RPE cells are able to secrete a diversified panel of pro-inflammatory cytokines, for example, IL-6, IL-8, chemoattractant monocyte protein-1 (MCP-1), and interferon- β (IFN- β), as well as anti-inflammatory factors, e.g., IL-11 and TGF- β . Intravitreal administration of MSC has been shown to exert a significant effect on the host's immune response by suppressing the production of pro-inflammatory cytokines, such as IFN- β and TNF- α through IL-1 receptor antagonist (IL-1RA) and prostaglandin E2 receptor (PGE2R) activation [36]. The therapeutic effect of MSCs is corroborated by the neurotrophic action of ciliary neurotrophic factor (CNTF) and brain-derived neurotrophic factor (BDNF): in culturing retinal ganglion cells, under conditions of oxidative stress, MSC expels the last factor that helps reduce pro-inflammation cytokine release, e.g., tumor necrosis factor- α (TNF- α) and interleukin-1 (IL-1) [33]. M-CSF, GM-CSF, and IL exercise an anti-inflammatory function and recruit macrophages by chemotaxis that help remove intraretinal cell debris [37]. The antiapoptotic activity is regulated by cytokines with an inhibiting (antiapoptotic) or inducing apoptosis (pro-apoptotic) action [38].

Proteins of the Bcl-2 family are particularly known for their regulation of apoptosis by interacting with caspases, a family of cysteine-containing protease enzymes (proteinases or caspases specific to cysteine's aspartate). RPE and Müller cells produce a wide heterogeneity of factors, e.g., fibroblast growth factors (FGF-1, FGF-2, and FGF-5), transforming growth factor- β (TGF- β), insulin-like growth factor 1 (IGF-1), ciliary neurotrophic factor (CNTF), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), some members of the Interleukin family, and the

pigmented epithelium factor (PEDF). This multitude of growth factors, released in the retinal cytosol, is able to produce a wide trophic action on adjacent structures. As a consequence, the progressive loss of RPE and Müller cells hinders the increment of these bioactive agents: their antiapoptotic action is therefore slowed down or completely blocked. The administration of mesenchymal cells can interfere with the apoptotic process involved in retinal degeneration. The growth factors excreted by the grafted mesenchymal cells perform a variety of functions; in particular they are able to facilitate the expression of the Bcl-2 gene in order to avoid the inexorable death of the cells, regardless of the root causes [17]. The cytoprotective activity of the GF contributes to neuroprotection by regulating the metabolic activity of the photoreceptors, which is widely compromised in diseases of the retina. Like bFGF, PEDF has been found to exert neurotrophic activity, inducing the overall survival of photoreceptors [39]. Significant data currently exist to suggest that certain factors such as EGF play a role in potentiating the neuroprotective action of Müller cells by stimulating their intracellular transcription and bFGF expression [40]. The VEGF released by the PRP has been shown to stimulate the proliferation of ADSCs which therefore promote the survival of grafted autologous fat and adipocytes [41]. BFGF is known to directly promote the survival of photoreceptors [42]. Synergy with electrical stimulation (ES) addresses four main aspects: survival of native cells, survival of transplanted cells, integration of transplanted cells, and functional formation of synapses/axon regeneration [43]. In recent years, the synergy between cell therapies and electrical stimulation has started to be considered as a possible treatment for degenerative diseases. Rat retinas treated with ES showed a reduction in apoptosis [44]. It has also been shown that in light-induced retinal degeneration models, stimulation with ES contains the death of photoreceptors and preserves the length of the external segment [45]. Consequently, it can be assumed that ES treatment can create a more balanced and less hostile environment by modifying the secretion of neurotrophic factors. ES affects the upregulation of neurotrophic factors in Müller cells normally involved in this protection mechanism. After ES, increased expression of *in vivo* beta fibroblast growth factor (b-FGF), insulin growth factor 1 (IGF-1), and brain-derived neurotrophic factor (BDNF) was observed. Conversely, ES reduces the production of pro-inflammatory cytokines such as tumor necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β), and the pro-apoptotic gene Bax. The release of neurotrophic factors from the postsynaptic membrane made possible by neuromodulation, together with the enrichment of the same factors in the extracellular environment managed by autologous grafts, determines the formation of synapses at a presynaptic level, facilitating and strengthening neurotransmission [44, 45].

4.3 Cell therapy and routes of administration

The effectiveness of cellular treatments for atrophic pathologies of the retina, in order to stabilize and enhance the visual function, is based on two key elements: on the one hand, the surgical implant techniques and the cell lines used, and on the other by the quantity and quality of residual retinal cells, in other words from the earliness of the treatment. The technique must be simple, totally risk-free, and painless, and the exploited cells must not cause further damage to the residual retinal cell or to the person. Cytokines and growth factors, released paracrinically from the cells administered, must bind to membrane receptors to trigger the pathway of intracellular signal transduction. They still require retinal cells that are still alive.

From the studies carried out so far, it seems that the greater the number of residual cells, the greater the interaction between GF and chorioretinal cell membrane receptors, cellular activity, and, ultimately, the improvement of visual performance (VP) [27]. The release of growth factors in a retina with very low

cellularity hardly causes detectable neuroenhancement. To achieve this goal, different approaches have been explored by inserting these cells in the subtenonian space and in the intravitreal or subretinal space. But it seems that positioning the implant in the suprachoroidal space can satisfy efficacy and safety. In fact, the graft under the tenon, although it has therapeutic significance, does not allow the growth factors produced to reach the neuroretinal tissues inside the sclera in important quantities.

Intravitreal injections of cellular material are effective and simple to perform, but it is necessary to pierce the bulb and leave this material free in the vitreous chamber. Serious complications such as infection, vitreoretinal tractions, and bleeding are also possible.

The release in the subretinal area seems to be the best for the possibility of a potential modification of cell lines due to the direct contact of MSCs with neuronal cells, but their grafting is even more dangerous when the retina is compromised by atrophic diseases [46]. The suprachoroidal graft maximizes the supply of growth factors that flow directly to the choroidal level and through the choroid to the entire retina without creating bulbar perforation. In our experience, in order to have the therapeutic action of growth factors in the retinal environment, we have explored the possibility of treating the dystrophic retina with the implantation of the cell types of mesenchymal origin mentioned above, in detail adipose stromal cells (ASC), stem cells derived from adipose tissue (ADSC) contained in the stromal-vascular fraction (SVF) of adipose tissue, and platelets (PLT) recovered in platelet-rich plasma (PRP) [47–49]. To this end, we used a surgical technique called Limoli retinal restoration technique (LRRT), described in previous works (**Figure 2**) [27, 50, 51]. The autotransplantation of ADSC, ACS, and PLT above the choroid plane improves the incretion of the bioactive factors produced in the choroidal flow and, consequently, promotes their widespread diffusion through the

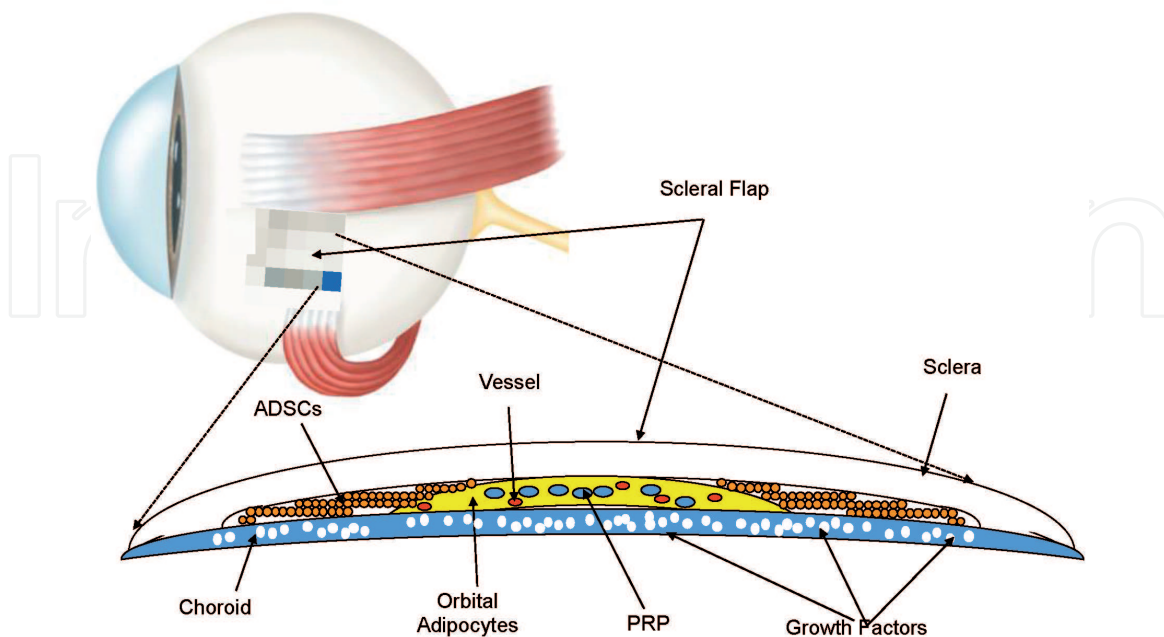


Figure 2. Autotransplantation of adipose tissue, ADSCs from vascular-stromal fraction, and PRP according to the Limoli retinal restoration technique (LRRT). The production of growth factors (GF), characteristic of these cells, is poured directly into the choroidal flow in paracrine mode, helping to maintain the trophism of the retinal cells. The GF, through the choroidal flux, have a direct action on the choroid, on the Müller cells, on the RPE cells with improvement of the physiology of the external segments (OS), on the rods, and on the cones. Image courtesy of P. Limoli-Milan Low Vision Center.

retinal tissue, finally exuding in the vitreous body. This action positively influences some functional parameters after interaction with the residual cells.

The relapses of cell therapy favor a better choroidal perfusion and a higher trophism of the photoreceptors, both directly (GF) and mediated by the RPE and Müller cells. It is therefore believed that the interaction between retinal cells and growth factors plays a crucial role in leading to an improvement in the prospects of degenerative retinopathy, to prevent and/or delay its progression.

The possible goals with cell therapy are schematically:

- Restoration and neuroenhancement on residual cells (**Figure 3**)
- The partial reduction of the scotoma (**Figure 4**)
- The improvement of the reading performance by stabilizing the fixation obtained with neuroenhancement (**Figure 5**)
- The improvement of the choroidal flow (**Figure 6**)
- The conservation of useful areas such as the fovea (when it is still present) or the preferential reading field (**Figure 7**)
- The slowing down of retinal disease (**Figure 8**)

In our study, greater foveal or retinal thickness is associated with a better prognosis. On the other hand, the lack of cells cannot make interactions between growth factors and membrane receptors possible [27, 51]. For this reason, cell therapies

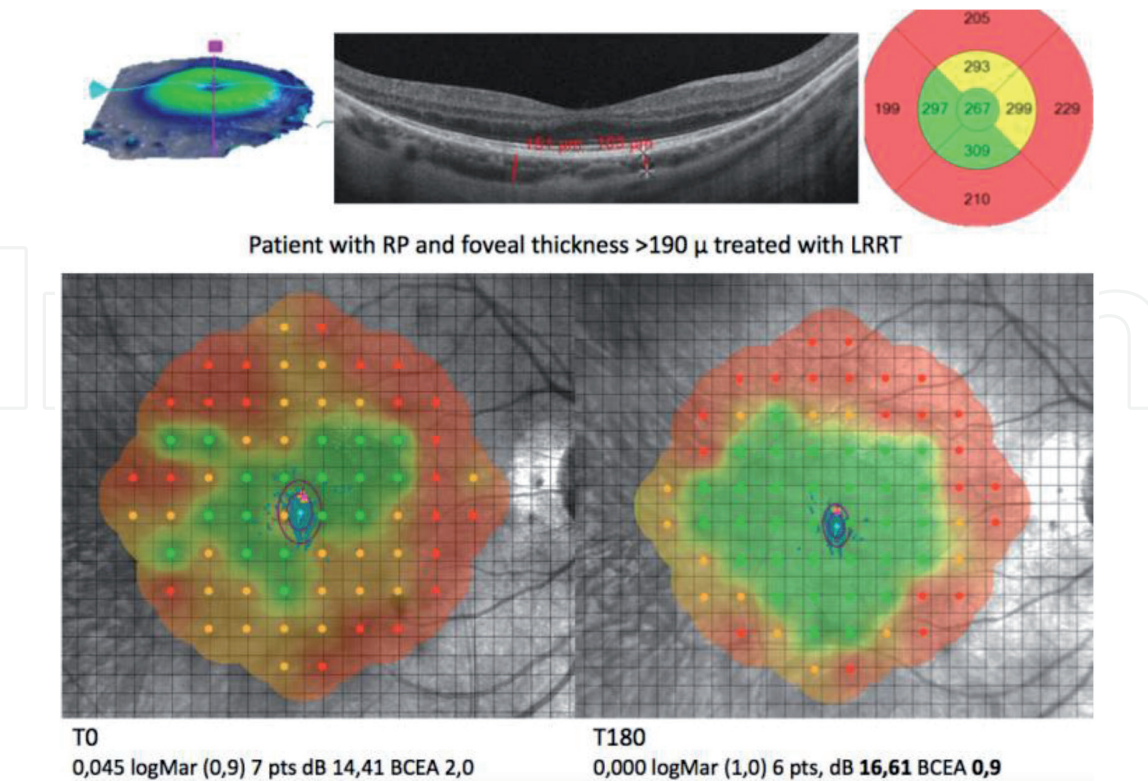


Figure 3. The image shows the effect of a suprachoroidal implantation of autologous mesenchymal cells in a patient with retinitis pigmentosa. The wealth of cells in the foveal area, documented by OCT, and therefore the high number of interactions between growth factors produced and specific membrane receptors, has allowed (T180) an increase in visual performance (BCVA, dB, pts). Image courtesy of P. Limoli-Milan Studies Center.

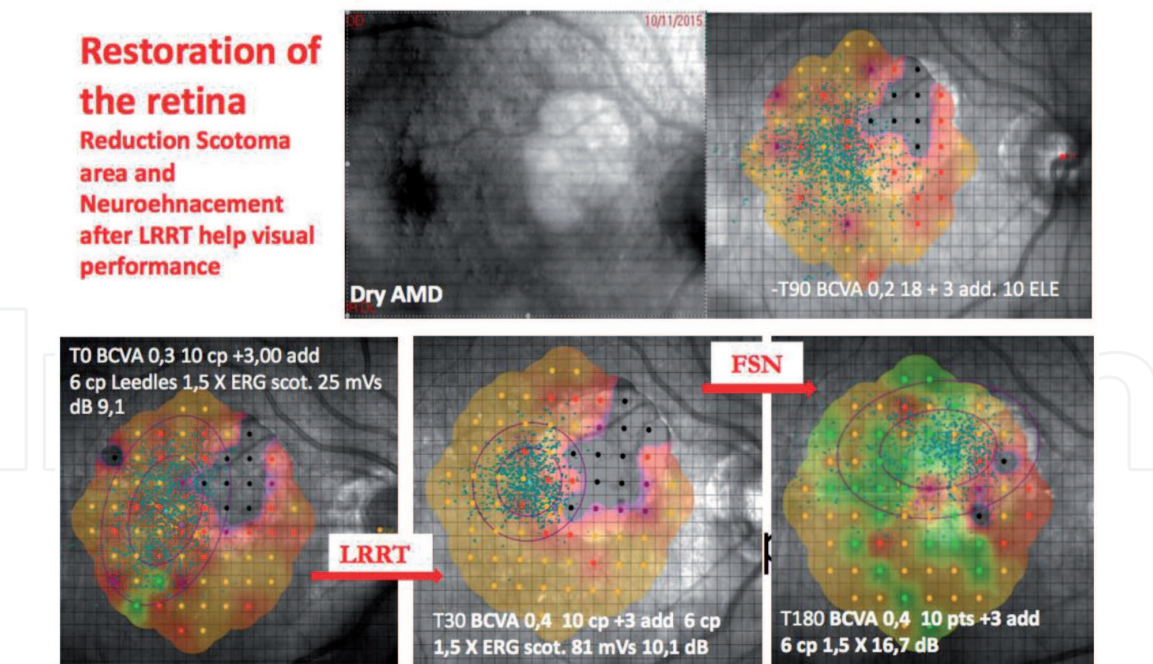


Figure 4.
Patient suffering from dry AMD with areolar evolution (retinography top left). The patient treated for 3 months with supplements did not show any increase in sensitivity even if we recorded a near viscous increase (picture below left). After suprachoroid implantation of autologous mesenchymal cells (T30 and T180), we observed an increase in sensitivity outside the atrophic area and a further improvement in the visual acuity. The near vision passes from 18 points of the initial evaluation to 10 points of the final evaluation which took place 9 months later. Image courtesy of P. Limoli-Milan Low Vision Center.

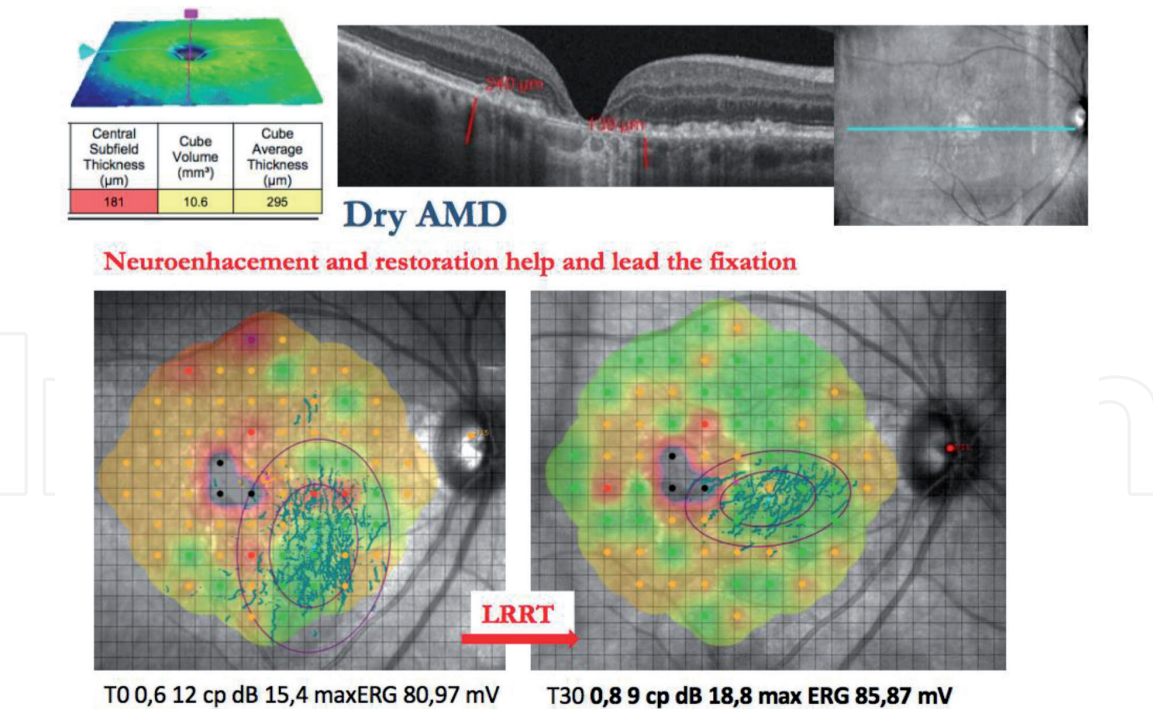


Figure 5.
The figure shows a case of dry AMD with a small atrophic area. An autologous suprachoroidal implant was performed and from the first month (bottom right) an improvement in visual performance was observed in terms of sensitivity, electrical activity, and visual acuity for far and near. Retinal neuroenhancement favored the re-centering and stabilization of fixations. Image courtesy of P. Limoli-Milan Low Vision Center.

must be proposed as soon as the disease starts to progress, when the cells are still numerous and the patient realizes the functional change. If a disease is stable and its impact on vision is accepted by the patient, it is not advisable to propose cellular

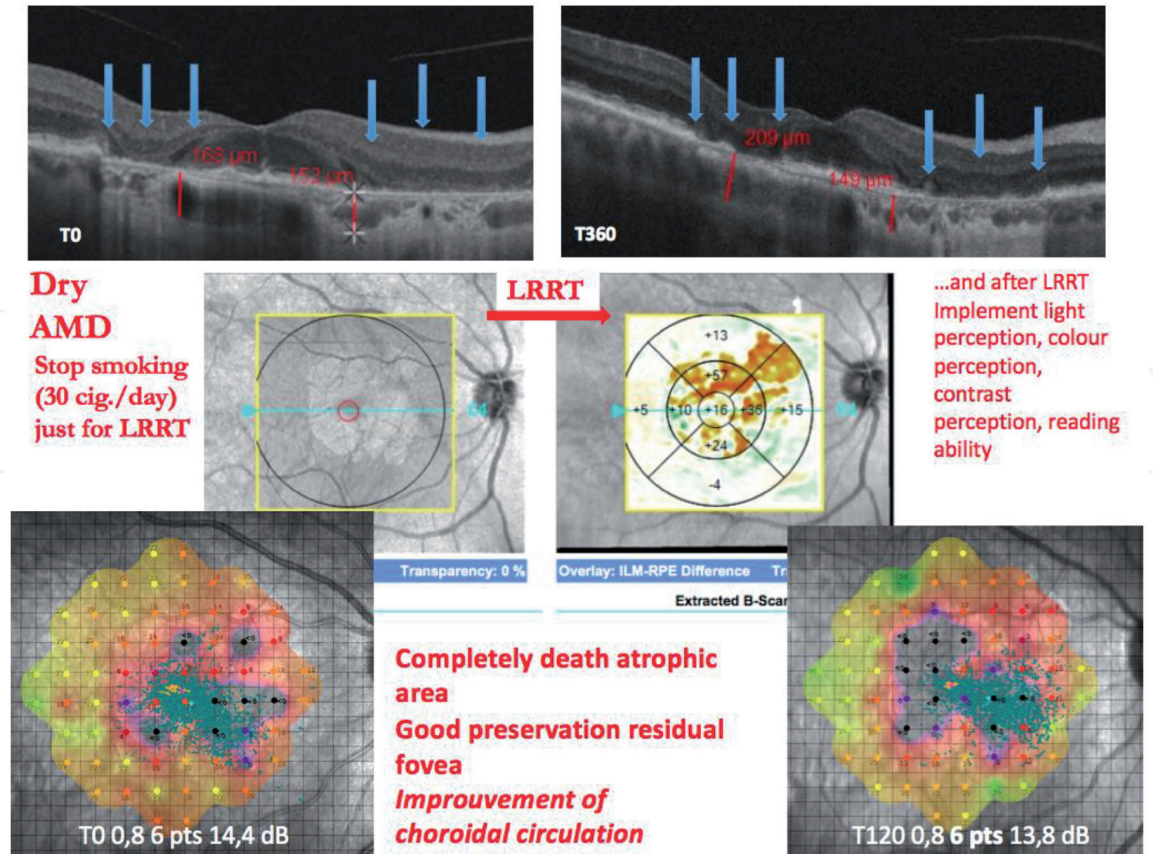


Figure 6. Patient suffering from dry AMD with a peripheral areolar evolution (blue arrows at the top left). Sensitivity appears to be compromised by a paracentral scotoma, but survival of the fovea allows for good visual ability (bottom left). After autologous suprachoroidal graft of mesenchymal cells (T180), we observed an increase in the thickness of the choroid (top right and center right). Despite the increase in the paracentral scotoma which has become more profound as an expression of a now dying area, the improvement of the choroidal circulation has contributed to the maintenance of the foveal area and the stabilization of visual performance. Image courtesy of P. Limoli-Milan Low Vision Center.

surgery. Knowledge of the overall amount of retinal cells is of particular importance: the rehabilitator and surgeon should be aware of this as a precise predictor of outcome for patients treated with cell therapy.

5. Conclusions

Stem cell therapy is going to change the natural history of various ophthalmologic conditions. For example, several studies have highlighted the chance to repair corneal damage through the implantation of conjunctival cells grown on the amniotic membrane with a good clinical outcome. In addition, an improvement in clinical parameters was observed in patients with limbal stem cell deficiency through the implantation of autologous conjunctival stem cells. This is evidenced by an increase in the visual acuity and a reduction of irritation. Despite biochemical evidences of the existence of stem cell populations in the remaining portions of the anterior segment (trabecular, iris, and crystalline), their clinical use is still under study.

Pioneering studies conducted on animal models have provided hopeful evidence for the hypothesis that stem cell therapy is a valid approach to sight-threatening degenerative retinal diseases, including retinitis pigmentosa, Stargardt's disease, dry age-related macular degeneration, and vascular occlusions. A number of phase I/II clinical trials on humans seem to have confirmed the effectiveness

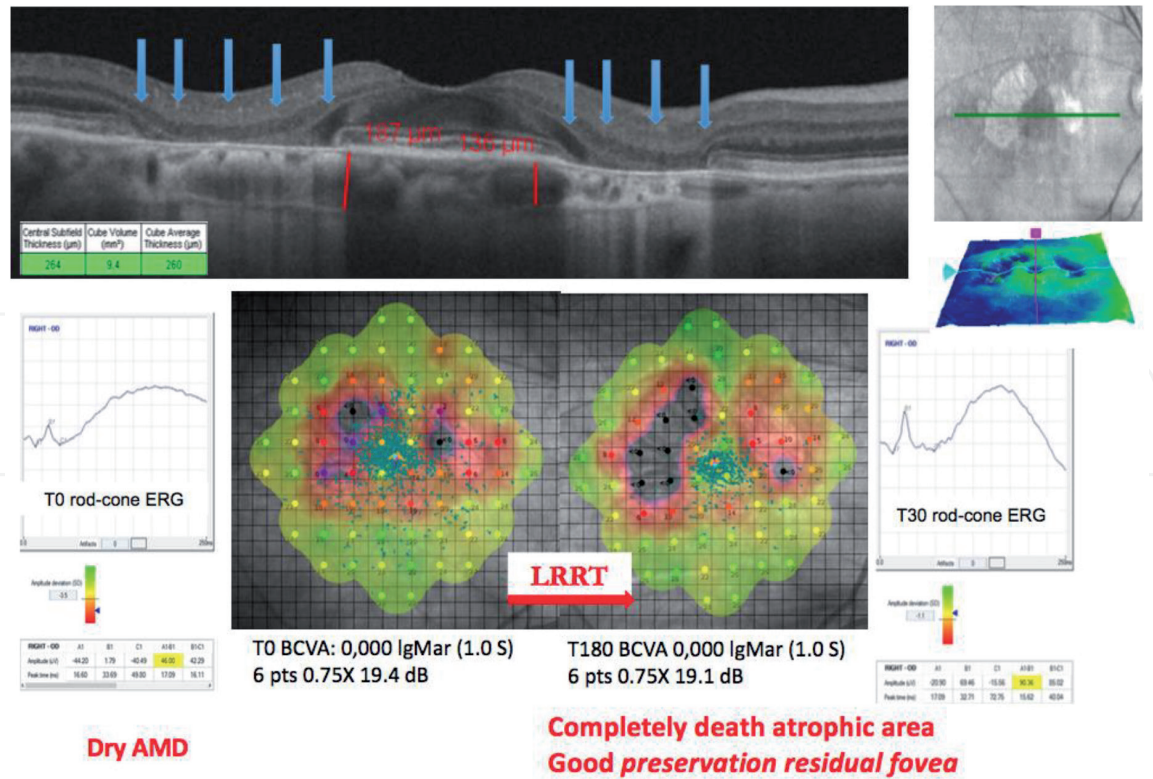


Figure 7.
Another case of dry AMD with saving of the foveal area (above) and To. Six months after LRRT, despite the progression of the scotoma within the paracentral atrophic areas, the fovea has maintained its sensitivity, and visual performance has been preserved (T180). ERG activity (bottom left) showed an increase (bottom right). Image courtesy of P. Limoli-Milan Low Vision Center.

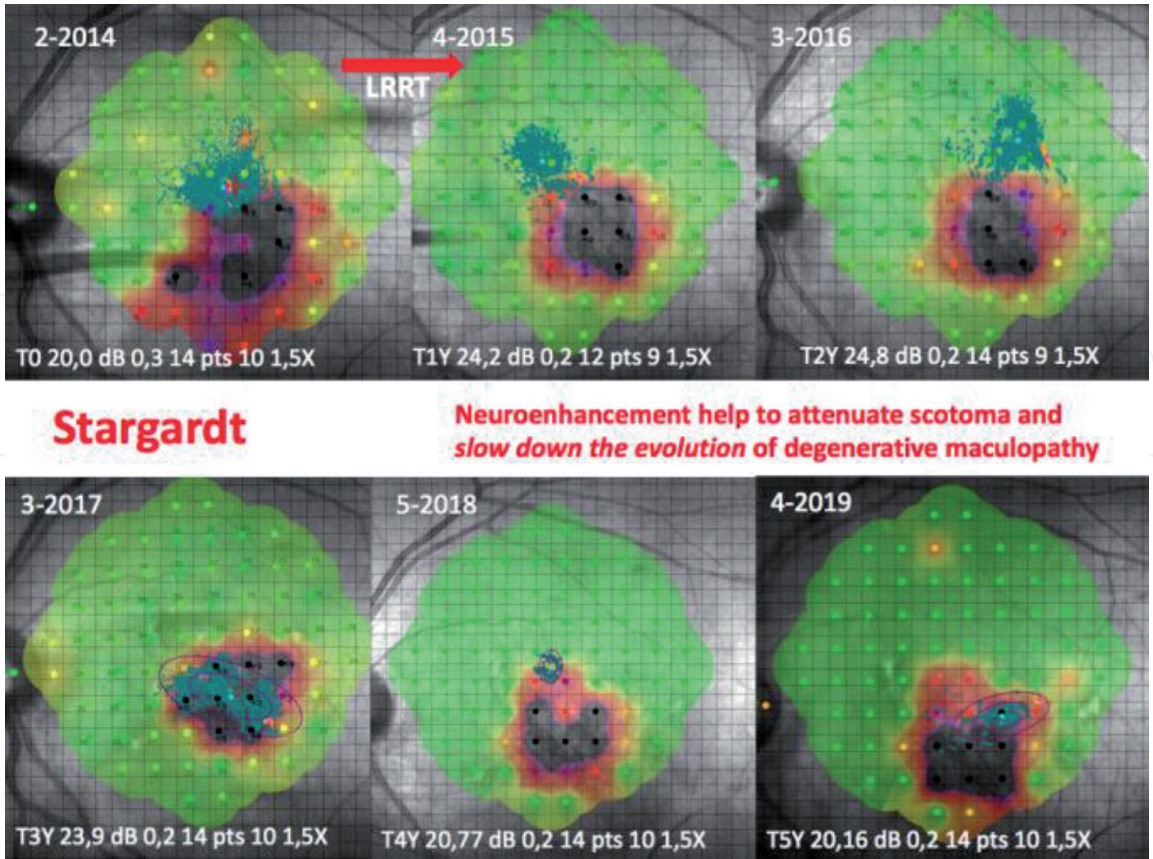


Figure 8.
Patient with molecular diagnosis of Stargardt's maculopathy. In 2014, a suprachoroidal graft of autologous mesenchymal cells was performed. Visual performance after 5 years appears unchanged. Image courtesy of P. Limoli-Milan Low Vision Center.

of this method. We now know for sure that when placed in an appropriate tissue niche stem cells not only survive and proliferate but are capable of differentiating into proper retinal cells which exhibit functional characteristics of real photoreceptors, resulting in the development of a retina-like structure. Further studies are needed to put such promising experimental data into clinical practice and establish standardized procedures for the application of stem cell therapy in the ophthalmological field.

Abbreviations

LESCs	limbal epithelial stem cells
ADSC	adipose-derived stem cells
IPSCs	induced pluripotent stem cells
HPSC	human pluripotent stem cells
MSCs	mesenchymal stem cells
ESCs	embryonic stem cells
TAC	transit amplifying cells
LSCD	limbal stem cell deficiency
ABCG2	ATP-binding cassette family G member 2
CK	cytokeratin
AMD	age-related macular disease
RPE	retinal pigment epithelium
ERG	electroretinography
BCVA	best corrected visual acuity
RGC	retinal ganglion cells
RP	retinitis pigmentosa
DR	diabetic retinopathy
FGF	fibroblast growth factor
VEGF	vascular endothelial growth factor
GM-CSF	granulocyte-macrophage colony stimulating factor
M-CSF	macrophage colony stimulating factor
PIGF	placental growth factor
TGFβ	transforming growth factor beta
HG	hepatocyte growth factor
IGF-1	insulin growth factor
IL	interleukin
CNTF	ciliary neurotrophic factor
BDNF	brain-derived neurotrophic factor
EGF	epidermal growth factor
PEDF	pigmented epithelium-derived factor
PDGF	platelet-derived growth factor
PDAF	platelet-derived angiogenesis factor
TSP	thrombospondin
MC	Muller cells
SOD	superoxide dismutase
IFN-β	interferon beta
IL-1 RA	interleukin 1 receptor antagonist
MCP-1	chemoattractant monocyte protein 1
PGE2R	prostaglandin E2 receptor
CNTF	ciliary neurotrophic factor
ES	electrical stimulation
VP	visual performance

LRRT	Limoli retinal restoration technique
OS	external segment
ASC	adipose stromal cells
PLT	adipose tissue and platelets
PRP	platelet rich plasma

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