

## Possible mechanisms of retinal function recovery with the use of cell therapy with bone marrow-derived stem cells

### *Possíveis mecanismos de recuperação da função da retina com uso de terapia celular com células tronco derivadas da medula óssea*

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#### ABSTRACT

Bone marrow has been proposed as a potential source of stem cells for regenerative medicine. In the eye, degeneration of neural cells in the retina is a hallmark of such widespread ocular diseases as age-related macular degeneration (AMD) and retinitis pigmentosa. Bone marrow is an ideal tissue for studying stem cells mainly because of its accessibility. Furthermore, there are a number of well-defined mouse models and cell surface markers that allow effective study of hematopoiesis in healthy and injured mice. Because of these characteristics and the experience of bone marrow transplantation in the treatment of hematological disease such as leukemia, bone marrow-derived stem cells have also become a major tool in regenerative medicine. Those cells may be able to restore the retina function through different mechanisms: A) cellular differentiation, B) paracrine effect, and C) retinal pigment epithelium repair. In this review, we described these possible mechanisms of recovery of retinal function with the use of cell therapy with bone marrow-derived stem cells.

**Keywords:** Retinitis pigmentosa; Retinal degeneration; Stem cell; Bone marrow

#### RESUMO

As células tronco derivadas da medula óssea têm sido propostas como uma fonte em potencial de células para medicina regenerativa. No olho, a degeneração de células neurais da retina são a marca de doenças difusas, como a degeneração macular relacionada com a idade (DMRI) e a retinose pigmentar. A medula óssea é um tecido ideal para estudar as células tronco por causa da sua acessibilidade. Devido a estas características e a experiência do transplante de medula óssea no tratamento de doenças hematológicas, como as leucemias, as células tronco derivadas da medula óssea têm se tornado a maior ferramenta na medicina regenerativa. Essas células podem ser capazes de restaurar a função da retina através dos seguintes mecanismos: A) diferenciação celular; B) efeito parácrino; C) reparo do epitélio pigmentado da retina. Nesta revisão nós descrevemos os possíveis mecanismos de recuperação da função da retina com uso de terapia celular com células tronco derivadas da medula óssea.

**Descritores:** Retinose pigmentar; Degeneração retiniana; Células tronco; Medula óssea

#### INTRODUCTION

Stem cell therapy is not a new concept. Aftermath of the bombings in Hiroshima and Nagasaki in 1945, researchers discovered that bone marrow transplanted into irradiated mice produced haematopoiesis<sup>(1)</sup>. Haematopoietic stem cells were first identified in 1961 and their ability to migrate and differentiate into multiple cell types was documented<sup>(2)</sup>. Bone marrow transplants are still used today as an adjunct therapy, which enables physicians to increase chemotherapeutic doses in cancer patients<sup>(3)</sup>.

Distinct stem cell types have been established from embryos and identified in the fetal tissues and umbilical cord blood (UCB) as well as in specific niches in many adult mammalian tissues and organs such as bone marrow (BM), brain, skin, eyes, heart, kidneys, lungs, gastrointestinal tract, pancreas, liver, breast, ovaries, prostate and testis<sup>(2)</sup>. All stem cells

are undifferentiated cells that exhibit unlimited self renewal and can generate multiple cell lineages or more restricted progenitor populations which can contribute to tissue homeostasis by replenishing the cells or tissue regeneration after injuries<sup>(4)</sup>.

Several investigations (Mimeault M)<sup>(4)</sup> (Ortiz-Gonzalez XR)<sup>(5)</sup> (Trounson A)<sup>(6)</sup> have been carried out with isolated embryonic, fetal and adult stem cells in a well-defined culture microenvironment to define the sequential steps and intracellular pathways that are involved in their differentiation into the specific cell lineages. More particularly, different methods for *in vitro* culture of stem cells have been developed, including the use of cell feeder layers, cell-free conditions, extracellular matrix (ECM) molecules such as collagen, gelatin and laminin and diverse growth factors and cytokines<sup>(4-7)</sup>.

Adult stem cells are present in most organs and tissues such as brain, bone marrow, blood vessels, skin, teeth, and heart. These stem cells are in the tissues that they are going to become, an area called the "stem cell niche"<sup>(8-14)</sup>. The inherent variety of stem cells has caused much debate on what constitutes a stem cell. In an ongoing effort to better classify stem cells and to understand their patterns of gene expression such that they might later be manipulated for gene therapies, scientists have begun genetically mapping stem cells. In general terms, a stem cell may be defined as an undifferentiated cell capable of self-renewal and of giving rise to one or more differentiated cell types<sup>(4)</sup>.

#### BONE MARROW DERIVED STEM CELL

BM-derived SCs have been proposed as a potential source of cells for regenerative<sup>(8-9)</sup>. This was based on the assumption

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that HSCs isolated from BM are plastic and are able to “trans-differentiate” into tissue-committed stem cells (TCSCs) for other organs (e.g., heart, liver, or brain). Unfortunately, the concept of SC plasticity was not confirmed in recent studies and previously encouraging data demonstrating this phenomenon *in vitro* could be explained by a phenomenon of cell fusion or, as postulated by our group, by the presence, of heterogeneous populations of SCs in BM<sup>(11-12)</sup>. The identification of VSELSCs (primitive, very small, embryonic-like) in BM supports the notion that this tissue contains a population of primitive stem cell, which, if transplanted together with HSCs, was able to regenerate damaged tissues in certain experimental settings. Cells from BM could be easily and safely aspirated. After administering local anesthesia, about 10 ml of the bone marrow is aspirated from the iliac crest using a sterile bone marrow aspiration needle, and mononuclear bone marrow stem cells is separated using the Ficoll density separation method<sup>(14-17)</sup> (Figure 1). Stem cell-based therapy has been tested in animal models for several diseases, including neurodegenerative disorders, such as Parkinson disease, spinal cord injury, and multiple sclerosis. The replacement of lost neurons that are not physiologically replaced is pivotal for therapeutic success. In the eye, degeneration of neural cells in the retina is a hallmark of such widespread ocular diseases as age-related macular degeneration (AMD) and retinitis pigmentosa (RP). In these cases the loss of photoreceptors that occurs as a primary event (RP) or secondary to loss of RPE (AMD) leads to blindness<sup>(8-9)</sup>.

Bone marrow is an ideal tissue for studying stem cells because of its accessibility and because proliferative dose-responses of bone marrow-derived stem cells can be readily investigated. Furthermore, there are a number of well-defined mouse models and cell surface markers that allow effective study of hematopoiesis in healthy and injured mice. Because of these characteristics and the experience of bone marrow transplantation in the treatment of hematological cancers, bone marrow-derived stem cells have also become a major tool in regenerative medicine. The bone marrow harbors at least two distinct stem cell populations: hematopoietic stem cells (HSC) and multipotent marrow stromal cells (MSC).

## 1) HEMATOPOIETIC STEM CELLS (HSCs)

Hematopoietic stem cells (HSCs) are multipotent stem cells that give rise to all the blood cell types including myeloid (monocytes and macrophages, neutrophils, basophils, eosinophils, erythrocytes, megakaryocytes/platelets, dendritic cells), and lymphoid lineages (T-cells, B-cells, NK-cells).

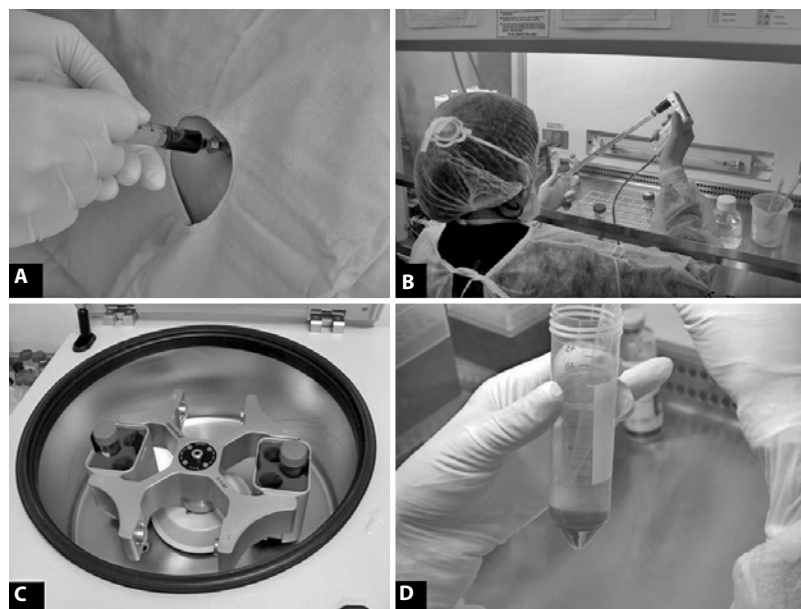
HSCs are found in the bone marrow of adults, which includes femurs, hip, ribs, sternum, and other bones. Cells can be obtained directly by removal from the hip using a needle and syringe (Figure 1), or from the blood following pre-treatment with cytokines, such as G-CSF (granulocyte colony-stimulating factors), that induce cells to be released from the bone marrow compartment. Other sources for clinical and scientific use include umbilical cord blood and placenta<sup>(10-11)</sup>.

In reference to phenotype, hematopoietic stem cells are identified by their small size, lack of lineage (lin) markers, low staining (side population) with vital dyes such as rhodamine 123 (rhodamine<sup>DULL</sup>, also called rho<sup>o</sup>) or Hoechst 33342, and presence of various antigenic markers on their surface, many of which belong to the cluster of differentiation series: CD34, CD38, CD90, CD133, CD105, CD45 and also c-kit, the receptor for stem cell factor<sup>(12-17)</sup>.

## 2) MULTIPOTENT MESENCHYMAL STROMAL CELLS (MESENCHYMAL STEM CELLS)

Mesenchymal stem cells (MSCs) are progenitors of all connective tissue cells. In adults of multiple vertebrate species, MSCs have been isolated from bone marrow (BM) and other tissues, expanded in culture, and differentiated into several tissue-forming cells such as bone, cartilage, fat, muscle, tendon, liver, kidney, heart, and even brain cells.

Accordingly to the International Society for Cellular Therapy<sup>(18)</sup> there are three minimum requirements for a population of cells be classified as MSC. The first is that MSCs are isolated from a population of mononuclear cells on the basis of their selective adherence to the surface of the plastic of culture dishes, differing in this respect with bone marrow hematopoietic cells, a disadvantage of this method is a possible contamination by hematopoietic cells and cellular hetero-



**Figure 1.** Sequence of photos showing the collection of bone marrow (A) and initial separation of the mononuclear cells using Ficoll/Hypaque gradient centrifugation (B)(C)(D).

geneity with respect to the potential for differentiation. The second criteria is that the expressions of CD105, CD73 and CD90 are present, and that CD34, CD45, CD14 or CD11b, CD79, or CD19 and HLA-DR are not expressed in more than 95% of the cells in culture. Finally, the cells can be differentiated into bone, fat and cartilage<sup>(19)</sup>.

**APPLICATION OF BONE MARROW (BM)-DERIVED STEM CELLS IN RETINAL DISEASES**

Bone marrow (BM)-derived stem cells may be able to restore the functioning of the retina through different mechanisms: A) cellular differentiation, B) paracrine effect, and C) retinal pigment epithelium repair.

**A) CELLULAR DIFFERENTIATION**

The mechanisms for SC-mediated differentiation events, including documented functional recovery, are still under considerable scientific debate. For adult SCs, the controversy between transdifferentiation and fusion has still to be solved<sup>(11,14)</sup>. Recently, it was reported that BMSCs are able to “transdifferentiate” or change commitment into cells that express early heart, skeletal muscle, neural, or liver cell markers<sup>(8-9,11,14-15)</sup>. Similarly, SCs from the BM contributed to the regeneration of infarcted myocardium. This was supported by the observations in humans that transplantation of SCs from mobilized peripheral blood expressing the early hematopoietic CD34<sup>+</sup> antigen led to the appearance of donor-derived hepatocytes, epithelial cells, and neurons<sup>(8-10)</sup>. Therefore, it was initially presumed the repair seen in damaged host tissues following SC transplantation or homing was due to incorporation and transdifferentiation of the BMSCs at the sites of damage. However, a number of studies have challenged this concept, providing evidence that BMSCs may instead incorporate into host tissues via fusion with host cells. Intravitreally injected, lineage-negative (Lin<sup>-</sup>) hematopoietic stem cells (HSCs) have been reported to rescue retinal degeneration in *rd1* and *rd10* mice. In the study, exogenous Lin<sup>-</sup> HSCs prevented retinal vascular degeneration and this vascular rescue correlated with neuronal rescue<sup>(20)</sup>. Although this approach showed a dramatic rescue effect, there was a limitation in that intravitreally injected bone marrow (BM)-derived stem cells were effectively incorporated into the retina only during an early, postnatal developmental stage, but not in adult mice. Directly injected exogenous Lin<sup>-</sup> HSCs only targeted activated astrocytes that are observed in neonatal mice or in an injury-induced model in the adult<sup>(20-22)</sup>.

**A1) THE ROLE OF BM DERIVED MICROGLIA**

Microglial activation in the retina provides an early response against infection, injury, ischemia, and degeneration.

In retinal degeneration, activated microglia migrate into the deeper retina with the expression of tumor necrosis factor- $\alpha$  before the onset of photoreceptor cell death, suggesting that microglial activation may trigger neuronal cell death<sup>(22)</sup>. On the other hand, microglia secrete neurotrophic factors and promote photoreceptor survival in a light-induced retinal degeneration model, and promote vascular repair in an oxygen-induced retinopathy model<sup>(22-23)</sup>.

The precise mechanisms of retinal protection by BM derived microglia remain elusive. One hypothesis is that microglia phagocytose cellular debris and clear the degenerative environment. Another possible mechanism is that microglia secrete neurotrophic factors to promote residual cell survival<sup>(22-23)</sup>. In the light-induced retinal degeneration model, microglia secrete nerve growth factor or ciliary neurotrophic factor and modulate secondary neurotrophic factor expression in Muller glia, contributing to the protection of photoreceptor cells<sup>(22-24)</sup>.

**B) PARACRINE EFFECT**

Paracrine signaling is a form of cell signaling in which the target cell is near (“para” = near) the signal-releasing cell.

A distinction is sometimes made between paracrine and autocrine signaling. Both affect neighboring cells, but whereas autocrine signaling occurs among the same types of cells, paracrine signaling affects other types of (adjacent) cells.

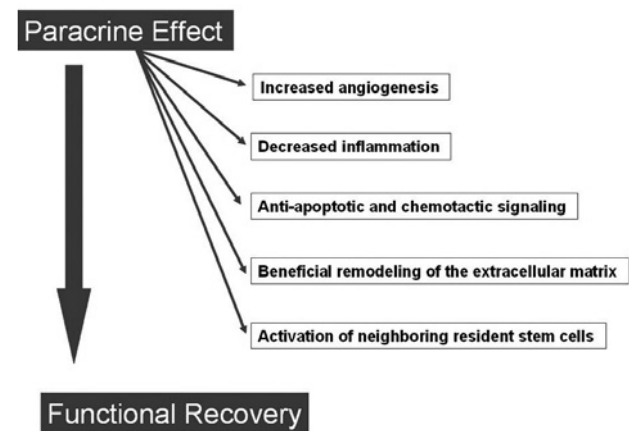
Cells communicate with each other via direct contact (juxtacrine signaling), over short distances (paracrine signaling), or over large distances and/or scales (endocrine signaling).

Some cell-to-cell communication requires direct cell-cell contact. Some cells can form gap junctions that connect their cytoplasm to the cytoplasm of adjacent cells. In cardiac muscle, gap junctions between adjacent cells allows for action potential propagation from the cardiac pacemaker region of the heart to spread and coordinately cause contraction of the heart.

Below we will mention the possible paracrine effects of stem cells (Figure 2) and their mechanisms in accordance with the classification proposed by Crisostomo et al. (2008)<sup>(25)</sup>.

**B1) INCREASED ANGIOGENESIS**

First, stem cells produce local signaling molecules that may improve perfusion and enhance angiogenesis to chronically ischemic tissue. Although the particular growth factors contributing to this neovascular effect remain to be defined, the list includes vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), and basic fibroblast growth factor (FGF2)<sup>(2-3)</sup>. VEGF is a strong promoter of angiogenesis. Although originally associated with liver regeneration, HGF also exerts beneficial effects on neovascularization and tissue remodeling. FGF2, a specific member of the FGF signaling family is involved intimately with endothelial cell proliferation and may be a more potent angiogenic factor than VEGF<sup>(25)</sup>. When exposed to either insult or stress, mesenchymal stem cells (MSC) in cell culture and in vivo significantly increase release of VEGF, HGF, and FGF2, which may improve regional blood flow as well as promote autocrine self survival. Increased perfusion due to the production of stem cell angiogenic growth factor has also been associated with improved end organ function. Further, VEGF overexpressing bone marrow stem cells demonstrate greater protection of injured tissue than controls. Thus, VEGF, HGF, and FGF2 may be important paracrine signaling molecules in stem cell-mediated angiogenesis, protection, and survival<sup>(26-30)</sup>.



**Figure 2.** Diagram showing the paths of the paracrine effect.

## B2) DECREASED INFLAMMATION

Stem cells appear to attenuate infarct size and injury by modulating local inflammation. When transplanted into injured tissue, the stem cell faces a hostile, nutrient-deficient, inflammatory environment and may release substances which limit local inflammation in order to enhance its survival. Recent studies implicate the release of the anti-inflammatory cytokine IL-10 as playing an integral role in modulating the activity of innate and adaptive immune cells, such as dendritic cells, T cells, and B cells. Transforming growth factor beta (TGF- $\beta$ ) appears to be involved in suppression of inflammation by stem cells. TGF- $\beta$ 1 plays a role in T cell suppression, and its anti-inflammatory effect may be further potentiated by concomitant HGF.

Modulation of local tissue levels of pro-inflammatory cytokines by anti-inflammatory paracrine factors released by stem cells, thus, are important in conferring improved outcome after stem cell therapy<sup>(25-31)</sup>.

## B3) ANTI-APOPTOTIC AND CHEMOTACTIC SIGNALING

Stem cells in a third pathway promote salvage of tenuous or malfunctioning cell types at the infarct border zone. Injection of MSC into a cryo-induced infarct reduces myocardial scar width 10 weeks later<sup>(29-30)</sup>. MSCs appear to activate an anti-apoptosis signaling system at the infarct border zone which effectively protects ischemia-threatened cell types from apoptosis.

Evidence also exists that both endogenous and exogenous stem cells are able to "home" or migrate into the area of injury

from the site of injection or infusion<sup>(26,30,32-38)</sup>. MSC in the bone marrow can be mobilized, target the areas of infarction, and differentiate into target tissue type.

Furthermore, expression profiling of adult progenitor cells reveals characteristic expression of genes associated with enhanced DNA repair, upregulated anti-oxidant enzymes, and increased detoxifier systems. HGF has been observed to improve cell growth and to reduce cell apoptosis<sup>(33)</sup>.

Granulocyte colony-stimulating factor (G-CSF) has been studied widely and promotes the mobilization of bone marrow-derived stem cells in the setting of acute injury. This homing mechanism may also depend on expression of stromal cell-derived factor 1 (SDF-1), monocyte chemoattractant protein-3 (MCP-3), stem cell factor (SCF), and / or IL-8<sup>(25,32-33)</sup>.

## B4) BENEFICIAL REMODELING OF THE EXTRACELLULAR MATRIX

Fourth, stem cell transplantation alters the extracellular matrix, resulting in more favorable post-infarct remodeling, strengthening of the infarct scar, and prevention of deterioration in organ function<sup>(34-37)</sup>. Acute human and murine MSC infusion prior to ischemia improve myocardial developed pressure, contractility, and compliance after ischemia/reperfusion (I/R) injury and decrease end diastolic pressure. Similarly, direct injection of human MSC into ischemic hearts decreased fibrosis, left ventricular dilation, apoptosis, and increased myocardial thickness with preservation of systolic and diastolic cardiac function without evidence of myocardial regeneration. MSCs appear to achieve this improved function by increasing acutely the cellularity and decreasing production of extracellular

**Table 1. Table showing clinics and experimental studies using cell therapy for retinal diseases**

	Type of study	Type of injury or illness	Route used	Type and source of cells
Atsushi Otani et al. <sup>(18)</sup>	Experimental study in animals	Mice with retinal degenerative disease	Intravitreal transplantation	Adult bonemarrow-derived lineage-negative hematopoietic stem cells
Wang S et al. <sup>(39)</sup>	Experimental study in animals	Retinitis pigmentosa	Tail vein	Pluripotent bone marrow-derived mesenchymal stem cells (MSCs)
Li Na & Li Xiao-rong & Yuan Jia-qin <sup>(27)</sup>	Experimental study in animals	Rat injured by ischemia/reperfusion	Intravitreal transplantation	Bone-marrow mesenchymal stem cells
Uteza Y, Rouillot JS, Kobetz A, et al. <sup>(41)</sup>	Experimental study in animals	Photoreceptor cell degeneration in Royal College of Surgeons rats	Intravitreal transplantation	Encapsulated fibroblasts
tZhang Y, Wang W <sup>(40)</sup>	Experimental study in animals	Light-damaged retinal structure	Subretinal space	Bone marrow mesenchymal stem cells
Tomita M <sup>(42)</sup>	Experimental study in animals	Retinas mechanically injured using a hooked needle	Intravitreal transplantation	Bone marrow-derived stem cells
Meyer JS et al. <sup>(43)</sup>	Experimental study in animals	Retinal degeneration	Intravitreal transplantation	Embryonic stem (ES) cells
Siqueira RC et al. <sup>(44-45)</sup>	Experimental study in animals	Chorioretinal injuries caused by laser red diode 670N-M	Intravitreal transplantation	Bone marrow-derived stem cells
Wang HC et al. <sup>(46)</sup>	Experimental study in animals	Mice with laser-induced retinal injury	Intravitreal transplantation	Bone marrow-derived stem cells
Johnson TV et al. <sup>(47)</sup>	Experimental study in animals	Glaucoma	Intravitreal transplantation	Bone marrow-derived mesenchymal stem cell (MSC)
Castanheira P et al. <sup>(48)</sup>	Experimental study in animals	Rat retinas submitted to laser damage	Intravitreal transplantation	Bone marrow-derived mesenchymal stem cell (MSC)
Jonas JB et al. <sup>(49)</sup>	Case report	Patient with atrophy of the retina and optic nerve	Intravitreal transplantation	Bone marrow-derived mononuclear cell transplantation
Jonas JB et al. <sup>(50)</sup>	Case report	3 patients with diabetic retinopathy, age related macular degeneration and optic nerve atrophy (glaucoma)	Intravitreal transplantation	Bone marrow-derived mononuclear cell transplantation
Siqueira RC et al. Clinical trial.gov NCT01068561 <sup>(51-52)</sup>	Clinical Trial Phase I	5 patients with retinitis pigmentosa	Intravitreal transplantation	Bone marrow-derived mononuclear cell transplantation

matrix proteins such as collagen type I, collagen type III, and TIMP-1 which result in positive remodeling and function<sup>(25,35)</sup>.

### B5) ACTIVATION OF NEIGHBORING RESIDENT STEM CELLS

Finally, exogenous stem cell transplantation may activate neighboring resident tissue stem cells. Recent work demonstrated the existence of endogenous, stem cell-like populations in adult heart, liver, brain, and kidney<sup>(33-39)</sup>.

These resident stem cells may possess growth factor receptors that can be activated to induce their migration and proliferation and promote both the restoration of dead tissue and the improved function in damaged tissue. Mesenchymal stem cells have also released HGF and IGF-1 in response to injury and when transplanted into ischemic myocardial tissue may activate subsequently the resident cardiac stem cells.

Although the definitive mechanisms for protection via stem cells remains unclear, stem cells mediate enhanced angiogenesis, suppression of inflammation, and improved function via paracrine actions on injured cells, neighboring resident stem cells, the extracellular matrix, and the infarct zone. Improved understanding of these paracrine mechanisms may allow earlier and more effective clinical therapies<sup>(25,36-37)</sup>.

### C) RETINAL PIGMENT EPITHELIUM (RPE) REPAIR WITH BM DERIVED STEM CELL

RPE dysfunction has been linked to many devastating eye disorders, including age-related macular degeneration, and to hereditary disorders, such as Stargardt disease and retinitis pigmentosa<sup>(38-40)</sup>. Attempts to repair the RPE include transplantation of RPE cells into the subretinal space<sup>(39-44)</sup>. Animal studies, RPE transplantation in humans, and macular relocation surgery have all shown that replacing diseased RPE with healthier RPE can rescue photoreceptors, prevent further visual loss, and even promote visual<sup>(45)</sup>. Also, recent work on human RPE patch graft transplantation demonstrates survival and rescue of photoreceptors for a substantial time after grafting and holds some promise<sup>(38)</sup>. Rescue of RPE and photoreceptors beyond the area of donor cell distribution suggests that diffusible factors are also involved in the rescue process. However, some problems exist, including the ability to obtain an adequate source of autologous RPE and that homologous cells have been associated with rejection. Fetal or adult transplanted RPE cells attach to Bruch's membrane with poor efficiency and do not proliferate. These transplantation procedures are complex, associated with high complication rates, and often result in only short-term<sup>(45)</sup>.

Recently, it has been reported that the bone marrow-derived cells regenerated RPE in two different acute injury models<sup>(39-44,46-48)</sup>.

Based on the above mentioned mechanisms, experimental and human studies with intravitreal bone-marrow derived stem cells have begun (Table 1).

Recently, some reports demonstrated the clinical feasibility of intravitreal administration of autologous bone marrow-derived mononuclear cells (ABMC) in patients with advanced degenerative retinopathies<sup>(49-52)</sup>. More recently, our group conducted a prospective phase I trial to investigate the safety of intravitreal ABMC in patients with RP or cone-rod dystrophy, with promising results<sup>(53-55)</sup>. The history starts to be written in this very promising therapeutic field. Welcome!

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## XIV Simpósio da Sociedade Brasileira de Glaucoma

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