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MESTRADO INTEGRADO EM MEDICINA

Stem Cell Therapy in Age-Related Macular Degeneration

Eduardo Fonseca Conde

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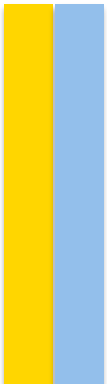

Stem Cell Therapy in Age-Related Macular Degeneration

Dissertação de candidatura ao grau de Mestre em Medicina, submetida ao Instituto de Ciências Biomédicas Abel Salazar – Universidade do Porto

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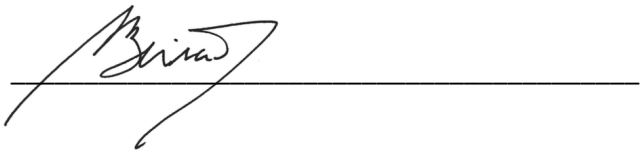

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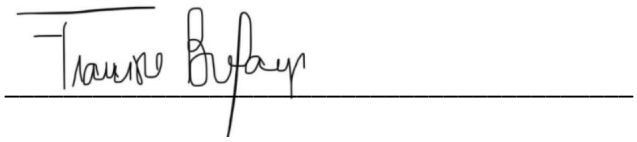

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Resumo

Introdução: A degenerescência macular associada à idade (DMI) é uma das principais causas de perda de visão em doentes com mais de 55 anos, com uma prevalência que se espere que continue a aumentar com o aumento da esperança média de vida. A DMI, caracterizada pela degeneração do epitélio pigmentar da retina (EPR), manifesta-se em duas formas tardias: atrófica e neovascular. Os tratamentos atuais, como as injeções de anti-VEGF, destinam-se principalmente à DMI neovascular e visam recuperar a acuidade visual e desacelerar a progressão da doença. A terapia com células estaminais, particularmente envolvendo células estaminais pluripotentes induzidas (iPSCs), têm potencial para tratamento regenerativo ao substituir as células danificadas do EPR. As iPSCs, capazes de se diferenciar em vários tipos de células, oferecem uma alternativa promissora devido ao facto de serem derivadas das próprias células do paciente, minimizando assim os riscos de rejeição imunológica. Avanços recentes na transplantação de células do EPR derivadas de iPSCs têm mostrado potencial em ensaios clínicos. No entanto, permanecem desafios na otimização dos protocolos de diferenciação, mecanismos de entrega e garantia de segurança e eficácia a longo prazo.

Palavras-chave: Stem-cells, therapeutics, age-related macular degeneration, retinal diseases.

Objetivos: Esta revisão discute o entendimento atual da DMI, avalia os tratamentos existentes e destaca os avanços e as futuras direções nas terapias baseadas em iPSCs para a DMI.

Metodologia: De forma a redigir esta revisão narrativa, foi realizada uma pesquisa minuciosa de publicações científicas relacionadas com a terapia com células estaminais para a degenerescência macular associada à idade utilizando a base de dados bibliográfica PubMed. O estudo focou-se exclusivamente em artigos publicados em inglês, sem restrições quanto às datas de publicação; no entanto, foi dada preferência a publicações mais recentes, quando aplicável.

Abstract

Introduction: ge-related macular degeneration (AMD) is a leading cause of vision loss in patients over 55, with its prevalence expected to rise with the increasing median life expectancy. AMD, characterized by the degeneration of retinal pigmented epithelium (RPE) cells, manifests in two late forms: non-neovascular and neovascular. Current treatments, such as anti-VEGF injections, primarily target neovascular AMD and aim to recover visual acuity and slow down disease progression.

Stem cell therapy, particularly involving induced pluripotent stem cells (iPSCs), holds potential for regenerative treatment by replacing damaged RPE cells. iPSCs, capable of differentiating into many different cell types, offer a promising alternative due to the fact that they can be derived from the patient's own cells, minimizing immune rejection risks. Recent advancements in iPSC-derived RPE cell transplantation have shown potential in clinical trials. However, challenges remain in optimizing differentiation protocols, delivery mechanisms and ensuring long-term safety and efficacy.

Keywords: Stem-cells, therapeutics, age-related macular degeneration, retinal diseases.

Objectives: This literary review discusses the current understanding of AMD, evaluates existing treatments and highlights the advancements and future directions in iPSC-based therapies for AMD.

Methods: To write this bibliographic review article, a thorough search for scientific publications related to stem cell therapy for age-related macular degeneration was conducted using the PubMed bibliographic database. The study exclusively targeted articles published in the English language, with no constraints on publication dates; however, preference was given to more contemporary publications where applicable.

List of Abbreviations

AMD - age-related macular degeneration

anti-VEGF - anti-vascular endothelial growth factor

BM - Bruch's membrane

ESCs - embryonic stem cells

iPSCs - induced pluripotent stem cells

PSCs - pluripotent stem cells

MSCs - mesenchymal stem cells

RPE - retinal pigmented epithelium

YLD - years lived with disability

SDI - socio demographic index

OCT - optical coherence tomography

CHF gene - complement factor H gene

ARMS2 gene - age related maculopathy susceptibility 2 gene

TIMP3 gene - tissue inhibitor of metalloproteinase 3 gene

MMPs - matrix metalloproteinases

EGF - epidermal growth factor

TNF - tumor necrosis factor

BRB - blood retinal barrier

PR - photoreceptors

MAC - membrane attack complex

ROS - reactive oxygen species

CTMPs - cell therapy medicinal products

GTMPs - gene therapy medicinal products

TEPs - tissue engineered products

GA - geographic atrophy

SMD - Stargardt macular dystrophy

ERM - epiretinal membranes

FGF - fibroblast growth factor

IGF - insulin-like growth factor

VIP - vasoactive intestinal peptide

NIC - nicotinamide

MHC - major histocompatibility complex

HIV - human immunodeficiency virus

Index

Introduction

Age-related macular degeneration (AMD) is a prevalent eye condition that primarily affects the elderly and substantially burdens both individuals and health care systems around the world.[1] As the global life expectancy continues to rise, its prevalence is also bound to increase.[2] AMD is considered the main cause of central vision loss in developed countries in patients over 55 years old. It is characterized by the degeneration and loss of the retinal pigmented epithelium in the macula and can be described as having many different subtypes or stages. [1][2]

Late AMD is presented in two forms, non-neovascular (dry) and neovascular (wet). Conventional therapies for wet AMD, such as intravitreal injections of anti-VEGF (anti-vascular endothelial growth factor), aim to not only improve vision but also to slow down the progression of the disease. However, a long-term benefit would be achieved by repairing and regenerating the damaged retinal tissue. Dry AMD, on the other hand, does not respond to current therapies and currently no effective treatments can reverse it, although there are several under investigation. [3] These include antioxidant therapy, drugs targeting multiple pathways (angiogenesis, complement and inflammatory pathways) and advanced therapy medicine (such as cell and gene therapy) as well as retinal implants.

Given the retina's inherent lack of intrinsic regenerative capacity, stem cell therapies have been held as a possible therapeutic tool. Stem cells are non-specialized immature cells without complex structures with limitless self-renewal ability and are branded by their ability to differentiate into numerous types of cells in the body. [4] In 2006, Shinya Yamanaka, a Japanese Nobel prize laureate and his team discovered that somatic cells could be reprogrammed into embryonic stem cell (ESC)-like cells, called induced pluripotent stem cells (iPSCs). [5] Stem cell therapies, specifically iPSC derived retinal pigmented epithelial (RPE) cell transplantation, have shown great promise in treating AMD and hereditary retinal diseases. Clinical studies have evaluated the safety and potential benefits of this treatment, and several preclinical and clinical studies have demonstrated that the transplantation of stem cells and derived products produces clinically significant outcomes and bypass many issues and ethics when compared to using ESCs[6], such as with harvesting stem cells from embryonic structures. While some clinical trials using somatic stem cells have had controversial outcomes, retinal regenerative medicine using stem cells is expected to progress with the incorporation of new technologies and protocols. This literary review aims to provide a brief up-to-date review of AMD and discuss and summarize its current treatment advancements, more specifically in the field of iPSC-RPEs transplantation.

The Macula

The macula is a specialized circular region in the center of the posterior retina, approximately 5.5 mm in diameter, temporal to the optic disc. The term macula is derived from the Latin macula lutea (yellow spot) that describes the appearance of the macula in real life due to the accumulation of two carotenoid pigments, lutein and zeaxanthin. Lutein and zeaxanthin are thought to function as antioxidants filters of high-energy blue light, thus protecting the macula from light-induced oxidative damage. The center of the macula has a tiny, central pit called the fovea centralis and is the region of the retina where cone photoreceptor density is highest (up to 200 000 cells mm^2) and visual acuity is maximal.[7] All layers of the retina are pushed aside concentrically in the fovea to allow the light to reach the densely packed sensory cones with minimum scatter from overlying tissues.

The Bruch's membrane (BM) is attached to the basal surface of the RPE and consists of an elastic semi-permeable barrier for major metabolic transport and exchange. One factor that may contribute to macular sensitivity is the fovea's relative lack of vasculature compared to the rest of the retina. These photoreceptors appear to rely mainly on the choroidal capillary bed, the choriocapillaris, as their primary source for oxygen and nutrients. The choriocapillaris lies adjacent to BM and is composed of fenestrated capillaries. The macula is most susceptible to direct light damage and age-related decreases in choroidal vasculature flow and volume. [7]

Age-related Macular Degeneration (AMD)

Age-related macular degeneration is correlated to the dysfunction of the retinal pigmented epithelial (RPE) cells, which cover Bruch's membrane and support the survival and function of photoreceptor cells. AMD is the primary cause of permanent central visual loss globally, impacting patient's quality of life and their ability to perform daily activities, such as recognizing faces, reading or even driving. As long as peripheral vision is preserved, AMD does not cause total blindness in patients. Over the years scientists have continuously reported the relationship between age and macular degeneration, probably as a result of the complicated interaction of genetics, metabolic and inflammatory mechanisms as well as several environmental factors, including smoking, lifestyle and nutritional disorders. [8]

Aging has become an epidemic worldwide, particularly in industrialized developed nations. As per the predictions of the United Nations, the proportion of individuals aged 60+ will increase three-fold (circa 2 billion) by 2050 and will constitute 33% of the total population of the developed world. [9] In this way, there is an increasing concern for the emergence of health conditions directly correlated with aging, such as age-related macular degeneration, and an increasing demand for the formulations

of new treatments aimed at these disorders. AMD is expected to affect around 288 million people by 2040. [10]

A multi-ethnic cohort study made in the United-States, reported that the incidence of early AMD was highest among white patients (5.3%), in comparison to Asian (4.5%) and Hispanic patients (3.3%) and lowest in the black ethnic group (1.6%). The incidence rate of late AMD was similar between patient groups.[11]

The 2019 Global Burden of Disease Study (GBD 2019), includes the most recent estimates of disease burden and influencing factors for researchers to study and is an incisive way to look at the prevalence and years lived with disability (YLDs) of AMD. Using this study, we can get a glimpse of the prevalence and YLDs from AMD between 1990 and 2019 and estimate the burden by age, sex, socio-demographic index (SDI), region and nation. In general, the number of prevalent cases and YLDs due to AMD increased over 30 years and were closely related to age, sex, socio-economic status and varied geographically. It is noteworthy that people in less developed countries seemed to be more likely to bear a heavier burden of AMD. The numbers were also higher in females than males. Visual impairment from advanced AMD is associated with a significant impairment of every day activities, depression and reduced quality of life. [13] It is estimated that future socioeconomic and medical challenges associated with AMD will be similar to those of acquired immunodeficiency syndrome, kidney failure and stroke. [13]

Early stages of age-related macular degeneration are characterized by the formation of the yellowish drusen deposits. As the number of drusen deposits increase, so does the depigmentation of the retinal pigment epithelial cells and their subsequent degeneration. The advanced stages of AMD are subdivided into two types: non-neovascular (dry) and neovascular (wet). Early AMD is characterized by the formation of drusen deposits which increase in quantity and size and lead to complement activation with chronic inflammation of the retinal pigmented epithelium. Advanced AMD is characterized by either predominant atrophic lesions (atrophic AMD) or by choroidal neovascularization. [14] The damaged RPE cells cannot clear debris efficiently and so condition a toxic environment for photoreceptors resulting in gradual loss of their function and decreased visual acuity. The most severe visual loss from AMD is caused by the late stages. [14] Dry AMD accounts for the great majority of these patients. Wet AMD accounts for about 10 to 15% of this type of degeneration.[14] In neovascular AMD, a choroidal vascularization proliferates and invades the retina, leaking blood, lipids and fluids and leading to the dysfunction of RPE cells, the formation of fibrous scars and ultimately loss of vision. Patients with drusen and mild pigmentary changes alone may have a visual acuity within the normal range.

Patients with AMD often report either acute or insidious worsening of vision in one or both eyes, which often becomes more apparent in dim light. The patient should be questioned about

distorted vision (metamorphopsia), which, if present, implies macular disease. However, patients may be asymptomatic for a long time. Clinical diagnosis of AMD is made based on observations from visual acuity testing, bilateral fundoscopy with dilated pupils, either by using a stereoscopic viewing method (slit-lamp biomicroscopy), which may show the presence of drusen, pigmentary, exudative, haemorrhagic or atrophic changes affecting the macula, or using Optical coherence tomography (OCT), which provides a high resolution image of the retina for a more detailed analysis.[15] OCT is noninvasive and can be performed easily on almost all patients. OCT-A (optical coherence tomography angiography) is a functional extension of OCT that provides additional information on retinal and choroidal circulation and neovascular membranes in wet AMD without the need for injected contrasts.[16] Additionally, in wet AMD, fluorescein angiography may be used to directly reveal active exudation from pathologic blood vessels.

Etiology of AMD

Genetic factors are thought to play an important role in AMD. Several studies have described the role of genetic variants in the development and progression of the disease, such as the complement factor H gene (CHF gene) or the age-related maculopathy susceptibility 2 gene (ARMS2 gene) and tissue inhibitor of metalloproteinase 3 gene (TIMP3 gene).[12] So far, the most important genetic traits associated with the disease are found on the CHF gene, which is an inhibitor of the inflammatory cascade. The ARMS2 protein, located in the mitochondria and involved in energy metabolism, is a strong predictor of AMD, although its exact function remains unclear. Also, a rare variant of the TIMP3 gene is highly associated with AMD development. [19] TIMP3 is thought to regulate not only the action of MMPs (matrix metalloproteinases) but also other molecules such as VEGF, EGF (epidermal growth factor) and TNF (tumour necrosis factor), playing a crucial role in maintaining the homeostasis of the RPE extracellular matrix and RPE metabolism.

Pathophysiology of AMD and the Retinal Pigment Epithelium (RPE)

The pathophysiology of AMD, to this day, is still not yet completely understood. Several factors are believed to play a role in the pathogenesis of AMD such as oxidative stress, chronic inflammation, the complement cascade, single-nucleotide polymorphisms in the complement factor H (CHF) gene, and even mitochondrial dysfunction. [16]

The defining pathology of AMD is damage to the RPE-Bruch's membrane complex. The retinal pigmented epithelium consists of a monolayer of non-regenerative polygonal cells [20] situated between the choriocapillaris and the sensory retina, forming the outer blood-retinal barrier (BRB).[21] The RPE plays a crucial role in maintaining the integrity of photoreceptors (PR). Its most important

functions include regulating the transport of ions, nutrients, water, and waste products to the choroidal vasculature through Bruch's membrane, phagocytosis of photoreceptor outer segments (crucial for photoreceptor renewal, high-energy light absorption and protection against light-induced oxidative damage), re-isomerization of all-trans-retinal into 11-cis-retinal, and maintaining the integrity of the RPE-retina structure. [20]

With age, Bruch's membrane becomes less permeable, leading to the accumulation of N-retinylidene-N-retinylethanolamine (A2E) and lipofuscin between the RPE and Bruch's membrane, resulting in the formation of drusen. [18] Photoreceptor cell dysfunction occurs as a result of the separation of photoreceptor cells from the underlying RPE cells and choroidal vessels. Drusen contains several proinflammatory factors such as complement components and tumour necrosis factors (TNF-α). [22] This build-up of drusen inhibits metabolite transport to the choroidal vessels and leads to chronic ischaemia and inflammation.[23] These conditions lead to the upregulation of inflammatory cytokines and growth factors, including vascular endothelial growth factor (VEGF), whose excessive local production induces the growth of neovascular membranes from the choriocapillaris.[24] Consequences of macular neovascularization include hemorrhage, exudation and subsequent central vision loss.

Activation of the complement pathway and the membrane attack complex (MAC) is suggested to play a critical role in the development of AMD.[25] Elevated levels of MAC in the outer layer of Bruch's membrane has been identified as another hallmark of early stage AMD. Production of reactive oxygen species (ROS) and free radicals such as superoxide, hydrogen peroxide and hydroxyl radicals in the RPE lead to chronic oxidative stress and damage to the retina. These mechanisms, though, are still not completely understood. [26][27] The accumulation of ROS results in the overproduction of lipofuscin and β-amyloid, which in turn begins a vicious cycle where overexpression of inflammatory factors lead to increased production of reactive oxygen intermediates which reduce the bioavailability of therapeutic antioxidants.[28]

Current Management of AMD

According to the multifactorial pathogenesis of AMD, multiple treatment options have been considered in clinical practice, such as oral supplementation, complement inhibition, neuroprotection and anti-inflammatory targets. Oral supplements may be beneficial in providing antioxidant protection against oxidative damage. According to the Age-Related Eye Disease Study 2 (AREDS 2),[29] antioxidant compounds, such as vitamin C, E, beta-carotene, lutein, zeaxanthin and zinc may help protect the progression of the disease. Curcumin, a naturally occurring substance present in turmeric, has also

been shown to exert antioxidant effects through inhibition of the NF-κB pathway.[30] Also, a higher intake of carotenoids, more specifically bioavailable lutein and zeaxanthin, seem to have no effects on intermediate AMD but are related to a long-term reduced risk of advanced AMD[31], therefore, an effort should be done to increase dietary consumptions of fruits and vegetables. Numerous studies have also suggested that statins may potentially play a part in the stabilizing of AMD, either through topical application as eye drops and as oral supplementation.[30]

The current treatment of wet AMD patients focuses on controlling the neovascular membrane's activity and restoring some of the retina's structural integrity, although it may not be possible to recover completely. First-line treatment involves the injection of anti-VEGF agents, such as brolucizumab (Beovu®), ranibizumab (Lucentis®) and aflibercept (Eylea®), which suppress endothelial cell proliferation, choroidal neovascularization formation and vascular permeability. Treat and extend (T&E)[32] is one of the different treatment regimens when it comes to anti-VEGF agents where the interval between administrations is either extended or reduced until the ideal interval for that patient is established, evidenced by stability of vision. These anti-VEGF agents are not always effective and are not curable as they only target one of the several different mechanisms inherent to the disease. Photodynamic therapy with or without intravitreal anti-VEGF agents is a second line option for patients where initial treatment with anti-VEGF was not effective, however, is very rarely recommended. [33]

Several therapeutic approaches are being investigated focusing on (1) disease prevention (including antioxidant and visual cycle modulators), (2) halting disease progression (including drugs with anti-inflammatory, oxidative stress, mitochondrial enhancer, β-amyloid inhibitor and neuroprotective properties) and eventually (3) vision restoration (including cell and gene therapy).

Advanced therapy medicinal products (ATMPs) are a group of complex and innovative biological products made for human use, consisting of cell therapy medicinal products (CTMPs), gene therapy medicinal products (GTMPs) and tissue engineered products (TEPs).[34] The eye is the perfect target for these therapeutic drugs due to its small dimensions, compartmentalization and immunologically privileged status, allowing for the introduction of foreign substances with minimal immune response.

Stem Cells

Stem cells are unspecialized cells in the human body that possess the remarkable skill to differentiate into many different types of specialized cells and therefore are held as the future in the scientific area of regenerative medicine. These cells, characterized by their self-renewal ability and pluripotency, have captivated curiosity for decades. Embryonic stem cells (ESCs) are characterized by their pluripotency, capable of generating any cell type within the three germ layers of the body. Pluripotent stem cells (PSCs) include embryonic stem cells and induced pluripotent stem cells

(iPSCs).[35] As mentioned before, iPSCs, reprogrammed from adult stem cells offer an enticing vision for personalized regenerative medicine, bypassing ethical concerns associated with harvesting stem cells from embryonic structures. Mesenchymal stem cells, which can be harvested from several different sources such as the umbilical cord, bone marrow tissue or adipose tissue, also exhibit multilineage differentiation and are also invaluable candidates for tissue repair.

Stem Cell Therapy

Stem cell based therapies provide a newfound hope for patients suffering from degenerative diseases, where treatment options so far have only been focused on the mitigation of symptoms. An important application of stem cells is known as tissue engineering, or the creation of cells and tissues for cell-based therapies. The demand for transplantable organs and tissues far surpasses the available supply. Stem cells offer the possibility of having a renewable source, but the amount of adult stem cells is very limited in each tissue and in their dividing capacity once removed from the body. On the other hand, pluripotent stem cells have more potential for renewal and are less restricted by starting material. Cell therapy includes the use of different types of stem cells, including embryonic cells, mesenchymal cells and induced pluripotent stem cells.

Stem cells for cell-based therapy can be either autologous, where the approach uses the patient's own cells, or allogenic, where the approach uses stem cells from a healthy donor. [36] To date, induced pluripotent stem cells (iPSCs), mesenchymal stem cells (MSCs), and retinal progenitor cells (RPCs) are under investigation in phase 1 and 2 clinical trials. These stem cells may be sourced from embryonic origins, known as embryonic stem cells or from adult sources, known as adult stem cells. [37][38]

So far, in the retina field, two mechanisms have been suggested, including replacing or regenerating the damaged RPE cells and introducing cells that can exert a supportive paracrine effect on photoreceptor function and survival. [39] Retinal cell transplantation, differentiated from various stem cells, is a promising therapeutic approach in ophthalmology. Current research is focused on determining optimal transplantation targets, time and methods of administration. Several different types of stem cells are being investigated for clinical cell therapy in AMD. Among these, RPE (retinal pigmented epithelial) cells are the most common target for AMD cell therapy studies. [40] Usual methods of replenishing RPE cells in AMD involve delivering these cells as a suspension or a patch to the subretinal space to restore the tissue's physiological function.

Clinical Trials with Stem Cells

Embryonic Stem Cells (ESCs)

A Phase I/IIa clinical trial currently underway, OpRegen®, for patients with progressive dry-AMD focusing on RPE cells derived from human ESCs is administering these cells as a cell suspension into the subretinal space in a single surgery.[46] Their goal is to assess the efficacy and tolerability of the subretinal delivery. In a preliminary analysis of this phase I/IIa clinical trial (NCT02286089) involving 12 patients afflicted with advanced dry age-related macular degeneration and geographic atrophy (GA), *Banin et al* documented that hESC-derived RPE cells, when administered with systemic immunosuppression prior to subretinal transplantation, exhibited good tolerance in recipients. Subretinal transplantation of 50,000-200,000 cells in suspension to the worse eye was administered using either pars plana vitrectomy and retinotomy under local anesthesia. Short course, perioperative systemic immunosuppression was used. Subsequent observations during extended follow-ups revealed the presence of these cells in the subretinal space and noted enhancements at the borders of the GA in the RPE layer. [41] A more recent update from *Riemann et al* concerning the fourth patient cohort in the same Phase I/IIa clinical trial (NCT02286089) highlighted visual enhancements and changes in drusen appearance post subretinal transplantation. Despite these positive outcomes, some patients developed epiretinal membranes (ERM) and retinal detachment, although these complications were effectively managed. [44]In a similar way, both in a London case study and in another study conducted in Asian patients, the transplantation of hESC-derived RPE cells in patients suffering from AMD and Stargardt macular dystrophy (SMD) was associated with clinically significant improvements in visual acuity, devoid of any abnormal cell proliferation. [42][43]

In a separate clinical experiment, *da Cruz et al* reported that the use of ESC-derived RPE cells for retinal degeneration led to improved visual acuity in two patients with severe exudative AMD following subretinal transplantation. These patients had previously been treated with anti-VEGF therapy before transplantation but did not continue these injections during the clinical trial. Nevertheless, challenges persisted as one patient experienced a decline in photoreceptor function over time, and another suffered a retinal detachment. [45]

The use of the ESCs technique has been associated with immunological complications upon allogenic transplantation,[47] such as massive T cell-mediated immune responses and rejection of transplanted cells. This occurs after T cell activation by antigen presenting cells through interaction with immunogenic antigens such as major histocompatibility complex (MHC) Class 1 molecules present in ESCs.[47]

The main ethical dilemma involves the question of whether it is morally acceptable to pursue these novel therapies at the expense of destroying an early human embryo, since isolation of ESCs

requires the destruction of a human embryo. This brings out individual opinions so deeply rooted in basic moral beliefs that developing a general consensus and policy acceptable to everyone seems unlikely.[48]

Mesenchymal Stem Cells (MSCs)

Mesenchymal stem cells (MSCs) and their derivatives have been extensively researched in a variety of preclinical and clinical settings for the treatment of retinal disorders, mainly focusing on the treatment of retinitis pigmentosa and advanced glaucoma.[49]

Induced Pluripotent Stem Cells (iPSCs)

Whilst ESC-derived RPE cells proved functional and resulted in improvements in visual acuity, their transplantation requires either local or systemic immunosuppression. iPSCs are induced by reprogramming differentiated adult stem cells back into an embryonic-like pluripotent state. As mentioned earlier, this technology was developed by Nobel laureate Shinya Yamanaka and his team, who showed that the expression of four pluripotency transcription factors (KLF4, c-MYC, OCT4 and SOX2) could convert adult stem cells back into a pluripotent state, which could then be re-differentiated into a new and desired type of cell. [50] Since iPSCs do not exist naturally and any patient's cells can be transformed into iPSCs, these could provide an unlimited pool of autologous cells that could be used for transplants without the risk of immune rejection. Moreover, easily accessible tissues such as skin, blood and even urine can be used as a source of adult stem cells for iPSC derivation. [51][52] One of the main challenges of cell therapy lies in selecting an appropriate cell source and method to generate authentic retinal pigment epithelial cells. Both embryonic stem cells and induced pluripotent stem cells have the potential to differentiate into RPE cells. Despite improvements in protocols, the process remains to this day inefficient and time-consuming, with limited survival rates after in vivo transplantation. [53] Consequently, many laboratories and research teams around the world are striving to develop efficient and rapid protocols to produce large-scale RPE cells for clinical use.

Some studies have identified straightforward methods for differentiating iPSCs.[54] Human iPSCs can differentiate into pigmented RPE either spontaneously or through heavily directed protocols. [54] Pluripotent stem cells have an inherent ability to differentiate into different types of cells. When certain external growth factors are removed, such as basic fibroblast growth factor (bFGF), they may follow natural cues that lead them to become pigmented cells, consistent with RPE cells. This happens due to intrinsic properties of the iPSCs, their interactions with each other and their environment and the conditions of the culture medium. This process can be slow and inconsistent and lead to a mixed population of cells, not all of which are RPE cells, in a lower yield when compared to a carefully

controlled process. However, it is a natural process which requires only a few steps and little external manipulation. One research team reported a simple, spontaneous differentiation protocol for pluripotent stem cells (such as ESCs and iPSCs) into RPE, but the efficiency was low (less than 10%). [55] This protocol involved changing the medium to one deprived of fibroblast growth factor-2 (FGF-2). The spontaneous procedure is very slow, operator dependent and does not produce cells in sufficient scale. To overcome these challenges, researchers have been able to differentiate iPSCs into RPE directly by adding chemical molecules that influence signalling pathways that are crucial for RPE development and specification. [56] Many different growth factors and chemical molecules have been tested on their effectiveness, including WNT antagonists (Dkk-1), BMP antagonist (noggin), activin A, NODAL antagonists (such as lefty-A), insulin-like growth factor (IGF) and small chemical molecules like Vitamin B3, dorsomorphin, XAV939, SB431542, and heparin. [57] Scientists have, over the years, designed various protocols using different combinations of cytokines and small chemical molecules. [58] For instance, Leach et al. published a quick and reliable protocol on direct differentiation that surpasses spontaneous differentiation methods. This protocol allows for efficient differentiation of RPE cells from iPSCs by combining factors such as activin A and nicotinamide (NIC) with noggin, FGF-2, IGF-1, Dkk1, CHIR99021, N2 and B27 supplements, significantly improving efficiency. [59][60] Another optimized timing protocol accomplishes 60% differentiation of iPSCs into RPE within 14 days. This protocol involves adding noggin, Dkk1, IGF-1, nicotinamide or aminobenzamide at specific times to the iPSC culture medium before introducing activin A and VIP.[61]

There remains some controversy regarding the immunogenicity of induced pluripotent stem cells and their derivatives, despite reports suggesting that cells differentiated from iPSCs are unlikely to be rejected by the immune system. [62] Although they have been used in clinical trials with autologous cells, the high cost of cell production significantly limits their widespread use as a standard treatment. To address these issues, Takahashi et al. investigated allogenic retinal cell lines derived from iPSCs. Recognizing that MHC (major histocompatibility complex) molecules on RPE cells, including those from iPSCs, may be the primary antigen in allogenic inflammatory reactions, they created RPE cells from iPSCs in homozygous MHC donor animals for the transplantation. They used chemical signal inhibitors (SB431542, Y-27632, and CKI-7) in the culture medium for the direct differentiation. These allogeneic iPSC-derived RPE cells were then transplanted into the subretinal tissue of an MHC-controlled monkey model. Immunohistochemical analysis showed no rejection signs in MHC-matched animal models without immunosuppression, whereas MHC-mismatched animals exhibited immune responses around the graft and retinal tissue damage. [63]

Two-dimensional and three-dimensional cultures offer different advantages and are used for different types of research and applications. 2D structures, such as a monolayer patch, are simpler and easier to manipulate whereas 3D structures, such as a scaffold, provide a more realistic environment

that can better mimic the conditions within the body. Although many differentiation protocols involve the formation of 3D structures, it is worth noting they generally yield low quantities or retinal pigment epithelium cells. [64] In a recent study, Michelet et al. introduced a simplified 2D culture method combined with lipoprotein uptake-based sorting, known as the PLUS protocol, to derive an RPE monolayer from human induced pluripotent stem cells within 90 days. This protocol eliminates the need for growth factors and small chemical molecules, making RPE production more cost-effective. The lipoprotein uptake-based sorting exploits the cells' intrinsic differentiation pathways. Cells that naturally uptake lipoproteins are those that are progressing towards an RPE fate. This reduces or eliminates the need for external signals typically provided by growth factors to induce differentiation. Additionally, it employs a feeder-free culture system, which is preferred. [65] They concluded that pure functional RPE monolayers could be derived from pluripotent stem cells in 90 days and that the simplicity and rapidity of their protocol made it potentially scalable.

Other efforts to induce retinal pigment epithelium cells from iPSC have shown that the transplantation of healthy RPE cells woven in carefully crafted biomimetic scaffolds can better mimic the morphology of native tissues.[66] The ideal scaffold would have to be non-immunogenic, mechanically robust but also sufficiently thin to allow exchange of nutrients and metabolites between the retina and choriocapillaris. A scaffold-based approach can also allow for cells to maintain a basal and apical polarization via tight junctions before they are implanted. Different biomaterials have been tested to engineer scaffolds for retinal tissue engineering, and have shown enhanced cell survival and improved organization of RPE cell populations. [67]

In one study, in an effort to maximize sterility and reduce inflammatory risks associated with animal-derived scaffolds, scientists used nanofibrous scaffolds made from natural proteins. To differentiate the induced pluripotent stem cells into RPE cells, they used a neural induction media for 22 weeks, followed by a retinal differentiation media supplemented with B27, vitamin A, ROCK inhibitor, and Y-27632, along with other essential culture components. Eventually, an epithelial-like hexagonal packing structure with tight cellular junctions was observed under light microscopy. [68]
Although scaffold-based approaches may ensure better cellular performance, when compared to cell suspensions, some limitations still remain. The formation of scars by microglia on several scaffolds have been documented and many different scaffolds have not been evaluated in vivo.[69] New instruments will need to be designed to deliver the RPE-scaffold materials into the subretinal space, minimizing trauma

In another model, Ye et al. found that sequential treatment with signalling pathway inhibitors (LDN193189, A-83-01, IWR-1-endo, and Y-27632 for the first 6 days, followed by CHIR99021 and SU5402 for an additional 12 days) along with nicotinamide could enhance the purity and quality of the

generated sheet. In an effort to reduce the risk of inflammation, they avoided using artificial scaffolds and still reported no tumour formation or immune rejection after transplant.

Method of Delivery

There are two different methods for the delivery of RPE cells: subretinal injection of a RPE cell suspension and the insertion of a monolayer patch of RPE cells sewn on special scaffolds into the subretinal space. One method involves pars plana vitrectomy, where the vitreous humor is removed to provide better access to the retina, followed by a small incision in the temporal part of the macula where a viable RPE cell suspension (approximately 50,000 cells per injection) is injected into the subretinal space, specifically in the damaged area that requires treatment. [(61)] This delivery method, on the other hand, has a few setbacks, such as the risk that the injected RPE cells might flow into the vitreous cavity. Concerns have also been raised about the viability of RPE cells injected as suspensions, as they are less likely to form a functional RPE monolayer compared to a monolayer patch of RPE cells. [(70)] RPE cells must form a monolayer and establish tight junctions with adjacent cells. They should also be fully polarized and interact with photoreceptors to perform their physiological functions. [(71)]

The "RPE Patch" technique involves implanting a monolayer of RPE cell sheet, either alone or on a biocompatible scaffold, under the retina. This approach allows for an oriented, polarized, and mature RPE monolayer sheet to repair the damaged area. The biocompatible scaffold mimics the properties of Bruch's membrane, such as permeability to soluble substances from the choroidal vessels and supports retinal pigmented epithelial cell metabolism, adhesion, and polarity. Therefore, using scaffolds in the patch method enhances cellular viability and stability, regulates RPE cell differentiation and ensures effective cellular function.[(72)]

In this context, *Mandai et al*. have presented compelling data from clinical trials involving iPSC derived RPE cells. One notable case involved autologous transplantation of iPSC derived RPE cells in a patient with advanced neovascular AMD, which was well tolerated by the patient's body. Despite the integrity of the transplanted cell layer, no discernible improvement in visual acuity was reported one-year post-transplantation [(73)] but a subsequent evaluation four years later indicated that the transplanted cells maintained structural integrity, allowing for visual acuity stability without the need for subsequent anti-VEGF treatments. [(74)]

Other reported approaches for the delivery of stem cells in retinal diseases include intravenous administration, intravitreal injections and suprachoroidal space injections. Intravitreal injection of cell-based therapies has been studied but the results have been inconclusive.[(75)] Suprachoroidal delivery has also been investigated, although concerns about undesirable immune responses remain. [(76)]

Challenges and Controversy

Ever since stem cells were introduced with their unique mechanisms of self-renewal and differentiation, there has been a growing concern about their safety and marketing as magical cells. Several studies have shown that the morphology and function of iPSC derived RPE cells are similar to native RPE cells both in vivo and in vitro. [77] In spite of years of scientific research, scientists still face numerous challenges, mainly due to ethical and regulatory reasons.

The most notable case where stem cells were used prematurely and ended up having undesirable outcomes occurred at a private clinic in the United States where three patients with advanced macular degeneration developed severe bilateral vision loss after receiving intravitreal injections of autologous adipose tissue-derived stem cells. The patients' severe visual loss after the intravitreal injection was associated with glaucoma, hemorrhagic retinopathy, vitreous hemorrhage, combined traction and rhegmatogenous retinal detachment, or lens dislocation. [78]

Retinal pigment epithelial cell transplantation possesses significant challenges due to immune responses. Clinical trials have observed that RPE allografts often fail to survive because of immune rejection. The rejection of transplanted cells is typically linked to histocompatibility differences between the donor and recipient.[79] In many cases, immunosuppressants are required to prevent implant rejection.

The transplantation of undifferentiated pluripotent stem cells may also pose a risk of tumour formation since teratomas, tumours that contain all three germ layers, could in theory develop.[80] The pluripotency of hESCs and iPSCs is a double-edged sword, meaning the same plasticity that allows them to generate different cell types also makes them difficult to control after in vivo transplantation. Genetic mutations in iPSCs or their differentiated cells are of concern as they increase the risk of cancer formation. For instance, the initial Japanese study that used autologous iPSC derived RPE cells to treat AMD was halted after unexpected mutations were noticed in the iPSCs derived from the second patient. [77] Therefore, the only way to ensure that a teratoma will not develop after transplantation is to differentiate them before injection and screen them for the presence of undifferentiated cells.[81]

Finally, a variety of viral and non-viral vectors, specifically recombinant viral vectors, are not only used in gene therapy but also in the creation of induced pluripotent stem cells, therefore, any remaining vectors must be checked to ensure the safety of the reprogrammed cells. [82] Ensuring microbiological sterility of the stem cells is crucial. A comprehensive viral testing program is needed to screen for all human opportunistic agents (e.g. hepatitis B and C, HIV…).

Conclusion

Although treatment options exist for the neovascular form of AMD, opportunities to restore the patients' vision are limited once photoreceptor cells become dysfunctional. So far degenerative diseases like AMD have proved to be a burden on healthcare systems, but also a burden on one's quality of life.

In the past few years, induced pluripotent stem cells have shown promise, specifically due to their greater immunological tolerability and fewer ethical constraints. Following a decade of preclinical and clinical studies, iPSC derived retinal pigment epithelium cells have emerged as a novel treatment for patients suffering from AMD. There has been significant evidence from phase I/II clinical trials supporting the therapeutic potential of these approaches.

Different stem cell therapy approaches are currently under investigation for the treatment of AMD. However, no product has so far reached the market. Further research must be conducted to optimize cell differentiation and delivery mechanisms, as well as ensuring long-term ocular tolerability and effectiveness, if ever stem cell transplantation is to become standard treatment in AMD.

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