Chapter

Diabetic Retinopathy and Stem Cell Therapy

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Abstract

This overview was evaluated by the development of diabetic retinopathy (DR) and the stem cell therapy approach. DR is a microvascular complication of diabetes mellitus, characterized by damage to the retinal blood vessels leading to progressive loss of vision. However, the pathophysiological mechanisms are complicated and not completely understood yet. The current treatment strategies have included medical, laser, intravitreal, and surgical approaches. It is known that the use of mesenchymal stem cells (MSC), which has a great potential, is promising for the treatment of many degenerative disorders, including the eye. In retinal degenerative diseases, MSCs were ameliorated retinal neurons and retinal pigmented epithelial cells in both *in vitro* and *in vivo* studies. Stem cell therapies show promise in neurodegenerative diseases. However, it is very important to know which type of stem cell will be used in which situations, the amount of stem cells to be applied, the method of application, and its physiological/neurophysiological effects. Therefore, it is of great importance to evaluate this subject physiologically. After stem cell application, its safety and efficacy should be followed for a long time. In the near future, widespread application of regenerative stem cell therapy may be a standard treatment in DR.

Keywords: diabetic retinopathy, mesenchymal stem cells, cell therapy, regenerative stem cell therapy, neurodegenerative diseases

1. Introduction

The eye is an excellent structure, both an optical and a neuronal device. There are many diseases related to the eye. Each anatomical part of this organ may show a defect and cause an eye defect. Diabetic retinopathy is one of the most common complications of type I and type II diabetes. One of the main causes of blindness worldwide is diabetic retinopathy. Although glucose controls are helpful for other diabetic complications, they cannot prevent the development of retinopathy. While many studies have been done on the physiology of the retina, there are many unknown dark spots. Studies suggest that radicals derived from reactive oxygen play an important role in the development of diabetic retinopathy. Due to high oxygen consumption, the brain and retina are very sensitive to oxidative stress. Oxidative stress has been found to cause brain and retinal damage in both diabetic humans and experimentally diabetic rats. Although various hypoglycemic drugs have been developed for the treatment of diabetes mellitus (DM), complications associated with diabetes remain major medical problems. Therefore, the development of new treatments is of great interest. The mechanisms in the development and progression

of diabetic retinopathy are not yet fully understood as they are multifactorial and complex. Stem cell therapies for retinal diseases have been around for a long time. Few clinical trials are currently showing improvement [1].

The eye is the site of many acute or chronic physiopathological disorders, reversible or not, that can lead to partial or total vision loss or major changes in the quality of patients' life. The search for innovative therapeutic strategies to correct these disorders is an important current issue. Gene and cell therapies are powerful therapeutic tools, but controlling the properties and spread of the injected material is a parameter that limits its application in humans. Anatomical isolation of the eye and ease of access, on the other hand, enable the use of such treatments, which have been previously developed in tissues and whose clinical application is complex [2].

Hillard Lazarus used mesenchymal stem cell (MSC) for the first time in 1995. Today, there are more than 400 applications in a wide variety of clinical fields such as inflammatory pathologies or immunological, fibrotic, or neurological disorders [3].

The use of MSC, which has a great potential, is promising for the treatment of many degenerative disorders, including the eye. In retinal degenerative diseases, MSC ameliorated retinal neurons and retinal pigmented epithelial cells in both *in vitro* and *in vivo* studies [1]. Diabetes is among the largest medical emergencies in the world. Hyperglycemia is responsible for a wide number of complications, with the vascular ones representing the leading cause of mortality. Stem cells have the unique ability to originate any organ or tissue and are capable of self-renewal. Among stem cells, great clinical interest is reserved for MSC [4].

2. Diabetes and diabetic retinopathy

The development of modern life has brought with it an inactive life [5]. The human population is constantly increasing, and diseases are also increasing. In addition, the expectation of prolonging life, lifestyle, and dietary habits that support obesity creates possible conditions for the development of diabetes. Diabetes is shown as the third cause of death in industrialized countries after cardiovascular diseases and cancer. It is stated that about 110 million people on a global scale suffer from diabetes mellitus. This type of diabetes is also called diabetes mellitus. The main symptom of this disease is the presence of sugar in the urine. A diabetic patient occurs every 8 minutes according to the research of a health institution. DM is the inability of sugar to enter the cell and perform its function as a result of the insufficiency of pancreatic insulin secretion or the ineffectiveness of insulin or the inability of insulin to function due to structural defects in the insulin molecule. Insulin produced in the pancreas is responsible for the transition of blood glucose into cells. When insulin is deficient, the level of glucose in the blood increases and it increases the permeability of the vessel by causing defects in the inner surface and outer wall of the vessel in the vascular tissue. Diabetes damages the retina the most in the eye tissue. It is predicted that diabetes mellitus will rise sharply in the next decade. Patients with diabetes suffer from life-limiting and threatening complications and suffer from diseases such as stroke, peripheral arterial diseases, and retinopathy. [6]. Diabetic retinopathy is the most common microvascular complication of DM, resulting in blindness worldwide. Diabetic retinopathy (DR) is a global problem, affecting approximately 100 million people worldwide. Blindness is 25 times more common in diabetic patients than in non-diabetic patients. DR is the most common cause of blindness in patients aged 20–64 years in developed countries. The prevalence of the disease is related to the age of the cases and the duration of the disease. Biochemical changes detected in diabetic retinopathy increased

oxidative stress, nonenzymatic glycosylation, protein kinase-C activation, polyol pathway, and increased nitric oxide [7].

Retinal neurons provide normal visual function. Vision loss in diabetes should be explained as a disorder in the function of neurons. To date, most research has generally focused on retinal vascular changes rather than the effect of diabetes on the neural retina. As a result of many studies, it has been determined that changes in neuronal function and vitality are effective in the pathological mechanism of diabetic retinopathy that starts in the early stage of diabetes. Neurophysiological changes have been observed immediately after the onset of diabetes in both humans and experimental animals [8].

The most common cause of retinopathy is diabetes. Retinopathy is responsible for about a third of vision loss and blindness in children. Microaneurysms, nonperfusion capillaries, hemorrhages and/or lipoprotein exudates, which are the onset of DR, indicate that DR is primarily a microvascular disease [9]. There is ample evidence of early retinal neurodegenerations in diabetes. Neuronal degenerations and early retinal disorders were observed in some animal models and studies in humans before the onset of diabetic vasculopathy [10]. Neurodegeneration, which causes thinning of the retina layer in animal studies, is not only limited to cell death and tissue loss but also causes functional disorders in neurotransmitters [11]. The most prominent feature of neurodegenerative diseases is increased neuronal loss with apoptosis. Increasing neuron frequency is accepted as an important component of pathology in diabetic retinopathy. Early studies characterized vascular lesions in postmortem specimens of human retinas [12–14].

Indeed, neurophysiological changes have been observed immediately after the onset of diabetes in both humans and experimental animals. It has been reported that vascular changes such as permeability changes during diabetes occur 8 days after the onset of diabetes in rats. Capillary dilation and increased blood flow are the earliest signs of diabetes in both humans and animals. Capillaries begin to close within a few years in dogs whereas in about 1 year of diabetes in rats. Typical retinopathy begins to develop in humans at 5–10 years, with microaneurysm, hemorrhage, macular edema, and neovascularization. The neural retina is transparent and invisible, so it is not visible on clinical examination. Vascular changes provide information about the course of the disease and the possibility of blindness. Apart from insulin therapy, the only proven treatment is laser photocoagulation, which destroys retinal regions with overt vascular disorders. This manipulation reduces macular edema and can improve visual acuity, but it cannot restore normal vision and prevent neuronal loss. If neurodegeneration begins shortly after the onset of diabetes, irreversible neuron damage occurs during laser therapy. Early neurophysiological and neurodegenerative changes should be considered as targets for current DR treatments. Psychophysical measurements also showed changes in vision in the early stage of diabetes onset. Contrast sensitivity decreases especially at mid and low spatial frequencies [1, 15].

Obesity is a major health problem in the world that is responsible for type II diabetes mellitus (DM) and its serious complications, such as retinopathy, cardiovascular disease, and nephropathy. In diabetic eyes, neovascularization results in blindness through a vitreous hemorrhage, retinal detachment, or glaucoma. Retinal hypoxia is the crucial factor for these complications [16]. Diabetic retinopathy is one of the most common complications of type I and type II diabetes. One of the main causes of blindness worldwide is diabetic retinopathy. Although glucose controls are helpful for other diabetic complications, they cannot prevent the development of retinopathy. The pathology of retinopathy is due to the deterioration of the vessels of the eye, which occurs due to various metabolic disorders in diabetic patients. These metabolic disturbances range from the level of vascular endothelial growth

factor (VEGF) to the accumulation of end products of its glycosylation. The primarily tissue-damaging effects of chronic hyperglycemia cause a complex interplay of multiple mechanisms, which cause abnormal permeability within the retinal vessels, and occlusion with ischemia and subsequent neovascularization. Current treatments include laser photocoagulation and vitreotomy, but these treatments are not curative and do not target the pathological mechanism of the disease. Various studies have been conducted in diabetic rats and human models. Immunohistochemical studies were able to show that intravitreally injected stem cells were localized to the inner retina and it has been stated that this increases visual function. Human clinical trials are ongoing to evaluate the safety, success, and utility of hematopoietic stem cell (HSC) injection in treating retinal vascular diseases. Two patients with diabetic retinopathy injected with HCC showed improvement in visual acuity and ophthalmic measurements even 12 weeks after treatment. The mechanism of the behavior of HSC is unclear, but is thought to be dependent on paracrine signaling. In animal models, intravitreal HSC has been shown to improve retinal damage caused by light, ischemia, and diabetes. Apart from HSC, other stem cells such as mesenchymal stem cell (MSC), endothelial progenitor cell (EPC), and adipose stromal cell are also being investigated for their use in the treatment of diabetic retinopathy. Diabetes mellitus causes both functional and structural deficiencies by affecting both the peripheral and central nervous systems. Peripheral disorders develop within a few weeks after the onset of diabetes, while central disorders take months to develop [17]. Diabetic retinopathy is a major complication of diabetes. However, the effect of a prediabetic condition on the retina has not been clarified. Prediabetes refers to a metabolic disorder defined by glycemic variables lower than diabetes but higher than normoglycemia and considered a high-risk condition for the development of diabetes. It has been stated that the majority of prediabetic patients will eventually develop diabetes [18, 19]. Current treatments for DR as laser photocoagulation, intravitreal anti-VEGF agents, intravitreal corticosteroids, and vitreoretinal surgery are applicable only at advanced stages of the DR and are associated with significant adverse effects [20]. Therefore, new treatments for the early stages of the DR are needed. Retinal diseases are the leading cause of vision loss in the world. Because of the ability of stem cells to self-renewal and differentiation to various types of cells, stem cells are becoming an attractive source of cell therapy in repairing damaged cells as retina pigment epithelium or photoreceptors. Consequently, retinal stem cell therapy is one of the promising therapeutic alternatives to recover vision [21].

3. Stem cells

The organism begins to form from a cell and then develops into a complete organism with more than 200 cell types. This phylogenetic trend, the tendency to switch from pluripotent cells to mature cells is an integral part of human development. This process, in which cells differentiate and turn into cells without plasticity, is necessary to form all special tissues of the human and to minimize the risk of tumor proliferation. Basically, for a cell to be accepted as a stem cell, this cell should first be able to renew itself without losing its plasticity, and then lose its plasticity and differentiate into different sub-cell types [22]. A stem cell is an undifferentiated cell with the capacity to self-renewal and differentiate. These cells have also the capacity to differentiate into special cells that make up tissues and organs. Self-renewal is the capacity for a cell to reproduce indefinitely by maintaining its undifferentiated state. Differentiation potential is the capacity for a cell to differentiate into one or more types of mature cells.

In addition, stem cells are also characterized by maintaining a certain calm-stagnant state, apart from their capacity for self-renewal and differentiation. This quiescent phase is the G_0 phase of the cell cycle, in which cells enter in the absence of mitotic factors. With this calm phase, stem cells can protect themselves against possible "attacks" and maintain their vitality. However, none of these features are sufficient to define the 'root' character of the cell. In fact, it should have the potential to rebuild the tissue's excellent function in the long run. This means a large number of cellular divisions and differentiation *in vivo*. These properties also play a vital role in organogenesis and adult tissue regeneration. There are many stem cells used and studied in research. Particularly, some researchers can find new stem cell sources today [23–25].

The two largest types of stem cells in mammals are embryonic stem cells isolated from the inner cell mass of the blastocyst and adult stem cells found in most adult tissues.

3.1 Embryonic stem cells (ESC)

They are the first discovered and studied stem cells. They are cells that can renew themselves and have the capacity to differentiate into all cell types that can form a whole organism.

3.2 Adult stem cells

With the development of the embryo, embryonic pluripotent stem cells are replaced by stem cells with a more limited capacity, which will provide organ and tissue formation. Cells of different tissues are now specialized. Organs need a mechanism to regenerate cells by replacing cells lost by apoptosis or lesion, thus maintaining their homeostasis. An adult loses about 20 billion cells in a day. This requires a permanent restructuring system [26]. Some organs such as the brain, heart, and kidney are less regenerated [27]. On the other hand, tissues such as bone marrow, skin, and intestines are constantly renewed. To ensure the regeneration prosess, organs have a cell reservoir; adult stem cells which serve throughout life. Their stocks are provided by the balance between self-renewal and differentiation capacities. Adult stem cells are also known as somatic stem cells. It is assumed that all organs of the body have mature stem cells, and in most organs, they are active throughout life. They constantly form new cells to ensure tissue regeneration (skin, cornea, bone marrow, intestine) [28–30]. In some organs, these cells become active after birth and then go into dormancy. We see them in organs with slow or almost no cellular regeneration; it is seen only in organs such as the brain and liver, where stem cells divide only during serious injuries or rarely [31, 32]. Hematopoietic stem cells, stromal stem cells, and stem cells in organs are adult stem cells.

Bone marrow contains two types of cells: hematopoietic stem cells (HSC) and stromal mesenchymal stem cells (MSC). HSC can form all mature hematopoietic cells such as myeloid and lymphoid. MSC plays a supportive role in hematopoiesis. Bone marrow also contains other types of cells. Progenitor endothelial cells (PEC) are found in the marrow as well as adult multipotent progenitor cells [33]. When necessary, PEH enters the circulation and plays a role in angiogenesis. In addition, some studies refer to bone marrow stem cells (F-MSCs), which represent a heterogeneous stem cell population. These cells can differentiate into many different types of cells, such as hepatocytes, endothelial cells, epithelial cells, cardiac or skeletal muscle cells, neuronal cells, or astrocytes. This indicates that bone marrow cells have the potential to differentiate into cells of another tissue [34]. Stem cells are ubiquitous. Some niches are yet to be discovered. Although bone marrow-derived

stem cells have been cited as a potential resource for regenerative therapy, their potential and usefulness are still open to debate [35, 36].

Deterioration in tissues or organ functions for any reason constitutes a very important problem in terms of seriously affecting an individual's quality of life. For this reason, regenerative medicine is concerned with repairing the damage and restoring normal body functions through stem cell therapies. Advances in stem cell research have shown cell-based therapy as a useful option to treat medically incurable diseases [36]. Stem cells can migrate to damaged tissue. The effect and cellular mechanisms of stem cells vary according to their environment. They have excellent plasticity that allows these cells to adapt to their environment and act appropriately [33].

"Plasticity" is the ability of a stem cell to acquire different differentiation programs under certain microenvironmental conditions. Endogenous MSC or exogenously administered MSC can migrate to the injured tissue and participate in its healing. The therapeutic effects of MSC can be attributed to its ability to secrete a wide variety of paracrine factors. These mechanisms are likely independent, but they can also act together. In many cases, a combination of these protective mechanisms can work together to heal the damage [37]. However, the mechanism of the therapeutic effect of stem cell is still open to debate. There are two basic explanations; these are cell differentiation and the paracrine effect of stem cells. The combination of these two mechanisms seems to be a third theory [38].

4. Mesenchymal stem cells and retinal degenerations

Retinal degenerations are pathologies that affect the light-sensitive cells of the retina, photoreceptors, cones, and rods. Cell therapy is accepted as an interesting alternative for retinal degenerations. A mouse model of retinal degeneration has been shown to improve visual function after transplantation of photoreceptors [39]. Other cells, including MSC, also show great potential by altering photoreceptors or protecting against degenerations due to their paracrine effects [40]. Some researchers also emphasize that MSC can differentiate into retinal cells, especially photoreceptor-like cells. This plasticity feature of MSC has been observed *in vitro* [41]. It has also been observed *in vivo* by subretinal injection in a rat model with retinal degeneration [42]. These results reveal the possibility of regenerative therapy in pathologies involving photoreceptor losses.

In general, cellular therapy works in two ways: to replace dead cells in the tissue to restore tissue function or to prevent/attenuate/slow tissue degeneration by reducing inflammatory infiltration or reduce apoptosis and cell death phenomena.

5. Stem cell and eye

The eye is a small organ, and the number of stem cells required therapeutically is theoretically less than in larger organs. Compared to other internal organs, the retinal environment is easily accessible with small-gauge vitrectomy needles, greatly increasing the potential for stem cell-based therapy for the treatment of retinal degenerative diseases. The retina is layered and thin. It depends on the preservation of cells, nerve anatomy, and synaptic networks to maintain vision. Retinal neuron connectivity is an important therapeutic goal to alleviate blindness in millions of people worldwide for the preservation or restoration of the original neural structure of the retina and photoreceptor [43]. The emergence of studies shows the possibility of cell regeneration in the adult central nervous system, which makes it possible

to envision the implantation of stem cells or progenitor cells as an approach to cell therapy. The restorative approach offers strong hope, given that key questions about the biology of development need to be explored. The transplantation of differentiated or undifferentiated retinal tissue (embryonic, newborn) in the subretinal position of the graft poses the problem of its structural and functional organization.

It is seen that different types of stem cells are used considering transplantation studies. When we look at transplantation studies, in studies using different types of stem cells (retina progenitor cell, neural stem cell, bone marrow-derived stem cell, and embryonic stem cell), although they settle in the retina, they are not able to express retinal-specific markers and cannot establish synaptic connections are encountered [44].

The discovery of stem cells has caused great excitement in the hopes of using such treatments to restore vision. Already, stem cells in the anterior segment of the eye have a remarkable clinical effect. Stem cell therapy provides re-epithelialization of the cornea and improves vision. The trabecular meshwork, located on the inner side of the junction of the sclera and the cornea, can also be regenerated with stem cells [38]. However, the most interesting studies have been done in the posterior segment of the eye. Most retinal degenerations begin with the loss of a neuron or damage to a neuron. Therefore, these cells should be replaced with a cell layer that is differentiated and functional in the appropriate medium. Sometimes pathology develops and destroys many cells. In this case, a graft consisting of several layers is required. To perform a transplant treatment for blindness, progenitor neuronal cells are isolated and transferred to different cells of the retina.

Studies on neuronal cell cultures that can differentiate are done. Today, very few of these differentiation mechanisms have been fully elucidated. Therefore, the use of cell transplantation in the retina seems distant [45].

Considering that stem cell therapy is promising in retinal diseases, studies were started with embryonic stem cells, and induced pluripotent stem cells were obtained. Many retinal cells such as retinal pigment epithelium, photoreceptors, and ganglion cells were obtained from induced pluripotent stem cells [46].

It is stated that the neuroretina, which is attached to the pigment epithelium (RPE), has a complex structure. Therefore, it has been stated that there are three different cells that can be considered in cell therapy: neuroretina (photoreceptors, bipolar cells, ganglion cells, and glial cells), RPEs, and vascular endothelial cells. Depending on retinal diseases, strategies to place different cells need to be developed [47].

6. Conclusion

Diabetic retinopathy (DR) is one of the largest causes of vision loss worldwide. The use of autologous stem cells for organ reconstruction offers a potential solution for the replacement of tissue or whole organs mechanisms in the development and progression of DR are not fully understood yet. Although many studies have been done about retinal physiology, many unknown dark spots are available about it. Stem cell therapy appears to be a possible option both to prevent neurovascular damage and to repair the damaged retina. Mesenchymal stem cell attracts great attention in retinal degenerations due to their ability to differentiate into neurons. However, the way and amount of stem cell administration will create different effects, it is important to know the effect of cell therapy on body after administration in relation to its use in the clinical practice.

To date, no treatment has been developed for the regeneration of retinal vasculature damage resulting from prolonged hyperglycemia. Cell therapy seems

to be a possible option both to prevent neurovascular damage and to repair damaged retina [48]. Although clinical evaluations and retinal autopsies of diabetic patients provide information about the progression and features of diabetic retinopathy, its pathophysiological mechanism is not yet understood. Studies on animal models continue in order to better understand the development of diabetic retinopathy at the molecular and cellular level [49]. Retina, in the nervous system, provides a suitable environment to study the functions and distribution of stem cells. It is stated that intravenously administered mesenchymal stem cell transplantation can inhibit retinal apoptotic cells, reduce inflammatory responses, and limit the spread of damage [50].

In a study in which intravitreal mesenchymal stem cell application was performed, some physiological parameters were examined and it was seen that although there were decreases in body weight in diabetics, there was no change in body weight in the group administered intravitreal stem cells. These findings were interesting for us. While it was reported that body weight increased significantly in the mouse model in which the human adipose tissue-derived mesenchymal stem cell was transplanted *via* the tail. It has been stated that intravitreal stem cell application also reduces intraocular pressure and provides a better cognitive function in the diabetic model [51].

As a result, more clinical trials should evaluate the application methods, the timing of the practice, using cell count and repetition dose of stem cell and their results. In the near future, the regenerative stem cell therapy may be a standard treatment in many degenerative eye disorders.

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References

[1] Sevil K. The Effect of Mesenchymal Stem Cell Applications on Diabetes and Tamoxifen Retinopathy in Diabetic Rats. Kayseri, Turkey: Erciyes University; 2020

[2] Trapani I, Puppo A, Auricchio A. Vector platforms for gene therapy of inherited retinopathies. Progress in Retinal and Eye Research. 2014;**43**:108-128. DOI: 10.1016/j.preteyeres.2014.08.001

[3] Galderisi U, Peluso G, Di Bernardo G. Clinical trials based on mesenchymal stromal cells are exponentially increasing: Where are we in recent years? Stem Cell Reviews and Reports. 2021;**17**(4):1-14. DOI: 10.1007/s12015-021- 10231-w

[4] Bassi R, Trevisani A, Tezza S, Ben Nasr M, Gatti F, Vergani A, et al. Regenerative therapies for diabetic microangiopathy. Experimental Diabetes Research. 2012;**2012**:916560. DOI: 10.1155/2012/916560

[5] Mutlu S. The Effect of Exercise on Physical Profile and Some Physiological Parameters in Pregnant Women. Kayseri, Turkey: Erciyes University; 2019

[6] Duh EJ, Sun JK, Stitt AW. Diabetic retinopathy: Current understanding, mechanisms, and treatment strategies. JCI Insight. 2017;**2**(14):e93751. DOI: 10.1172/jci. insight.93751

[7] Berkit İ. The Effect of Intravitreal Triamcinalone Acetonide on Retinal Vascular Endothelial Growth Factor Release in Diabetic Rats. Aydın, Turkey: Adnan Menderes University; 2008

[8] Lieth E, Gardner TW, Barber AJ, Antonetti DA, Penn State Retina Research Group. Retinal neurodegeneration: Early pathology in diabetes. Clinical & Experimental Ophthalmology. 2000;**28**(1):3-8. DOI: 10.1046/j.1442-9071.2000.00222.x.(2000)

[9] Lecleire-Collet A, Audo I, Aout M, Girmens JF, Sofroni R, Erginay A, et al. Evaluation of retinal function and flicker light-induced retinal vascular response in normotensive patients with diabetes without retinopathy. Investigative Ophthalmology & Visual Science. 2011;**52**(6):2861-2867. DOI: 10.1167/iovs.10-5960

[10] Lasta M, Pemp B, Schmidl D, Boltz A, Kaya S, Palkovits S, et al. Neurovascular dysfunction precedes neural dysfunction in the retina of patients with type 1 diabetes. Investigative Ophthalmology & Visual Science. 2013;**54**(1):842-847. DOI: 10.1167/iovs.12-10873

[11] Barber AJ, Baccouche B. Neurodegeneration in diabetic retinopathy: Potential for novel therapies. Vision Research. 2017;**139**:82-92. DOI: 10.1016/j.visres.2017.06.014

[12] Bloodworth JM Jr. Diabetic microangiopathy. Diabetes. 1963;**12**:99-114. DOI: 10.2337/diab.12.2.99

[13] Bloodworth JM Jr, Molitor DL. Ultrastructural aspects of human and canine diabetic retinopathy. Investigative Ophthalmology. 1965 Dec;**4**(6):1037-1048

[14] Cunha-Vaz JG. Pathophysiology of diabetic retinopathy. The British Journal of Ophthalmology. 1978;**62**(6):351-355. DOI: 10.1136/bjo.62.6.351

[15] Chihara E, Matsuoka T, Ogura Y, Matsumura M. Retinal nerve fiber layer defect as an early manifestation of diabetic retinopathy. Ophthalmology. 1993;**100**(8):1147-1151. DOI: 10.1016/s0161-6420(93)31513-7

[16] Arjamaa O, Nikinmaa M. Oxygen-dependent diseases in the retina: role of hypoxia-inducible factors. Experimental Eye Research. 2006;**83**(3):473-483. DOI: 10.1016/j.exer.2006.01.016

[17] Biessels GJ, Cristino NA, Rutten GJ, Hamers FP, Erkelens DW, Gispen WH. Neurophysiological changes in the central and peripheral nervous system of streptozotocin-diabetic rats. Course of development and effects of insulin treatment. Brain. 1999;**122**(Pt 4):757-768. DOI: 10.1093/brain/122.4.757

[18] Alves MRP, Boia R, Campos EJ, Martins J, Nunes S, Madeira MH, et al. Subtle thinning of retinal layers without overt vascular and inflammatory alterations in a rat model of prediabetes. Molecular Vision. 2018;**24**:353-366

[19] Cai J, Boulton M. The pathogenesis of diabetic retinopathy: Old concepts and new questions. Eye (London, England). 2002;**16**(3):242-260. DOI: 10.1038/sj.eye.6700133

[20] Simó-Servat O, Hernández C, Simó R. Usefulness of the vitreous fluid analysis in the translational research of diabetic retinopathy. Mediators of Inflammation. 2012;**2012**:872978. DOI: 10.1155/2012/872978

[21] Feng X, Chen P, Zhao X, Wang J, Wang H. Transplanted embryonic retinal stem cells have the potential to repair the injured retina in mice. BMC Ophthalmology. 2021;**21**(1):26. DOI: 10.1186/s12886-020-01795-1

[22] Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy. 2006;**8**(4):315-317. DOI: 10.1080/14653240600855905

[23] Meng X, Ichim TE, Zhong J, Rogers A, Yin Z, Jackson J, et al. Endometrial regenerative cells: A novel stem cell population. Journal of Translational Medicine. 2007;**5**:57. DOI: 10.1186/1479-5876-5-57

[24] Reinisch A, Hofmann NA, Obenauf AC, Kashofer K, Rohde E, Schallmoser K, et al. Humanized large-scale expanded endothelial colony-forming cells function in vitro and in vivo. Blood. 2009;**113**(26):6716-6725. DOI: 10.1182/blood-2008-09-181362

[25] Humphries A, Graham TA, McDonald SA. Stem cells and inflammation in the intestine. Recent Results in Cancer Research. 2011;**185**:51-63. DOI: 10.1007/978-3-642-03503-6_3

[26] Fuchs E. The tortoise and the hair: Slow-cycling cells in the stem cell race. Cell. 2009;**137**(5):811-819. DOI: 10.1016/j.cell.2009.05.002

[27] Bergmann O, Bhardwaj RD, Bernard S, Zdunek S, Barnabé-Heider F, Walsh S, et al. Evidence for cardiomyocyte renewal in humans. Science. 2009;**324**(5923):98-102. DOI: 10.1126/science.1164680

[28] Barker N, van Es JH, Kuipers J, Kujala P, van den Born M, Cozijnsen M, et al. Identification of stem cells in small intestine and colon by marker gene Lgr5. Nature. 2007;**449**(7165):1003-1007. DOI: 10.1038/nature06196

[29] Greco V, Chen T, Rendl M, Schober M, Pasolli HA, Stokes N, et al. A two-step mechanism for stem cell activation during hair regeneration. Cell Stem Cell. 2009;**4**(2):155-169. DOI: 10.1016/j.stem.2008.12.009

[30] Takizawa H, Regoes RR, Boddupalli CS, Bonhoeffer S, Manz MG. Dynamic variation in cycling of hematopoietic stem cells in steady state and inflammation. The Journal of Experimental Medicine.

2011;**208**(2):273-284. DOI: 10.1084/jem.20101643

[31] Lugert S, Basak O, Knuckles P, Haussler U, Fabel K, Götz M, et al. Quiescent and active hippocampal neural stem cells with distinct morphologies respond selectively to physiological and pathological stimuli and aging. Cell Stem Cell. 2010;**6**(5):445-456. DOI: 10.1016/j.stem.2010.03.017

[32] Furuyama K, Kawaguchi Y, Akiyama H, Horiguchi M, Kodama S, Kuhara T, et al. Continuous cell supply from a Sox9-expressing progenitor zone in adult liver, exocrine pancreas and intestine. Nature Genetics. 2011;**43**(1):34-41. DOI: 10.1038/ng.722

[33] van Haaften T, Thébaud B. Adult bone marrow-derived stem cells for the lung: Implications for pediatric lung diseases. Pediatric Research. 2006;**59**(4 Pt 2):94R-99R. DOI: 10.1203/01.pdr.0000203550.50258.5a

[34] Tomita M, Adachi Y, Yamada H, Takahashi K, Kiuchi K, Oyaizu H, et al. Bone marrow-derived stem cells can differentiate into retinal cells in injured rat retina. Stem Cells. 2002;**20**(4):279-283. DOI: 10.1634/stemcells.20-4-279

[35] Machalińska A, Baumert B, Kuprjanowicz L, Wiszniewska B, Karczewicz D, Machaliński B. Potential application of adult stem cells in retinal repair--challenge for regenerative medicine. Current Eye Research. 2009;**34**(9):748-760. DOI: 10.1080/02713680903050592

[36] Enzmann V, Yolcu E, Kaplan HJ, Ildstad ST. Stem cells as tools in regenerative therapy for retinal degeneration. Archives of Ophthalmology. 2009;**127**(4):563-571. DOI: 10.1001/archophthalmol.2009.65

[37] Li H, Fu X. Mechanisms of action of mesenchymal stem cells in cutaneous wound repair and regeneration. Cell and Tissue Research. 2012;**348**(3):371-377. DOI: 10.1007/s00441-012-1393-9

[38] Marchetti V, Krohne TU, Friedlander DF, Friedlander M. Stemming vision loss with stem cells. The Journal of Clinical Investigation. 2010;**120**(9):3012-3021. DOI: 10.1172/JCI42951

[39] Pearson RA, Barber AC, Rizzi M, Hippert C, Xue T, West EL, et al. Restoration of vision after transplantation of photoreceptors. Nature. 2012;**485**(7396):99-103. DOI: 10.1038/nature10997

[40] Ng TK, Fortino VR, Pelaez D, Cheung HS. Progress of mesenchymal stem cell therapy for neural and retinal diseases. World Journal of Stem Cells. 2014;**6**(2):111-119. DOI: 10.4252/wjsc.v6.i2.111

[41] Nadri S, Yazdani S, Arefian E, Gohari Z, Eslaminejad MB, Kazemi B, et al. Mesenchymal stem cells from trabecular meshwork become photoreceptor-like cells on amniotic membrane. Neuroscience Letters; **541**:43-48. DOI: 10.1016/j.neulet.2012.12.055

[42] Huo DM, Dong FT, Yu WH, Gao F. Differentiation of mesenchymal stem cell in the microenviroment of retinitis pigmentosa. International Journal of Ophthalmology. 2010;**3**(3):216-219. DOI: 10.3980/j.issn.2222-3959.2010.03.08

[43] Singh R, Cuzzani O, Binette F, Sternberg H, West MD, Nasonkin IO. Pluripotent stem cells for retinal tissue engineering: Current status and future prospects. Stem Cell Reviews and Reports. 2018;**14**(4):463-483. DOI: 10.1007/s12015-018-9802-4

[44] Goureau O, Sahel JA. Cellules souches rétiniennes: mécanisme de différenciation et potentiel

thérapeutique [Retinal stem cells: Mechanism of differentiation and therapeutic application]. Pathologie Biologie (Paris). 2006;**54**(2):64-71. French. DOİ: 10.1016/j.patbio.2005.02.002.

[45] Limb GA, Daniels JT, Cambrey AD, Secker GA, Shortt AJ, Lawrence JM, et al. Current prospects for adult stem cell-based therapies in ocular repair and regeneration. Current Eye Research. 2006;**31**(5):381-390. DOI: 10.1080/02713680600681210

[46] Garg A, Yang J, Lee W, Tsang SH. Stem cell therapies in retinal disorders. Cells. 2017;**6**(1):4. DOI: 10.3390/cells6010004

[47] Siqueira RC. Stem cell therapy in retinal diseases? Revista Brasileira de Hematologia e Hemoterapia. 2012;**34**(3):222-226. DOI: 10.5581/1516-8484.20120054

[48] Kramerov AA, Ljubimov AV. Stem cell therapies in the treatment of diabetic retinopathy and keratopathy. Experimental Biology and Medicine (Maywood, N.J.). 2016;**241**(6):559-568. DOI: 10.1177/1535370215609692

[49] Lai AK, Lo AC. Animal models of diabetic retinopathy: Summary and comparison. Journal Diabetes Research. 2013;**2013**:106594. DOI: 10.1155/2013/106594

[50] Jiang Y, Zhang Y, Zhang L, Wang M, Zhang X, Li X. Therapeutic effect of bone marrow mesenchymal stem cells on laser-induced retinal injury in mice. International Journal of Molecular Sciences. 2014;**15**(6):9372-9385. DOI: 10.3390/ijms15069372

[51] Sevil K, Bekir C. Effect of mesenchymal stem cell on vep in dıabetıc rat. International Journal of Advances in Science, Engineering and Technology (IJASEAT). 2009, 2019;**7**(3):5-11 DOIONLINE NO - IJASEAT-IRAJ-DOIONLINE-16111