Potential Therapeutic Application of Mesenchymal Stem Cells in Ophthalmology

E.Yu. Zakirova[a*], A.N. Valeeva[a,b], A.M. Aimaletdinov[a], L.V. Nefedovskaya[a], R.F. Akhmetshin[b], C.S. Rutland[c*], A.A. Rizvanov[a*]

[a]Kazan Federal University, Kazan, Russia
[b]Kazan State Medical University, Kazan, Russia
[c]University of Nottingham, Nottingham, UK

*Corresponding authors: Dr E. Zakirova: lenahamzina@yandex.ru, Dr C.S Rutland: catrin.rutland@nottingham.ac.uk: Prof. A. Rizvanov, rizvanov@gmail.com

Abstract

At present a wide variety of methods have been proposed to treat eye disorders, drug therapies are most commonly used. It should be noted that effective treatment modalities especially for degeneration of the retina and optic nerve are lacking. In the last few years stem cell transplantation has been proposed as an alternative method. The opportunities that stem cells provide within clinical use are almost unlimited. These cells are presently applied to treat various traumatic and degenerative disorders due to their unique biologic properties. Stem cells have high proliferative capabilities and are a self-maintained population of cells capable of differentiating into different cell types. Thus, they are represent a very primary stage of a cell lineage. Their ability to differentiate into different pathways provides animals with great plasticity in the renewal of somatic cells in postnatal ontogenesis. Pre-clinical and clinical ophthalmology studies where mesenchymal stem cells are applied and various methods of their administration are discussed herein. In addition the safety and efficacy of using bone marrow- and adipose tissue-derived mesenchymal stem cells have been discussed.

Key words: mesenchymal stem cells; ophthalmology; eye disorders; clinical trial; retina; cornea.

1. Introduction

The development and introduction of modern treatment modalities for different eye disorders are a challenge in medicine. In recent years ophthalmologists have placed a greater focus on stem cells (SCs) as the renewal and regeneration of any tissue in the adult body depends on somatic SCs, with eye tissues being no exception (Holan et al., 2015). The cornea is a protective barrier and consists of three layers with a different germinal origin: the epithelium originates from superficial ectoderm and the stroma and endothelium arise from neural crest cells (mesechymal tissue). Experimental studies have shown that a variety of SCs are present in each of these layers (Amano et al., 2006). For example, limbal SCs maintain epithelial homeostasis and regenerate the cornea, with their deficiency being the main cause of blindness all over the world (Ksander et al., 2014). However, clinical use of cultured stromal and endothelial SCs is hindered as it is difficult to isolate them in sufficient numbers and optimized culture media are lacking. Therefore, it is a high priority to search for an alternative, readily available source of SCs which can be provided in sufficient amounts especially as stem cells have such high potential in the treatment of eye disorders characterized by permanent loss of cells such as glaucoma, age-related macular degeneration, degeneration of photoreceptor cells, hereditary retinopathy, mechanic and ischemic retinal lesions (Joe and Gregory-Evans, 2010; Oner, 2018; Song et al., 2015; Zarbin, 2016).

2. Overview of stem cells

Stem cells from adults can be subdivided into three main groups; hematopoietic, multipotent mesenchymal (stromal) and tissue-specific progenitor cells. Hematopoietic stem cells are multipotent stem cells that give rise to all blood cells of myeloid (monocytes, macrophages, neutrophils, basophils, eosinophils, red

blood cells, megakaryocytes and platelets, dendritic cells) and lymphoid (T- and B cells and natural killers) lineages (Eaves, 2015).

Tissue-specific progenitor cells are poorly differentiated cells located in various tissue types and organs and are responsible for the renewal of their cell populations, in essence to replace dead cells. An example of tissue-specific progenitors includes myosatellite cells, which reside in the myocardium (Le and Chong, 2016). These cells are oligo- and unipotent. That progenitor cells can divide only a restricted number of times whilst other stem cells are capable of unlimited self-renewal is their main difference from other SCs (Klimczak and Kozlowska, 2016). Therefore in this context mesenchymal stem cells (MSCs) are of great interest. Firstly, MSCs give rise to several tissue types. They can differentiate into epithelial neuron-like cells, retinal ganglion cells, glial and photoreceptor cells (Phinney and Prockop, 2007). MSCs are also known to successfully differentiate into keratocytes and corneal epithelial cells (Sun et al., 2018; Zhang et al., 2015).

Adipose derived (AD) and bone marrow (BM) derived are the most common and available sources of MSCs. MSCs derived from adipose and bone marrow have significant potential for tissue regeneration - they secrete signaling molecules such as neurotrophic factors, growth factors or cytokines which can diffuse in a local tissue medium and interact with nearby cells (Lin et al., 2009). Immunophenotyping is one of the most relevant methods used to differentiate BM and AD MSCs from other cells. On their surfaces both MSC types carry similar positive membrane markers such as CD105 (endoglin), CD73 (5'-nucleotidase) and CD90 (Thy-1), whilst remaining negative for markers including CD45, CD34, CD14, CD11b (Dominici et al., 2006).

Mulipotent abilities of BM and AD MSCs are normally assessed by their multi-lineage differentiation in three pathways such as osteogenic, adipogenic and chondrogenic ones. The differentiation into these lineages is a gold standard for MSC identification and any cell-based product should at least meet this requirement in order to be classified as MSCs (Dominici et al., 2006). For this purpose MSCs are cultured in a specific induction medium for 2-3 weeks to induce differentiation into

particular types of cells. Then they are stained for calcium, lipids and proteoglycans to demonstrate whether the cells have functionally specialized into osteocytes, adipocytes and chondrocytes, respectively (Fig. 1). The potential of MSCs for neuronal differentiation and cardiomyoblasts has also been used as criteria in some studies; however, these differentiation types are not used as a routine method to determine biologic activity of MSCs.

3. Stem cell treatments for ocular disorders and injuries

3.1 Stem cell use in retinal and optic nerve disorders

When treating degenerative eye disorders, MSCs are known to have a protective effect on ganglion cells of the retina and to stimulate regeneration of their axons in the optic nerve with paracrine factors they secrete. MSCs mainly provide a trophic supply of axonal neuroprotection and regeneration in damaged cells of the retina either by direct secretion of neurotrophic factors or by possible stimulation of its endogenous cells which provide additional paracrine supply and/or effects of cell replacement when activated (Mead et al., 2015). To date positive effects of MSCs have been conclusively established in the treatment of retina endothelial defects (Zhang et al., 2015).

The administration of MSCs is also one of modern methods to treat diabetic retinopathy. A pilot clinical study conducted in China (No. ChiCTR-ONC-16008055; chictr.org.cn) showed intravenous administration of BM MSCs to be safe and effective in this pathology (Gu et al., 2018). Two of the seventeen patients showed differing adverse effects, one showed an increase in creatine kinase levels and the other patient showed increased creatinine levels but these decreased after transfusion, unfortunately the study did not have a placebo control group and it also had a relatively short patient follow-up period of six months. Interestingly, AD MSCs implanted into the vitreous cavity in a diabetic retinopathy murine model mainly differentiated into pericytes when associating and maintaining the retinal vasculature which indicates a unique role of ADSCs in the treatment of diabetic

retinopathy (Mendel et al., 2013). Adipose-derived stem cells were also shown to stabilize retinal microvasculature in a murine model of diabetic retinopathy and went on further to show that the cells from diabetic mice were less effective than those from healthy donors (Cronk et al., 2015)

Age-related macular degeneration is the most common cause of blindness in developed countries. BM MSCs were shown in pre-clinical models of this degenerative disorder of human retina to have a protective effect on photoreceptor cells (Inoue et al., 2007; Wang et al., 2010). Although BM MSCs when injected into a subretinal area can differentiate into photoreceptor protein expressing cells, their ability to differentiate into functionally useful retinal cells is questionable. Their action is considered to be largely related to paracrine effects due to a release of neurotrophic factors (NTFs). NTFs are a family of proteins that are involved in regulating the growth, functioning and survival of neurons and other cells of the nervous system. Thus, BM MSC-based therapy can exert positive effects on the recipient's cells by producing cytokines and neurotrophic factors and alter a neurodegenerative process by means of immunomodulatory activity (Chichagova et al., 2018). Phase 2 results from eight patients (review number 56733164/203) with AD MSC implantation treatment for dry-type age-related macular degeneration and Stargardt’s macular dystrophy showed no ocular or systemic complications and all experienced enhanced vision improvement (Oner et al., 2018). These disorders are of particular importance as there are no approved therapy to cure them presently, therefore finding alternative therapies is essential. Although treatments such as injections of anti-VEGF, verteporfin photodynamic therapy and steroids slow progression of age-related macular degeneration, other technologies need to be explored. In addition these methods still carry the risk of adverse events including endophthalmites, cataract and retinal detachment and levels of 50% of patients discontinuing treatment have been reported in a retrospective study, with 47% of these due to poor treatment responses (Kataja et al., 2018). Therefore there is a great need to improve treatment types, efficacy and outcomes.

MSCs can be used to treat both acute and chronic ocular disorders. For example, an injection of BM MSCs into the anterior chamber in rodent models effectively decreased the intraocular pressure in experimental open-angle glaucoma. Researchers thought this profound effect to be due to paracrine factors of MSCs. Moreover, MSCs and factors they secreted induced reactivation of a pool of progenitor cells in the ciliary body and enhanced cell proliferation. Proliferating cells were observed within the camber angle for at least a month (Manuguerra-Gagne et al., 2013).

3.3 Ocular injuries

BM MSCs are capable of differentiating into specific keratocytes of the cornea which has been confirmed by low expression levels of markers specific for cornea epithelial cell phenotypes (Harkin et al., 2015). In cornea chemical burn models, MSCs exert their paracrine activity in a damaged cornea as anti-inflammatory and anti-angiogenic effects. Autologous BM MSCs subconjunctively injected to rats with a chemical burn of the cornea are known to promote regeneration of the corneal epithelium, to reduce inflammation and neovascularization, and to increase the expression of anti-inflammatory cytokines (Ke et al., 2015; Sharma et al., 2018). There was a similar positive effect (Fig 2) when allogenic BM MSCs were subconjunctively injected into cats with traumatic corneal ulcers (Zakirova et al., 2015). Intravenous administration of BM-MSCs to mice with corneal chemical burns also stimulated regenerative processes in the site of injury as compared to the control group without cell transplantation (Lan et al., 2012). Similarly in a rabbits with alkali burns those treated with AD MSCs or BM MSCs showed that antioxidant enzymes were restored, corneal reepithelialization was observed alongside reduced neovascularization after 15 days in comparison to controls (Cejka et al., 2016). Allogenic AD MSCs implanted subconjunctively also improved clinical manifestations of eosinophilic keratitis in cats. Feline eosinophilic keratitis is a chronic disease of the cornea caused by an immune response to an unknown antigenic challenge. None of the 5 implanted cats had systemic or local

complications over the 11-month follow-up. The state of the cornea and conjunctiva improved without any signs of regression or worsening (Villatoro et al., 2018). It is also known that limbal epithelial stem cells play several roles in ensuring that the corneal epithelium is maintained, including repopulation of the cells (Yoon et al., 2014). Work in vitro and on rabbit cornea burns models has shown that use of collagenase enzymes promotes a more suitable environment for limbal stem cells following cornea damage (Gouveia et al., 2019). By preventing YAP activation, the natural phenotype of these cells was maintained whilst having no negative effects on healthy cells, although naturally more clinical trials need to be undertaken to further assess efficacy and safety in people.

The cornea is not the only area of interest for MSC treatment. Light-induced retinal injuries in rats have also shown MSC responses including increased production of neutrotropic factor expression within the cells in response to injury, with basic fibroblast growth factor likely to be involved in this mechanism (Xu et al., 2013). Lacrimal glands in rabbits show progenitor cell reactions and epithelial-mesenchymal transitions following ligation-injury of the excretory duct (Lin et al., 2017). A study on canine keratoconjunctivitis sicca treatment using allogeneic AD MSCs showed that in mild-moderate cases eyes reverted to a healthy state (Bittencourt et al., 2016). Most of the severe cases showed improvements in tear production with one patient regaining clinically normal levels however one patient did not respond to treatment at all. In addition no short term (7-28 days) or long term (6 and 12 months) adverse effects were observed in any of the 15 patients.

3.4 Cornea and ocular surface disorders

Dry eye syndrome is one of the most common eye disorders. Its worldwide prevalence ranges from 7% to 33% depending on disease diagnosis management and the population demographics investigated (Lin et al., 2003; Moss et al., 2000). Causes of this syndrome are multiple, however, inflammation on the eye surface plays an essential role in its pathogenesis. The therapeutic potential of MSCs was studied in a dry eye syndrome murine model caused by an intraorbital injection of

concanavalin A (Lee et al., 2015). The results showed that periorbitally injected MSCs reduced an infiltration with CD4 (+) T-cells and decreased inflammatory cytokine levels in the intraorbital gland and on the eye surface. In addition, MSCs stimulated the formation of a lacrimal fluid and significantly increased the number of goblet cells in the conjunctiva. The study demonstrated the integrity of the corneal epithelium when injecting MSCs. No adverse effects were reported but the authors did highlight that some immune-modulatory effects of MSCs are species-specific which highlights the need to undertake trials in differing species. A separate study in rats similarly showed increased secretory granules and goblet cell numbers using topically applied MSCs (Beyazyildiz et al., 2014; Lee et al., 2015). These results formed the conclusion that MSCs can be used to treat a number of ocular surface diseases when inflammation plays a key role in the pathogenesis (Beyazyildiz et al., 2014; Lee et al., 2015). The implantation of allogenic AD MSCs around lacrimal glands significantly improved the manifestation of clinical symptoms in dogs with a dry eye syndrome, with the effect being stable over the study period. There were no negative effects on the cornea or signs of regression or deterioration. The animals tolerated cell administration well and none of them had any systemic or local complications during the study (Villatoro et al., 2017; Villatoro et al., 2015).

4. Advancing stem cell therapy in veterinary medicine

The use of MSCs is rapidly expanding in veterinary medicine outside of ophthalmology, which may also be applicable to ocular disorders and injuries. One example is the use of AD MSC in equine bone spavin treatment trials in comparison to horses undergoing convention steroid treatment and a control group who were limited only in their movement (Nicpon et al., 2013). The AD MSCs cultivated in vitro showed progressive reductions in lameness, as did the steroid group in contrast to the control group, in addition the treatment proved to be safe. Similar results were observed in horses with superficial flexor tendon injuries treated with AD MSC combined with autologous platelet concentrate in comparison to controls (Marycz et

al., 2012). Indeed it may be possible that conventional treatments or even more recent advances in gene therapy such as those observed in equine tendon injuries (Kovac et al., 2017, 2018), could be utilized in conjunction with the stem cell therapies discussed above in order to enhance proliferation, increase wound healing and reduce recovery time.

The use of MSC has advanced dramatically over the years as have the methods used to increase and collect them. A study on mice and people showed that exercise increases hematopoietic stem/progenitor cells (HSPCs) and very small embryonic-like stem cells mobilization (Kroepfl et al., 2012; Marycz et al., 2016). Interestingly this mimics the observations seen in people following acute myocardial infarction, strokes and other disorders (Paczkowska et al., 2009; Wojakowski et al., 2009). Linking these together, application of stem cells, increased mobilization of stem cells, understanding the cascades and mechanisms behind these processes and applying complimentary therapies may offer ways in which MSC therapy can be enhanced in the future for all applications including ocular. Other mechanisms of improving stem cell therapies have been suggested including the use conventional treatments alongside stem cells, assessing proximity of delivery to the site of treatment, and overcoming immune rejection by using host cells, thus overcoming traditional graft complications (Cislo-Pakuluk and Marycz, 2017).

Therapeutic administration of MSCs can be applied to the eye in a number of modes including topical, subconjunctival, intraperitoneal [IP] and intravenous [IV]. A recent paper looking at administration following corneal injury in mice has indicated that in this incidence subconjunctival and IV showed superior therapeutic efficacies in terms of epithelial integrity, accelerated tissue restoration, and reduced fibrosis, corneal opacity and inflammation in comparison to topical or IP (Shukla et al., 2019). Therefore although topical delivery may be easily accessible for the cornea, it may not provide the highest efficacy in comparison to other methods. Cell viability and function, achieving cells retention in the affected area and providing a cell matrix may also affect efficacy. Techniques such as delivery within fibrin glue in rats and the use of a biologically active 3D matrix with MSCs modified for

increased production of VEGF165 and FGF2 in dogs have provided methods in which to enhance efficacy (Masgutov et al., 2019; Zakirova et al., 2019). Understanding the mechanisms has also resulted in MSC derived characteristics or structures such as extracellular vesicles being used in pre-clinical and clinical trials (Galieva et al., 2019). Once example is a macular hole clinical trial using extracellular vesicles which has been approved by the Food and Drug Administration (NCT03437759). Most animal clinical trials are still ongoing and the many hundreds of human clinical trials are mostly in early stages therefore the full range of clinical outcomes, including adverse effects are yet to be determined. It is widely regarded that the main hurdles to overcome when considering MSC therapies are donor heterogeneity, ex vivo expansion, immunogenicity, and cryopreservation (Galderisi and Giordano, 2014; Galipeau, 2013), this is likely to be the case in both people and veterinary patients.

5. Concluding remarks

The last decade has demonstrated that methods based on MSC transplantation are safe and effective. The diversity of approaches to isolate, culture and administer SCs determines the development of cell-based technologies for the treatment of numerous eye disorders depending upon the pathology. The use of AD- and BM-derived MSCs to treat certain ocular diseases is scientifically proven and evidence-based. Ensuring that techniques are simple and reproducible are especially important as this will provide a quick and effective introduction into routine clinical practice. Thus, ongoing scientific experiments/research and activities to introduce MSCs into a clinical practice offer great opportunities for cell-based therapy and highlight an essential role of MSCs in the evolution of medicine and biology.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgments

This study was supported by the Russian Government Program of Competitive Growth of Kazan Federal University. Professor Albert A. Rizvanov was supported by state assignment 20.5175.2017/6.7 of the Ministry of Science and Higher Education of Russian Federation.

Author contributions

All authors wrote the manuscript and reviewed the final draft.

Figure legends

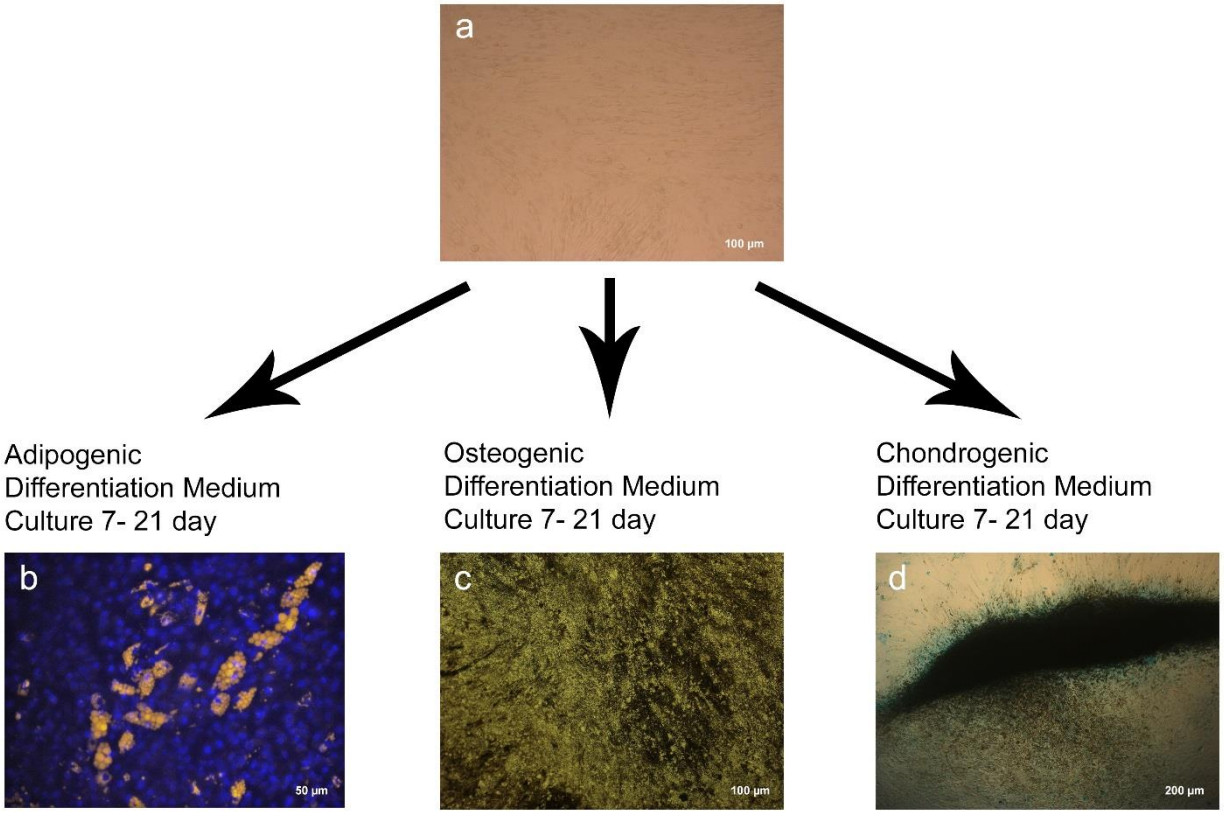

Figure. 1 Morphology of human mesenchymal stem cells derived from adipose tissue during the differentiation protocols. a) Human mesenchymal stem cells derived from adipose tissue. b) Adipogenic differentiation - cell nuclei are stained with Dapi (blue), adipose tissue inclusions within cells dyed in Nile Red (yellow). c) Osteogenic differentiation - Von Kossa staining, the calcium containing area is stained in black. d) Chondrogenic differentiation- acid mucopolysaccharides of the extracellular matrix formed within the differentiation of AD MSCs into chondrocytes are stained with Alcian blue.

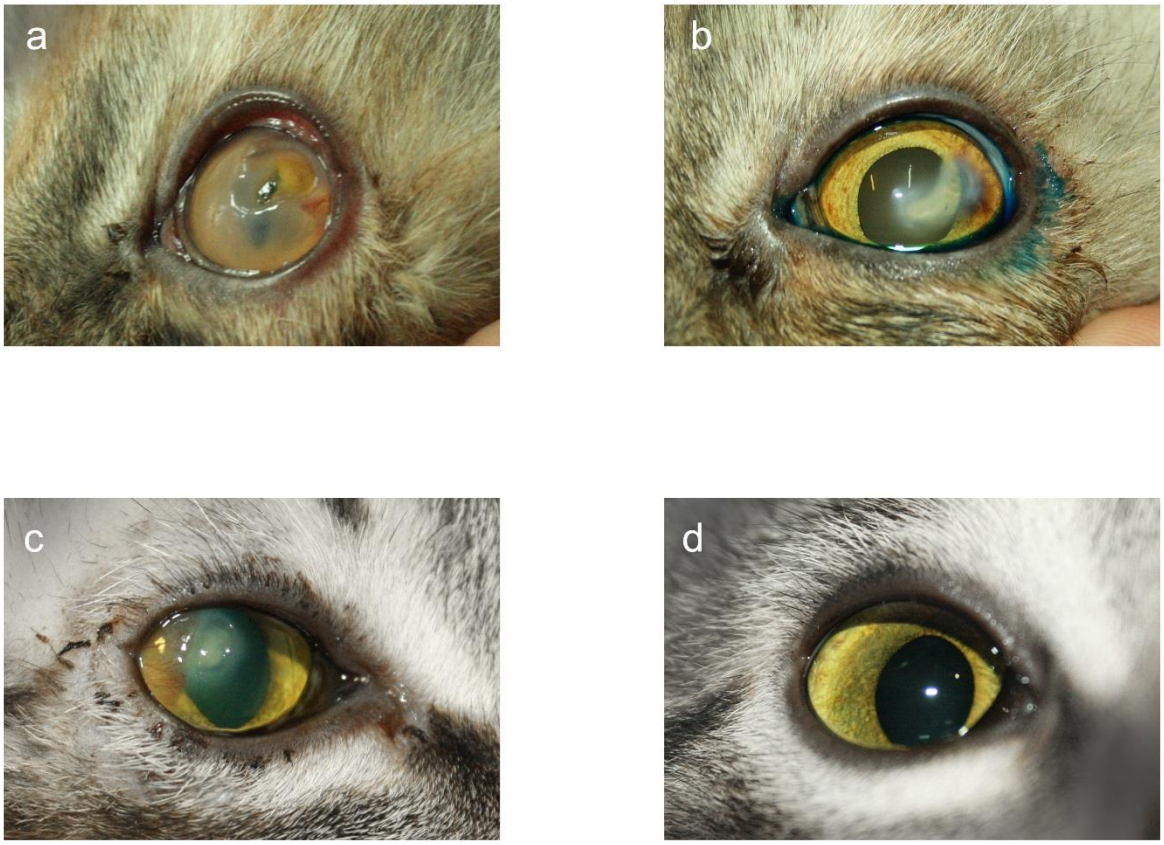

Figure 2. Mesenchymal stem cell treatment of corneal ulcers. Traumatic corneal ulcers in cats treated with routine methods (a, b) and subconjunctival transplantation of allogenic AD MSCs (c, d). Deep ulcers of the cornea accompanied with pronounced blepharospasm, edema of the eyelids, tenderness, and purulent secretion were diagnosed in cats (a, c). b) At 25 days in animals treated with classical methods symptoms such as tenderness, blepharitis, blepharospasm, slight purulent and mucous secretion, keratitis and deep vascularity of the cornea were present. d) At 25 days the animals transplanted with AD MSCs had edema of the eyelids, tenderness and blepharospasm; there was no purulent and mucous secretion, a small scar rather that a corneal defect was visualized.

References

Amano, S., Yamagami, S., Mimura, T., Uchida, S., Yokoo, S., 2006. Corneal stromal and endothelial cell precursors. Cornea 25, S73-77.

Beyazyildiz, E., Pinarli, F.A., Beyazyildiz, O., Hekimoglu, E.R., Acar, U., Demir, M.N., Albayrak, A., Kaymaz, F., Sobaci, G., Delibasi, T., 2014. Efficacy of Topical Mesenchymal Stem Cell Therapy in the Treatment of Experimental Dry Eye Syndrome Model. Stem Cells International.

Bittencourt, M.K.W., Barros, M.A., Martins, J.F.P., Vasconcellos, J.P.C., Morais, B.P., Pompeia, C., Bittencourt, M.D., Evangelho, K.D., Kerkis, I., Wenceslau, C.V., 2016. Allogeneic Mesenchymal Stem Cell Transplantation in Dogs With Keratoconjunctivitis Sicca. Cell Med 8, 63-77.

Cejka, C., Holan, V., Trosan, P., Zajicova, A., Javorkova, E., Cejkova, J., 2016. The Favorable Effect of Mesenchymal Stem Cell Treatment on the Antioxidant Protective Mechanism in the Corneal Epithelium and Renewal of Corneal Optical Properties Changed after Alkali Burns. Oxid Med Cell Longev.

Chichagova, V., Hallam, D., Collin, J., Zerti, D., Dorgau, B., Felemban, M., Lako, M., Steel, D.H., 2018. Cellular regeneration strategies for macular degeneration: past, present and future. Eye (Lond) 32, 946-971.

Cislo-Pakuluk, A., Marycz, K., 2017. A Promising Tool in Retina Regeneration: Current Perspectives and Challenges When Using Mesenchymal Progenitor Stem Cells in Veterinary and Human Ophthalmological Applications. Stem Cell Rev Rep 13, 598-602.

Cronk, S.M., Kelly-Goss, M.R., Ray, H.C., Mendel, T.A., Hoehn, K.L., Bruce, A.C., Dey, B.K., Guendel, A.M., Tavakol, D.N., Herman, I.M., Peirce, S.M., Yates, P.A., 2015. Adipose-Derived Stem Cells From Diabetic Mice Show Impaired Vascular Stabilization in a Murine Model of Diabetic Retinopathy. Stem Cell Transl Med 4, 459-467.

Dominici, M., Le Blanc, K., Mueller, I., Slaper-Cortenbach, I., Marini, F., Krause, D., Deans, R., Keating, A., Prockop, D., Horwitz, E., 2006. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 8, 315-317.

Eaves, C.J., 2015. Hematopoietic stem cells: concepts, definitions, and the new reality. Blood 125, 2605-2613.

Galderisi, U., Giordano, A., 2014. The Gap Between the Physiological and Therapeutic Roles of Mesenchymal Stem Cells. Med Res Rev 34, 1100-1126.

Galieva, L.R., James, V., Mukhamedshina, Y.O., Rizvanov, A.A., 2019. Therapeutic Potential of Extracellular Vesicles for the Treatment of Nerve Disorders. Front Neurosci 13, 163.

Galipeau, J., 2013. The mesenchymal stromal cells dilemma-does a negative phase III trial of random donor mesenchymal stromal cells in steroid-resistant graft-versus-host disease represent a death knell or a bump in the road? Cytotherapy 15, 2-8.

Gouveia, R.M., Lepert, G., Gupta, S., Mohan, R.R., Paterson, C., Connon, C.J., 2019. Assessment of corneal substrate biomechanics and its effect on epithelial stem cell maintenance and differentiation. Nat Commun 10, 1496.

Gu, X., Yu, X., Zhao, C., Duan, P., Zhao, T., Liu, Y., Li, S., Yang, Z., Li, Y., Qian, C., Yin, Z., Wang, Y., 2018. Efficacy and Safety of Autologous Bone Marrow Mesenchymal Stem Cell Transplantation in Patients with Diabetic Retinopathy. Cell Physiol Biochem 49, 40-52.

Harkin, D.G., Foyn, L., Bray, L.J., Sutherland, A.J., Li, F.J., Cronin, B.G., 2015. Concise reviews: can mesenchymal stromal cells differentiate into corneal cells? A systematic review of published data. Stem Cells 33, 785-791.

Holan, V., Trosan, P., Cejka, C., Javorkova, E., Zajicova, A., Hermankova, B., Chudickova, M., Cejkova, J., 2015. A Comparative Study of the Therapeutic Potential of Mesenchymal Stem Cells and Limbal Epithelial Stem Cells for Ocular Surface Reconstruction. Stem Cells Transl Med 4, 1052-1063.

Inoue, Y., Iriyama, A., Ueno, S., Takahashi, H., Kondo, M., Tamaki, Y., Araie, M., Yanagi, Y., 2007. Subretinal transplantation of bone marrow mesenchymal stem cells delays retinal degeneration in the RCS rat model of retinal degeneration. Exp Eye Res 85, 234-241.

Joe, A.W., Gregory-Evans, K., 2010. Mesenchymal stem cells and potential applications in treating ocular disease. Curr Eye Res 35, 941-952.

Kataja, M., Hujanen, P., Huhtala, H., Kaarniranta, K., Tuulonen, A., Uusitalo-Jarvinen, H., 2018. Outcome of anti-vascular endothelial growth factor therapy for neovascular age-related macular degeneration in real-life setting. Brit J Ophthalmol 102, 959-965.
Ke, Y., Wu, Y., Cui, X., Liu, X., Yu, M., Yang, C., Li, X., 2015. Polysaccharide hydrogel combined with mesenchymal stem cells promotes the healing of corneal alkali burn in rats. PLoS One 10, e0119725.
Klimczak, A., Kozlowska, U., 2016. Mesenchymal Stromal Cells and Tissue-Specific Progenitor Cells: Their Role in Tissue Homeostasis. Stem Cells Int 2016, 4285215.
Kovac, M., Litvin, Y.A., Aliev, R.O., Zakirova, E.Y., Rutland, C.S., Kiyasov, A.P., Rizvanov, A.A., 2017. Gene Therapy Using Plasmid DNA Encoding Vascular Endothelial Growth Factor 164 and Fibroblast Growth Factor 2 Genes for the Treatment of Horse Tendinitis and Desmitis: Case Reports. Front Vet Sci 4.
Kovac, M., Litvin, Y.A., Aliev, R.O., Zakirova, E.Y., Rutland, C.S., Kiyasov, A.P., Rizvanov, A.A., 2018. Gene Therapy Using Plasmid DNA Encoding VEGF164 and FGF2 Genes: A Novel Treatment of Naturally Occurring Tendinitis and Desmitis in Horses. Front Pharmacol 9.
Kroepfl, J.M., Pekovits, K., Stelzer, I., Fuchs, R., Zelzer, S., Hofmann, P., Sedlmayr, P., Dohr, G., Wallner-Liebmann, S., Domej, W., Mueller, W., 2012. Exercise Increases the Frequency of Circulating Hematopoietic Progenitor Cells, But Reduces Hematopoietic Colony-Forming Capacity. Stem Cells and Development 21, 2915-2925.
Ksander, B.R., Kolovou, P.E., Wilson, B.J., Saab, K.R., Guo, Q., Ma, J., McGuire, S.P., Gregory, M.S., Vincent, W.J., Perez, V.L., Cruz-Guilloty, F., Kao, W.W., Call, M.K., Tucker, B.A., Zhan, Q., Murphy, G.F., Lathrop, K.L., Alt, C., Mortensen, L.J., Lin, C.P., Zieske, J.D., Frank, M.H., Frank, N.Y., 2014. ABCB5 is a limbal stem cell gene required for corneal development and repair. Nature 511, 353-357.
Lan, Y., Kodati, S., Lee, H.S., Omoto, M., Jin, Y., Chauhan, S.K., 2012. Kinetics and function of mesenchymal stem cells in corneal injury. Invest Ophthalmol Vis Sci 53, 3638-3644.
Le, T., Chong, J., 2016. Cardiac progenitor cells for heart repair. Cell Death Discov 2, 16052.
Lee, M.J., Ko, A.Y., Ko, J.H., Lee, H.J., Kim, M.K., Wee, W.R., Khwarg, S.I., Oh, J.Y., 2015. Mesenchymal stem/stromal cells protect the ocular surface by suppressing inflammation in an experimental dry eye. Mol Ther 23, 139-146.
Lin, H., Liu, Y., He, H., Botsford, B., Yiu, S., 2017. Lacrimal Gland Repair after Short-term Obstruction of Excretory Duct in Rabbits. Sci Rep 7, 8290.
Lin, N., Hu, K., Chen, S., Xie, S., Tang, Z., Lin, J., Xu, R., 2009. Nerve growth factor-mediated paracrine regulation of hepatic stellate cells by multipotent mesenchymal stromal cells. Life Sci 85, 291-295.
Lin, P.Y., Tsai, S.Y., Cheng, C.Y., Liu, J.H., Chou, P., Hsu, W.M., 2003. Prevalence of dry eye among an elderly chinese population in Taiwan - The Shihpai eye study. Ophthalmology 110, 1096-1101.
Manuguerra-Gagne, R., Boulos, P.R., Ammar, A., Leblond, F.A., Krosl, G., Pichette, V., Lesk, M.R., Roy, D.C., 2013. Transplantation of mesenchymal stem cells promotes tissue regeneration in a glaucoma model through laser-induced paracrine factor secretion and progenitor cell recruitment. Stem Cells 31, 1136-1148.
Marycz, K., Mierzejewska, K., Smieszek, A., Suszynska, E., Malicka, I., Kucia, M., Ratajczak, M.Z., 2016. Endurance Exercise Mobilizes Developmentally Early Stem Cells into Peripheral Blood and Increases Their Number in Bone Marrow: Implications for Tissue Regeneration. Stem Cells International.
Marycz, K., Toker, N.Y., Grzesiak, J., Wrzeszcz, K., Golonka, P., 2012. The Therapeutic Effect of Autogenic Adipose Derived Stem Cells Combined with Autogenic Platelet Rich Plasma in Tendons Disorders in Horses in vitro and in vivo Research. J Anim Vet Adv 11, 4324-4331.
Masgutov, R., Masgutova, G., Mullakhmetova, A., Zhuravleva, M., Shulman, A., Rogozhin, A., Syromiatnikova, V., Andreeva, D., Zeinalova, A., Idrisova, K., Allegrucci, C., Kiyasov, A., Rizvanov, A., 2019. Adipose-Derived Mesenchymal Stem Cells Applied in Fibrin Glue Stimulate Peripheral Nerve Regeneration. Front Med (Lausanne) 6, 68.
Mead, B., Berry, M., Logan, A., Scott, R.A., Leadbeater, W., Scheven, B.A., 2015. Stem cell treatment of degenerative eye disease. Stem Cell Res 14, 243-257.
Mendel, T.A., Clabough, E.B., Kao, D.S., Demidova-Rice, T.N., Durham, J.T., Zotter, B.C., Seaman, S.A., Cronk, S.M., Rakoczy, E.P., Katz, A.J., Herman, I.M., Peirce, S.M., Yates, P.A., 2013. Pericytes derived from adipose-derived stem cells protect against retinal vasculopathy. PLoS One 8, e65691.
Moss, S.E., Klein, R., Klein, B.E.K., 2000. Prevalence of and risk factors for dry eye syndrome. Arch Ophthalmol-Chic 118, 1264-1268.

Nicpon, J., Marycz, K., Grzesiak, J., 2013. Therapeutic effect of adipose-derived mesenchymal stem cell injection in horses suffering from bone spavin. Pol J Vet Sci 16, 753-754.
Oner, A., 2018. Stem Cell Treatment in Retinal Diseases: Recent Developments. Turk J Ophthalmol 48, 33-38.
Oner, A., Gonen, Z.B., Sevim, D.G., Kahraman, N.S., Unlu, M., 2018. Suprachoroidal Adipose Tissue-Derived Mesenchymal Stem Cell Implantation in Patients with Dry-Type Age-Related Macular Degeneration and Stargardt's Macular Dystrophy: 6-Month Follow-Up Results of a Phase 2 Study. Cell Reprogram 20, 329-336.
Paczkowska, E., Kucia, M., Koziarska, D., Halasa, M., Safranow, K., Masiuk, M., Karbicka, A., Nowik, M., Nowacki, P., Ratajczak, M.Z., Machalinski, B., 2009. Clinical Evidence That Very Small Embryonic-Like Stem Cells Are Mobilized Into Peripheral Blood in Patients After Stroke. Stroke 40, 1237-1244.
Phinney, D.G., Prockop, D.J., 2007. Concise review: mesenchymal stem/multipotent stromal cells: the state of transdifferentiation and modes of tissue repair--current views. Stem Cells 25, 2896-2902.
Sharma, N., Kaur, M., Agarwal, T., Sangwan, V.S., Vajpayee, R.B., 2018. Treatment of acute ocular chemical burns. Surv Ophthalmol 63, 214-235.
Shukla, S., Mittal, S.K., Foulsham, W., Elbasiony, E., Singhania, D., Sahu, S.K., Chauhan, S.K., 2019. Therapeutic efficacy of different routes of mesenchymal stem cell administration in corneal injury. Ocul Surf.
Song, W.K., Park, K.M., Kim, H.J., Lee, J.H., Choi, J., Chong, S.Y., Shim, S.H., Del Priore, L.V., Lanza, R., 2015. Treatment of macular degeneration using embryonic stem cell-derived retinal pigment epithelium: preliminary results in Asian patients. Stem Cell Reports 4, 860-872.
Sun, J., Liu, W.H., Deng, F.M., Luo, Y.H., Wen, K., Zhang, H., Liu, H.R., Wu, J., Su, B.Y., Liu, Y.L., 2018. Differentiation of rat adipose-derived mesenchymal stem cells into corneal-like epithelial cells driven by PAX6. Exp Ther Med 15, 1424-1432.
Villatoro, A.J., Claros, S., Fernandez, V., Alcoholado, C., Farinas, F., Moreno, A., Becerra, J., Andrades, J.A., 2018. Safety and efficacy of the mesenchymal stem cell in feline eosinophilic keratitis treatment. BMC Vet Res 14, 116.
Villatoro, A.J., Fernandez, V., Claros, S., Alcoholado, C., Cifuentes, M., Merayo-Lloves, J., Andrades, J.A., Becerra, J., 2017. Regenerative Therapies in Dry Eye Disease: From Growth Factors to Cell Therapy. Int J Mol Sci 18.
Villatoro, A.J., Fernandez, V., Claros, S., Rico-Llanos, G.A., Becerra, J., Andrades, J.A., 2015. Use of adipose-derived mesenchymal stem cells in keratoconjunctivitis sicca in a canine model. Biomed Res Int 2015, 527926.
Wang, S.M., Lu, B., Girman, S., Duan, J., McFarland, T., Zhang, Q.S., Grompe, M., Adamus, G., Appukuttan, B., Lund, R., 2010. Non-Invasive Stem Cell Therapy in a Rat Model for Retinal Degeneration and Vascular Pathology. Plos One 5.
Wojakowski, W., Tendera, M., Kucia, M., Zuba-Surma, E., Paczkowska, E., Ciosek, J., Halasa, M., Krol, M., Kazmierski, M., Buszman, P., Ochala, A., Ratajczak, J., Machalinski, B., Ratajczak, M.Z., 2009. Mobilization of Bone Marrow-Derived Oct-4(+) SSEA-4(+) Very Small Embryonic-Like Stem Cells in Patients With Acute Myocardial Infarction. J Am Coll Cardiol 53, 1-9.
Xu, W., Wang, X.T., Xu, G.X., Guo, J., 2013. Basic Fibroblast Growth Factor Expression is Implicated in Mesenchymal Stem Cells Response to Light-Induced Retinal Injury. Cellular and Molecular Neurobiology 33, 1171-1179.
Yoon, J.J., Ismail, S., Sherwin, T., 2014. Limbal stem cells: Central concepts of corneal epithelial homeostasis. World J Stem Cells 6, 391-403.
Zakirova, E.Y., Shalimov, D.V., Garanina, E.E., Zhuravleva, M.N., Rutland, C.S., Rizvanov, A.A., 2019. Use of Biologically Active 3D Matrix for Extensive Skin Defect Treatment in Veterinary Practice: Case Report. Front Vet Sci 6, 76.
Zakirova, E.Y., Valeeva, A.N., Faizullina, R.R., Akhmetshin, R.F., Nefedovskaya, L.V., Rizvanov, A.A., 2015. Transplantation of allogeneic mesenchymal stromal cell for treating corneal ulcers in cats. Gene&Cells 10, 49-55.
Zarbin, M., 2016. Cell-Based Therapy for Degenerative Retinal Disease. Trends Mol Med 22, 115-134.
Zhang, L., Coulson-Thomas, V.J., Ferreira, T.G., Kao, W.W., 2015. Mesenchymal stem cells for treating ocular surface diseases. BMC Ophthalmol 15 Suppl 1, 155.