Review Article

Stem Cell & Regenerative Medicine

Mesenchymal Stem Cells for Treating Ocular Surface Diseases

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Abbreviation

alpha-SMA: Alpha smooth muscle actin; AM: Amniotic membrane; BM: Basement membrane; BV: Blood vessel; CD: Cluster of differentiation; CECs: Corneal epithelial cells; DNP-63: Dinitrophenyl-63; eTAC: Early Transient Amplifier Cells; EMT: Epithelial to Mesenchyme Transition; GAGs: Glycosaminoglycans; HDF: Hepatocyte Growth Factor; HLA: Human Leukocytic Antigen; iPSC: Induced Pluripotent Stem Cells; KGF: Keratinocyte Growth Factor; LSCs: Limbal Stem Cells; MSC: Mesenchymal Stem Cells; MET: Mesenchyme to Epithelial Transition; MAPK-38: Mitogen Activated Protein Kinase 38; MPS VII: Mucopolysaccharidosis type VII; N: Nerve; NGF: Nerve Growth Factor; PMN: Polymorph Nuclear Leukocytes; PMC: Post Mitotic Cells; SC: Stem Cells; TDC: Terminally Differentiated Cells; RK: Radial Keratotomy.

Introduction

Significance: Ocular surface diseases represented one of the most common causes of impaired vision or even blindness. Mesenchymal stem cells (MSC) have gained a high interest in cell therapy and regenerative medicine. They are not only readily available, have powerful differentiation capabilities but also present immunosuppressive properties. They are widely used in allograft owing to their ability to escape from host immune rejection.

Recent Advances: Clinical trials are underway using MSC for the treatment of a range of diseases not treatable by conventional therapies. Numerous studies have been directed towards the use of MSC for treating corneal defects with very promising outcomes.

Critical Issues: Currently, the diversity in protocols of the isolation and expansion of MSC are hindering the assessment of cell treatment ability and the further development of treatment regimens.

Future Directions: Therefore, future studies should develop international standards for MSC isolation and characterization. In this review, we discuss recent advances in MSC for treating ocular surface diseases.

MSC identification and characterization

Isolated MSC are able to adhere to the plastic surface of culture dish and proliferate in vitro. They are fibroblast-like cells and express certain cell surface markers. Though no single cell marker or a set of markers is simply determined to define MSC, several characterization tests can be combined to identify MSC [1].

MSC immunophenotype character

Immunophenotype analysis is one of the essential tests for MSC. The minimal cell surface markers that should be examined include positive ones as: CD105 (endoglin), CD90 (Thy-1)) and CD73 (5'-nucleotidase; and negative markers as: CD34, CD45, CD14, CD11b (integrin αM chain), CD79α and HLA-DR surface molecules [2]. Many other markers have also been recognized to be indicative for the characterization of MSC, as CD13, CD44, CD106, CD29, CD166 expression, and the lack of CD38, CD31expression [3]. Fluorescence-activated cell sorting can be performed routinely in order to evaluate the purity of cell population [4].

MSC as a New Therapeutic Approach

MSC can play major roles in healing of tissue damage not only by directly differentiating into many of the resident cell types but also by secreting several growth factors as (VEGF, NGF, HGF) aiding in tissue repair and regeneration [5,6].

Besides of their reparative function, MSC have shown marked immunosuppressive and anti-inflammatory potentialities (Figure 1) [7]. This major function of MSC opens up new avenues for development of MSC-based immune-therapeutics to treat life threatening autoimmune diseases or severe chronic inflammatory conditions. MSCs can directly differentiate to mesenchymal cells [7], or to cells other than mesenchyme such as epithelial cell lineage or neural cell lineage, as shown formerly in bleomycin-induced lung injuries [8,9], or in skin injuries [10].

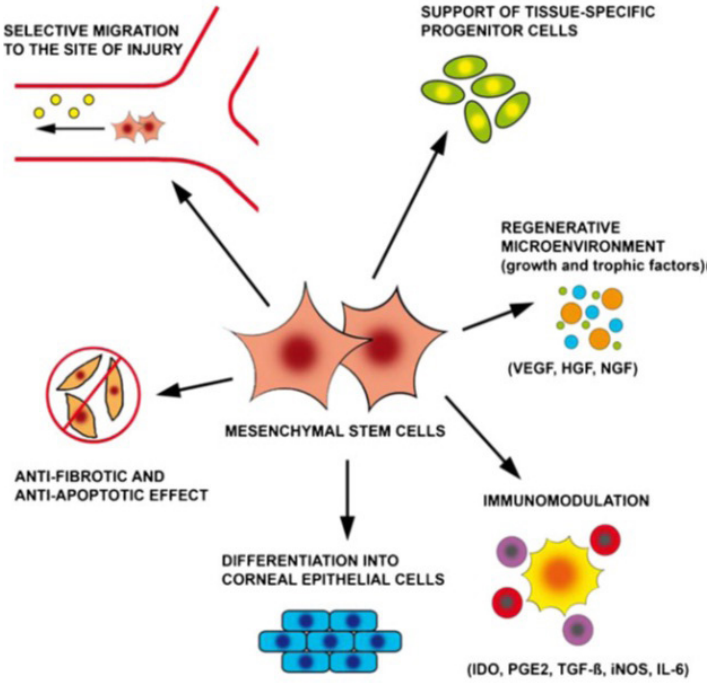

Figure 1: A schematic diagram for the therapeutic effect of MSCs. MSCs can contribute to the healing process through different mechanisms, as differentiation into epithelial-like cells, immune suppression potentialities and its ability to produce different trophic factors.

Mesenchyme to epithelial transition (MET) Process of MSC

The molecular mechanisms of mesenchyme to epithelial transition (MET) of MSC have not been yet elucidated. Currently, the relationship between EMT/MET and MSC are being highly investigated. Meanwhile, MET process commonly occurs in mucosal healing and in cancer metastasis [11]. For instances during organ morphogenesis, the epithelium-associated genes are up regulated and the mesenchymal ones are down regulated.

Former workers reported trans differentiation of MSC into corneal epithelial cells with typical MET phenomenon [12]. They suggested that, at day 5 of a corneal alkali burn, wound microenvironment could generate factors that have the ability to transform circulating MSCs into epithelial cells. Several phenotypic changes were observed such as, the reduction of actin stress fibers at the bottom of cells and the reduction of α-SMA (alpha -smooth muscle actin) a marker for mesenchymal origin. In addition to acquisition of epithelial characteristics, such as increased expression of E-cadherin in the cell-cell contact sites and the rearrangement of actin filaments (Figures 2 and 3).

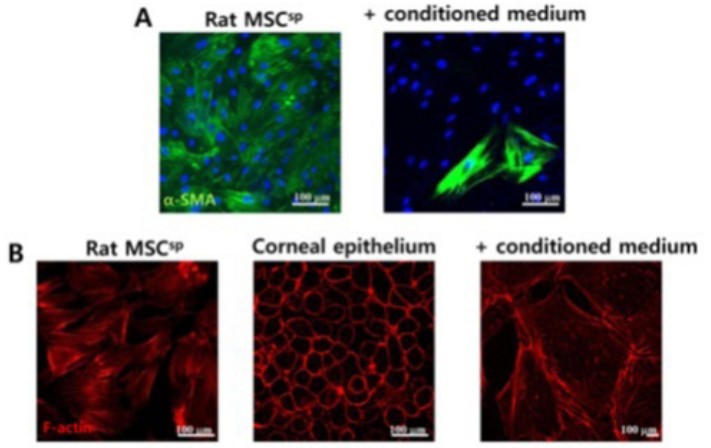

Figure 2: Administration of reconstituted wound microenvironment in vitro resulted in rearrangement of actin filaments in transformed MSC and reduction of α-SMA.
(A) Showing the spindle shaped MSC and epithelioid transformed MSC using immunostaining for α-SMA. Following administration of reconstituted wound microenvironment in vitro for 19 days, significant reduction of immuno-reactivity of α-SMA was observed (scale bar: 100 μm).
(B) Comparing the spindle-shaped MSC and epithelioid transformed MSC by immunostaining with actin-phalloidin for actin filaments.

The distinct patterns of actin filaments (F-actin) in MSC and corneal epithelial cells were shown as stress fibers at the bottom of all cells (left) and cortical actin ring at the cell-cell contact sites (middle), respectively. At 19 days after administration of conditioned medium, actin rearrangement from stress fibers to cortical actin ring was accompanied with morphological changes in transformed MSC (scale: 100 μm).

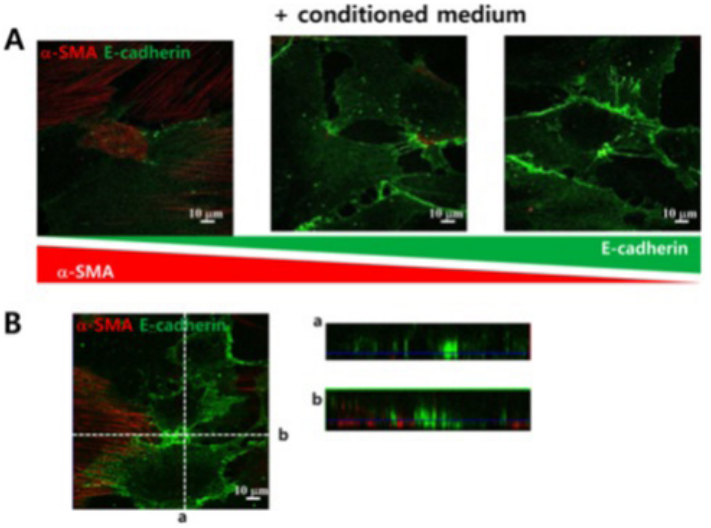

Figure 3: Induction of E-cadherin was accompanied in transformed MSC by administration of reconstituted wound microenvironment in vitro.
(A) The immunoreactivity of α-SMA, a mesenchymal marker, and E cadherin, an epithelial marker, displayed mutually exclusive expression patterns. Increased expression of Ecadherin in transformed MSC depicted typical EMT process (scale bar: 10 μm).
(B) Immuno-reactivity of E-cadherin at cell to cell contact site showed by z-scanning from confocal analysis (scale bar: 10 μm).

MSC and stem cell niche

Stem cells (SC) functions are determined by the extracellular tissue microenvironment known as the ‘‘niche’’ and by the intrinsic genetic programs in the SC; both of these have a major impact on the delicate balance of self-renewal and differentiation [13]. Studies have shown that, SC niche provided an extracellular matrix in which SC are maintained in an undifferentiated state. These niche characteristics are extremely essential during embryonic development, organogenesis and tissue regeneration [14,15].

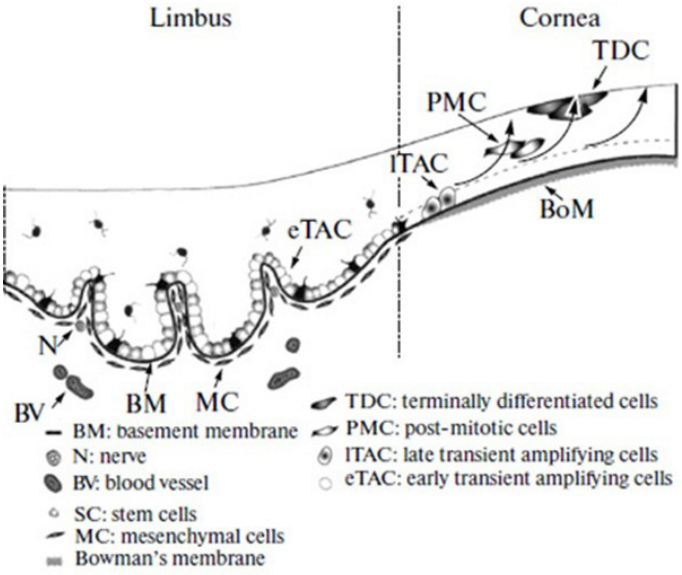

Figure 4: Scheme of niche of limbal stem cells of the corneal epithelium (modification of Li et al. 2007).

Structure difference

The SC-containing limbal epithelium is located at the Palisades of Vogt, which are surrounded by a vascular network and are highly pigmented by the presence of melanocytes [16-18]. limbal epithelium is highly infiltrated by antigen-presenting Langerhans’s cells [19], and suppressor T-lymphocytes [20]. The BM of the limbal epithelium differs from that of the cornea, for instance, it was found that, the percentage of limbal basal cell membranes with hemidesmosomes to be significantly less than those of the cornea [21]. The limbal BM is undulated with 'pegs’’ or papillae of stroma extending upward [21], and fenestrated by so called focal stromal projections and limbal crypts [22-24]. However, the cornea, lacks such papillae.

Former studies showed that, corneal ectasia induced by LASIK, keratoconus and radial keratotomy (RK wounds), may modulate the differentiation of corneal epithelial cells that resulted from disrupted BM, leading to dedifferentiation of corneal epithelial cells into transient amplifying cells (TACs) [21], (Figure 4). They propose that, such phenotypic changes in corneal epithelial cells, may result in expression of DNp63 markers and connexin43 (Cx43); which were also expressed by the basal layer of the corneal epithelium, on disruption of the BM, and by the Bowman’s membrane of the damaged cornea potentially through the p38-MAPK (mitogen activated protein kinase) pathway [21]. This might be due to alteration of their niche, including their extracellular matrix and BM. These results, and the anatomical features of the limbus, suggested that limbal stem cells might closely interact with cells in the underlying limbal stroma [21,23,24].

In addition to laminin-5 and laminin-1, the limbal BM also contains laminin a2b2 chains, while the corneal BM does not [25]. Moreover, a1, a2, and a5 chains of type IV collagen are present in the limbal BM, whereas a3 and a5 chains are present in its corneal counterpart [25-27]. All of these components hence contribute to the distribution of SC in this niche [30].

It was also suggested that, BM sequesters and modulates growth factors and cytokines that are released from limbal cells into the niche for efficient and precise targeting of limbal SC [29-33]. In other words, regulation of cytokines in cornea and limbus was found to be different [31]. These observations suggest that the corneal epithelial BM may affect the overlying epithelial phenotype. Ultimately, limbal SC contribute to the regeneration and repair of transparent corneas [32,33]. In an animal study [32], the rabbit cornea was destroyed by n-heptanol corneal epithelial debridement and surgical removal of the limbal zone. During Postoperative follow-up showed, progressive conjunctivalization, vascularization and signs of limbal SC deficiency, was observed for all corneas in a 6-month recovery [32]. Transplantation of SC-containing limbal tissue to treat limbal SCs deficiency showed that replaced limbal SC can provide new healthy corneal epithelial cells and restore the lost niche of the limbal stromal layer, leading to regression of the vessels and rearrangement of the corneal stromal lamellae that resulted from limbal SC deficiency (Figure 5).

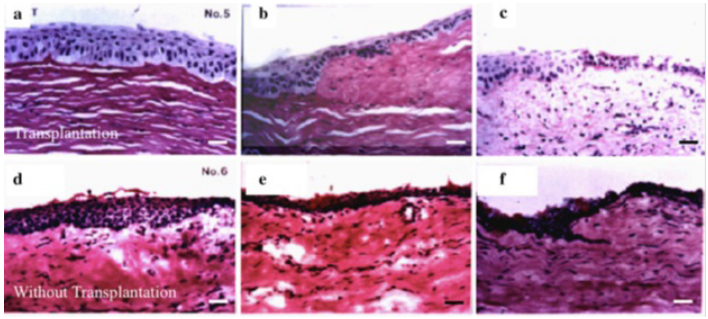

Figure 5: Autograft limbal tissue transplantation for limbal stem cells deficiency in a rabbit model. LSCs not only provide new healthy corneal epithelium but also reform the stromal layer by regression of the vessels and rearrangement of the stromal lamellae. a, b, and, c are images showing the pathology of corneal surfaces 6 months after LSCs transplantation. d, e, and, f are images from control groups without limbal SCs transplantation. a and d are areas of the central cornea, b and e are areas of the peripheral cornea, c and f are limbal areas.(H&E staining; Bar 25 lm).

Strategies for treatment of limbal SC deficiency by SC expansion technique represent a new approach intended for development of tissue-based therapeutics. The aim of such technique is to replace lost tissue function occurring as a result of aging, surgery, injury, inflammatory diseases, or hereditary causes.

Studies had been performed by Tsai et al. (2014) for cases with central corneal stromal opacity treated by limbal SC transplantation

followed by penetrating keratoplasty [34]. In one successful case of limbal SC reconstruction of the whole cornea, several layers of epithelial cells grew over an amniotic membrane (AM) on the central cornea, even 10 months after limbal SC transplantation (Figure 6a), similar to the rabbit model of limbal tissue reconstruction for limbal SCs deficiency (Figure 5a). In another case, 6 months after a failed allograft of LSCs reconstruction, the pathological specimen of the central cornea did not reveal normal epithelial and stromal phenotype; there was irregular lamellar structure with PMN leukocyte infiltration and fibrovascular ingrowth (Figure 6b), similar to the rabbit model of limbal SCs deficiency (Figure 5d). For this specimen the AM could not be identified. Immunohistological study of this successful specimen of central cornea with previous reconstruction by limbal SC with AM revealed DNp63- positive cells throughout the epithelial layers of the central cornea (Figure 6d). Identification of the AM by Alcian blue staining (Figure 6c) indicated that the AM can provide the niche environment for cultured LSCs and maintain the limbal-like environment for the transplanted area of the cornea. By ex-vivo expansion, these SC-like cells can be successfully grown on the human cornea and thereby help to maintain its clarity and the homeostasis between corneal epithelium proliferation and differentiation for at least 16 years [34,35]. This technique supports the importance of the intact AM as a unique niche for maintenance and expansion of LSCs in vitro.

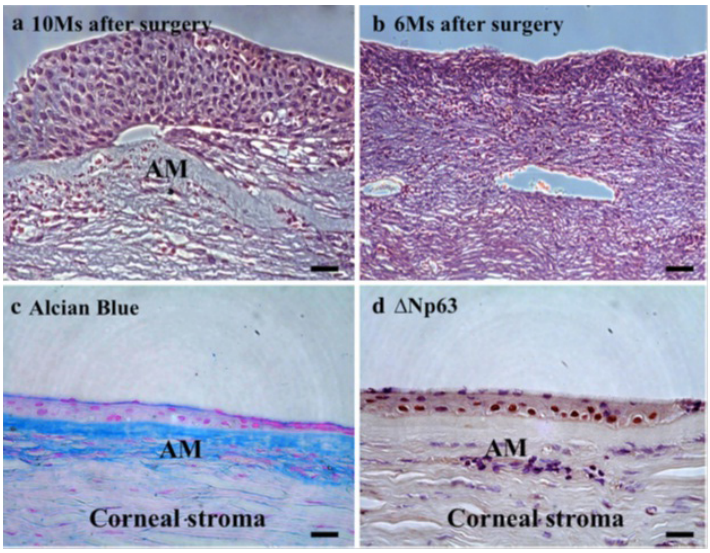

Figure 6: Pathology studies performed for cases with preexisting central corneal stromal opacity treated by LSCs transplantation followed by penetrating keratoplasty.

A. 10 months after LSC transplantation, epithelial cells regularly arranged into multiple layers and the AM could still be identified.

B. An unsuccessful case 6 months after LSC transplantation. Irregular lamellar structure with PMN leukocyte infiltration and fibrovascular ingrowth were observed. The epithelium and stroma had irregular phenotypes, and the AM could not be identified from this specimen.

C. A successful specimen of central cornea with previous reconstruction by LSCs with AM. The AM could be identified by positive Alcian blue staining.

D. Positive DNp63 cells are identified throughout the epithelial layer of the central cornea, indicating the AM can provide the niche environment for cultured LSCs and maintain the limbal-like environment for the transplanted area of the cornea. H&E staining; Bar 25 lm.

Role of Micro-environment, Stem Cell Niche and Paracrine Factors

It is obvious that, the stem cell microenvironment niche has a definitive role in maintaining stem cell properties (such as pluripotency, cell division and differentiation capacities) [36]. Paracrine factors secretion is one of the mechanisms by which the niche interacts with the stem cells [37], along with cell-cell and extra-cellular matrix interactions.

The supply of micro-environment elements can increase corneal stem cell survival and cell repair [38]. Saichanma and colleagues differentiated murine induced Pluripotent SC (iPSC) into corneal epithelium-like cells by co-culturing them with corneal limbal stroma, thus replicating the corneal epithelial stem cell niche [39]. The coculture was performed using Transwell plates, which means there was no physical contact between the iPSC and the corneal limbal stroma. The successful differentiation into corneal epithelium-like cells showed that factors secreted by the stroma seemed to be responsible for iPSC differentiation, revealing the importance of paracrine interactions.

Surprisingly, limbal microenvironment could influence stem cell fate as hair follicle stem cells could transdifferentiate directly into Corneal Epithelial cells (CECs) when cultured in a limbus-specific-like niche while remaining epidermal when grow in a hair follicle culture condition [27].

Furthermore, several studies revealed that the microenvironment was greatly involved in the success of stem cell transplant and the long-term survival of the graft in ocular surface therapy [40-43].

The paracrine factors secretion represents a major mechanism by which stem cells enhance wound repair and maintain homeostasis. Intercellular communication is partly mediated by secreted microvesicles (SMV), including exosomes, an emerging novel avenue with great potential [44]. Exosomes are defined as SMV ranging from 30 to 150 nm in diameter. They are released from multivesicular endosomes after fusion with the plasma membrane and are of endosomal origin [45]. Exosomes are enriched for cytoskeleton molecules, membrane trafficking, chaperones, signal transduction proteins and cytoplasmic enzymes. Remarkably, they can also deliver functional RNAs, mRNAs and long non-coding RNAs to recipient cells [46]. Exosomes have multiple biological functions, including modulation of vascular homeostasis, angiogenesis and immune activation [47].

Exosomes likely have a great potential in cell repair, and several studies have demonstrated their role in cell protection and wound healing, for example on cardiac, skin or skeletal muscle cells [48].

As exosomes derived from MSC [49], and from ESC have shown regenerative capacities and cell plasticity [50], and neural cell derived secreted microvesicles contain Pax-6 mRNA, there is

a strong possibility that exosomes, derived either from limbal stem cells or corneal cells could enhance corneal repair and even transdifferentiation. The use of exosomes could overcome the limitations and risks associated with stem cells, due to the limited size of donor tissues [42]. Moreover, stem cell transplantation requires the development of a safe and reproducible technique to ensure the successful engraftment of the tissue, whereas exosomes could be applied topically for corneal repair. Thus, the administration of exosomes would be safer than stem cell transplantation, with no need for surgery. Some exosomes have been shown to express molecules of the major histocompatibility complex (MHC) at their surface [50], so the question of immune reaction has to be considered in the same way that it is for stem cell transplantation. It is worth noting that the eye is an immuno-privileged site where the risk of immune reaction is relatively reduced (immune tolerance) [50]. Hence, exosomes are a very interesting alternative to stem cell therapies, the study of their effect on ocular surface regeneration could pave the way to their use as a new therapy for corneal pathologies such as LSCD.

Conclusion

Human clinical studies for treating LSCD currently apply limbal, conjunctival, and oral mucosal as no single cell type has proven clearly superior, and there is still a need for standardized procedures. Over the past few years, new techniques and cell sources have been developed, some of them having a very promising future. It has been shown that MSC could differentiate into corneal cells in vitro, which is very encouraging for possible clinical applications. However, several technical and ethical issues need to be resolved, and further pre-clinical studies are required before considering applications in human.

The microenvironment, including paracrine factors, has a central role in the survival and renewal of LSC. Exosomes are secreted vesicles with major potential; they are known to have the ability to carry proteins, enzymes and RNAs, thus having a crucial role in intercellular communications. Today, exosomes are considered as a novel pathway with a great potentiality in therapeutics, especially in regenerative medicine. Their use in ocular surface diseases could circumvent the limitations and risks of stem cell transplantation, and they would be easier to handle on a pharmaceutical aspect. A better understanding of the LSC niche and microenvironment, as well as the role of exosomes in corneal regeneration, could open the way to a brand new approach for the management of ocular surface diseases.

References

1. Zhang L, Coulson-Thomas VJ, Ferreira TG, et al. Mesenchymal stem cells for treating ocular surface diseases. BMC Ophthalmol. 2015; 15 Suppl 1: 155.
2. Dominici M, Le Blanc K, Mueller I, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy. 2006; 8: 315-317.
3. Lu LL, Liu YJ, Yang SG, et al. Isolation and characterization of human umbilical cord mesenchymal stem cells with hematopoiesis-supportive function and other potentials. Haematologica. 2006; 91: 1017-1026.
4. Zhang H, Zhang B, Tao Y, et al. Isolation and characterization of mesenchymal stem cells from whole human umbilical cord applying a single enzyme approach. Cell Biochem Funct. 2012; 30: 643-649.
5. Hong HS, Kim YH, Son Y. Perspectives on mesenchymal stem cells: tissue repair, immune modulation, and tumor homing. Arch Pharm Res. 2012; 35: 201-211.
6. Hong HS, Kim DY, Yoon KJ, et al. A new paradigm for stem cell therapy: substance-P as a stem cell-stimulating agent. Arch Pharm Res. 2011; 34: 2003-2006.
7. Holan V, Javorkova E. Mesenchymal stem cells, nanofiber scaffolds and ocular surface reconstruction. Stem Cell Rev. 2013; 9: 609-619.
8. YS Yoon, A Wecker, L Heyd, et al. Clonally expanded novel multipotent stem cells from human bone marrow regenerate myocardium after myocardial infarction. J Clin Invest. 2005; 115: 326.
9. Ortiz LA, Gambelli F, McBride C, et al. Mesenchymal stem cell engraftment in lung is enhanced in response to bleomycin exposure and ameliorates its fibrotic effects. Proc Natl Acad Sci U S A. 2003; 100: 8407-8411.
10. Rojas M, Xu J, Woods CR, et al. Bone marrow-derived mesenchymal stem cells in repair of the injured lung. Am J Respir Cell Mol Biol. 2005; 33: 145-152.
11. F Sipos, G Muzes. Isolated lymphoid follicles in colon: switch points between inflammation and colorectal cancer?, World J, Gastroenterol. 2011; 17: 1666.
12. Ahn W, Sook Hong , Zhang M, et al. Induction of Mesenchymal to Epithelial Transition of Circulating Mesenchymal Stem Cells by Conditioned Medium of Injured cornea. Tissue Engineering and Regenerative Medicine. 2013; 10: 86-92.
13. Li L, Xie T. Stem cell niche: structure and function. Annu Rev Cell Dev Biol. 2005; 21: 605-631.
14. Ohlstein B, Kai T, Decotto E, et al. The stem cell niche: theme and variations. Curr Opin Cell Biol. 2004; 16: 693-699.
15. Morrison SJ, Spradling AC. Stem cells and niches: mechanisms that promote stem cell maintenance throughout life. Cell. 2008; 132: 598-611.
16. Davanger M, Evensen A. Role of the pericorneal papillary structure in renewal of corneal epithelium. Nature. 1971; 229: 560-561.
17. Higa K, Shimmura S, Miyashita H, et al. Melanocytes in the corneal limbus interact with K19-positive basal epithelial cells. Exp Eye Res. 2005; 81: 218-223.
18. Goldberg MF, Bron AJ. Limbal palisades of Vogt. Trans Am Ophthalmol Soc. 1982; 80: 155-171.
19. Baum JL. Melanocyte and Langerhans cell population of the cornea and limbus in the albino animal. Am J Ophthalmol. 1970; 69: 669-676.
20. Vantrappen L, Geboes K, Missotten L, et al. Lymphocytes and Langerhans cells in the normal human cornea. Invest Ophthalmol Vis Sci. 1985; 26: 220-225.
21. Gipson IK. The epithelial basement membrane zone of the limbus. Eye (Lond). 1989; 3: 132-140.

22. Dua HS, Shanmuganathan VA, Powell-Richards AO, et al. Limbal epithelial crypts: a novel anatomical structure and a putative limbal stem cell niche. Br J Ophthalmol. 2005; 89: 529-532.
23. Wang IJ, Tsai RJ, Yeh LK, et al. Changes in corneal basal epithelial phenotypes in an altered basement membrane. PLoS One. 2011; 6: e14537.
24. Vantrappen L, Geboes K, Missotten L, et al. Lymphocytes and Langerhans cells in the normal human cornea. Invest Ophthalmol Vis Sci. 1985; 26: 220-225.
25. Tuori A, Uusitalo H, Burgeson RE, et al. The immunohistochemical composition of the human corneal basement membrane. Cornea. 1996; 15: 286-294.
26. Ljubimov AV, Burgeson RE, Butkowski RJ, et al. Human corneal basement membrane heterogeneity: topographical differences in the expression of type IV collagen and laminin isoforms. Lab Invest. 1995; 72: 461-473.
27. Schlötzer-Schrehardt U, Dietrich T, Saito K, et al. Characterization of extracellular matrix components in the limbal epithelial stem cell compartment. Exp Eye Res. 2007; 85: 845-860.
28. Kedinger M, Lefebvre O, Duluc I, et al. Cellular and molecular partners involved in gut morphogenesis and differentiation. Philos Trans R Soc Lond B Biol Sci. 1998; 353: 847-856.
29. Quesenberry PJ, Becker PS. Stem cell homing: rolling, crawling, and nesting. Proc Natl Acad Sci U S A. 1998; 95: 15155-15157.
30. Li DQ, Tseng SC. Differential regulation of keratinocyte growth factor and hepatocyte growth factor/scatter factor by different cytokines in human corneal and limbal fibroblasts. J Cell Physiol. 1997; 172: 361-372.
31. Tsai RJ, Sun TT, Tseng SC. Comparison of limbal and conjunctival autograft transplantation in corneal surface reconstruction in rabbits. Ophthalmology. 1990; 97: 446-455.
32. Tsai RJ, Tseng SC. Effect of stromal inflammation on the outcome of limbal transplantation for corneal surface reconstruction. Cornea 1995; 14: 439-449.
33. Tsai RJ, Tsai RY. From stem cell niche environments to engineering of corneal epithelium tissue. Jpn J Ophthalmol. 2014; 58: 111-119.
34. Tsai RJ, Tsai RY. From stem cell niche environments to engineering of corneal epithelium tissue. Jpn J Ophthalmol. 2014; 58: 111-119.
35. Watt FM, Hogan BL. Out of Eden: stem cells and their niches. Science. 2000; 287: 1427-1430.
36. Gnecchi M, Zhang Z, Ni A, et al. Paracrine mechanisms in adult stem cell signaling and therapy. Circulation Research. 2008; 103: 1204–1219.
37. Liu Z, Wan P, Duan H, et al. ES micro-environment enhances stemness and inhibits apoptosis in human limbal stem cells via the maintenance of telomerase activity. PloS One. 2013; 8: 1-14.
38. Yu D, Chen M, Sun X, et al. Differentiation of mouse induced pluripotent stem cells into corneal epithelial-like cells. Cell Biol Int. 2013; 37: 87-94.
39. Saichanma S, Bunyaratvej A, Sila-Asna M. In vitro transdifferentiation of corneal epithelial-like cells from human skin derived precursor cells. International Journal of Ophtalomogy. 2012; 5: 158-163.
40. Harkin DG, Foyn L, Bray LJ, et al. Concise reviews: can mesenchymal stromal cells differentiate into corneal cells? A systematic review of published data. Stem Cells. 2015; 33: 785-791.
41. Wan PX, Wang BW, Wang ZC. Importance of the stem cell microenvironment for ophthalmological cell-based therapy. World J Stem Cells. 2015; 7: 448-460.
42. Dai Y, Guo Y, Wang C, et al. Non-genetic direct reprogramming and biomimetic platforms in a preliminary study for adipose-derived stem cells into corneal endothelia-like cells. PLoS One. 2014; 9: e109856.
43. Théry C. Exosomes: secreted vesicles and intercellular communications. F1000 Biology Reports. 2011; 3: 15.
44. Colombo M, Raposo G, Théry C. Biogenesis, secretion, and intercellular interactions of exosomes and other extracellular vesicles. Annual Review of Cell and Developmental Biology. 2014; 30: 255-289.
45. Valadi H, Ekström K, Bossios A, et al. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nature Cell Biology. 2007; 9: 654-659.
46. Nazarenko I, Rana S, Baumann A, et al. Cell surface tetraspanin Tspan 8 contributes to molecular pathways of exosome-induced endothelial cell activation. Cancer Research. 2010; 70: 1668-1678.
47. Khan M, Nickoloff E, Abramova T, et al. Embryonic stem cell-derived exosomes promote endogenous repair mechanisms and enhance cardiac function following myocardial infarction. Circulation Research. 2015; 117: 52-64.
48. Yu B, Zhang X, Li X. Exosomes derived from mesenchymal stem cells. International Journal of Molecular Science. 2014; 15: 4142–4157.
49. Suntres ZE. Therapeutic uses of exosomes. Exosomes Microvescicles. 2013; 1: 1-8.
50. Streilein JW. Ocular immune privilege: the eye takes a dim but practical view of immunity and inflammation. Journal of Leukocyte Biology. 2003; 74; 179-185.