# A COMPARATIVE STUDY ON THE THERAPEUTIC POTENTIAL

*by* Evelyn Komaratih

**Submission date:** 03-Mar-2021 11:45AM (UTC+0800)
**Submission ID:** 1522872252
**File name:** 08._A_COMPARATIVE_STUDY_ON_THE_THERAPEUTIC_POTENTIAL_OF.pdf (436.87K)
**Word count:** 5225
**Character count:** 27958

DOI : 10.35124/bca.2019.19.S2.4825

*Biochem. Cell. Arch.* Vol. 19, Supplement 2, pp. 4825-4832, 2019 | www.connectjournals.com/bca | *ISSN 0972-5075*

# A COMPARATIVE STUDY ON THE THERAPEUTIC POTENTIAL OF OCULAR AND NON-OCULAR STEM CELL SECRETOME ON ALKALI-INDUCED LIMBAL STEM CELL NICHE DAMAGE

**Evelyn Komaratih[1], Yuyun Rindiastuti[1], Yohanes Widodo Wirohadidjojo[2], Delfitri Lutfi[1], Nurwasis[1], Fedik A. Rantam[3], Nora Ertanti[3] and Cita R. S. Prakoeswa[4*]**

[1]Department of Ophthalmology, Faculty of Medicine Universitas Airlangga/ dr.Soetomo, General Academic Hospital, Surabaya, Indonesia.

[2]Department of Dermatovenerology, Faculty of Medicine, Universitas GadjahMada/ dr. Sardjito, General Academic Hospital, Yogyakarta, Indonesia.

[3]Stem Cell Research and Development Center, UniversitasAirlangga, Surabaya, Indonesia.

[4]Department of Dermatovenerology, Faculty of Medicine Universitas Airlangga/ dr.Soetomo, General Academic Hospital, Surabaya, Indonesia.

*e-mail : drcita.rosita@gmail.com

**(Received 23 August 2019, Revised 14 October 2019, Accepted 25 October 2019)**

**ABSTRACT : The study of secretome from stem cells has become a promising area of research in limbal stem cell deficiency (LSCD). This study aimed to investigate the therapeutic potential of secretome from AD-MSCs and LSCs in a culture of alkali-damaged LSCs as a model of limbal niche damage. Secretome was derived from 4 passages of AD-MSCs and LSCs under serum deprivation conditions. LSCs were damaged using 6,25mM NaOH to generate a limbal niche damage model and were then divided into 5 groups consisting of a control group, as well as 50% AD-MSCs, 25% AD-MSCs, 50% LSCs and 25% LSCs as the treated groups. After 48 hours of treatment, cell proliferation was determined by MTT-assay and cell migration was determined by in vitro scratch assay. VEGF supernatant was measured to analyze anti-angiogenic properties. A concentration of EGF and HGF in secretome was measured using ELISA. The data were analyzed using Oneway ANOVA dan post-hoc Tukey. Secretome derived from AD-MSCs and LSCs successfully promoted the proliferation and migration of LSCs, compared to the control, with the highest proliferation and migration rate seen in the 50% LSC secretome group ((94.38±2.51%, *P* 0.000; 68.52 ± 3.51%, *P* 0.000, respectively). EGF (1216.67 pg/mL) and HGF (2943.33 pg/mL) levels were higher in LSC secretome compared to the secretome of AD-MSCs. A 50% AD-MSC secretome with a level of VEGF supernatant at 65 pg/mL showed better anti-angiogenic activity. Secretome from ocular and non-ocular stem cells demonstrated clear therapeutic benefits inlimbal niche damage.**

***Key words :*** Limbal stem cell deficiency, limbal stem cell, mesenchymal stem cell, ocular surface regeneration.

## INTRODUCTION

Damage to limbal stem cells (LSCs) and their niche leads to limbal stem cell deficiency (LSCD) and is most frequently related to severe chemical burns. This condition results in corneal opacification and neovascularization, leading to vision loss (Sejpal *et al*, 2013; Qihua *et al*, 2018). The therapeutic strategy for LSCD depends on the severity and can range from conservative to invasive treatment. In cases of severe LSCD with more than 75% damage to the LSC niche, limbal stem cell transplantation is the treatment of choice. While the specific properties of the limbal niche have not yet been fully characterized, it is likely to include both cellular and extracellular components. Interaction between the production of growth and cellular factors plays an important role in maintaining the limbal niche. Hence, introducing an external growth factor in LSCD cases should be considered one alternative for ocular surface regeneration.

Mesenchymal stem cell (MSC) populations have a multi-lineage differentiation capacity and a regenerative potential across many tissues and organs. Limbal stem cells (LSCs) and adipose derived-mesenchymal stem cells (AD-MSCs) can also differentiate into corneal epithelial-like cells in certain culture conditions. However, availability and cultivation of healthy donor LSCs remains a challenge. Consequently, other sources of stem cells with a similar potential to LSCs warrant exploration (Aslan *et al*, 2012;

Haagdorens *et al*, 2016; Qihua *et al*, 2018). Ma *et al* (2006) were the first to expand and transplant bone marrow MSCs within human amniotic membrane (HAM) in order to repair the ocular surface of rats with LSCD (Ma *et al*, 2006). Stem cells exert their therapeutic potential through homing-direct differentiation mechanisms and paracrine effects (Aslan *et al*, 2012; Haagdorens *et al*, 2016). Mesenchymal stem cells secrete various bioactive factors, known as secretome, in their conditioned media. The secretome from stem cells contains anti-inflammatory, growth and immunomodulatory factors, as well as cytokines, which are all thought to contribute to ocular surface regeneration. The use of secretome has many advantages compared to a stem cell itself, namely a lack of immune rejection, manufacturable potential and convenient transport (Chuang *et al*, 2012; Wuchter *et al*, 2014). Consequently, MSC secretome can be a useful as an alternative cell-free based therapy for ocular surface regeneration in LSCD. Previous studies have demonstrated the essential role of secretome derived from limbal fibroblasts in regulating epithelial proliferation, differentiation, and wound healing. Indeed, the cell-free nature of MSC secretome has gained particular interest with respect to its safety (Amirjamshidi *et al*, 2011; Saharadze *et al*, 2019).

This study aimed to investigate the therapeutic potential of secretome generated from AD-MSCs and LSCs in a culture of alkali-damaged LSCs, as a model of limbal niche damage. The potential effects derived from AD-MSC and LSC secretome are cell proliferation and migration. In addition, concentrations of epidermal growth factor (EGF) and hepatocyte growth factor (HGF) contained within secretome were measured, as they may contribute to the therapeutic potential that secretome has in limbal niche regeneration.

## MATERIALS AND METHODS

### Materials

The reagents included Type I collagenase (Roche USA), alpha modified eagle medium (á-MEM, Gibco-Life Technologies, USA), nonessential amino acid (NEAA, Sigma, USA), Trypsin-EDTA (Gibco - Life Technologies, USA), fetal bovine serum (FBS, Biowest, USA), NaOH (Gibco-Life Technologies, USA) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphyniltetrazolium bromide (MTT; Gibco-Life Technologies, USA). FITC conjugated antibodies for CD 105, CD 90, CD 73, P63 and CD 45 were purchased from BIOSS (USA). Human vascular endothelial growth factor (VEGF), EGF and HGF ELISA kits were obtained from the Bioassay Technology Laboratory in China.

### Methods

#### Study design

This *in vitro* study investigated the potential therapeutic effect of secretome generated from AD-MSCs and LSCs in a culture of LSCs that were damaged with NaOH, as a model of limbal niche damage. All experiments were conducted with approval from the institutional ethics review board at the Universitas Airlangga through the Stem Cell Research and Development Center.

#### Cell isolation and characterization

#### Adipose-derived MSC isolation and characterization

Adipose-derived MSCs were isolated from a single, healthy 27 year-old-woman donor under aseptic technique. Following an informed consent, 2×2 cm of adipose tissue was collected. Adipose-derived MSCs were isolated, with modification, using an enzymatic method based on protocol developed by Zhu *et al* (2013). Adipose tissue was briefly minced into approximately 1 $cm^3$ pieces and then washed with PBS. The tissue was then placed in sterile tubes containing a α-MEM medium with an enzymatic cocktail comprised of 0.075% Collagenase Type I. An incubation period at 37°C lasted for 30-60 minutes. Following incubation, the digested tissues were washed with culture medium, transferred into a new sterile tube and centrifuged at 300 g for 5 min. Supernatant was then discarded and cell pellets were resuspended in a culture medium composed of á-MEM, 1% penicillin-streptomycin, 1% amphotericin B, 1% NEAA, 5% FBS, and 5ng/mL of bFGF. Culture medium was stored in an incubator at 37°C and 5% $CO_2$ and changed every 3 days until a 90% confluence was reached. It was then passaged with a warm trypsination method. To confirm the MSC's phenotype, cells were monitored for the expression of CD 105, CD 90, CD 73, and CD 45 at the third passage. $5×10^3$ cells were then cultured in 96 multiwell plates until an 80% confluence was reached. The cells were then fixated with 10% formaldehyde for 15 minutes. Fixated cells were each stained with FITC conjugated antibody and incubated for 60 minutes at 37°C. This was followed by an overnight incubation at 4°C. Stained cells were then washed 3 times using PBS tween 0.2%, followed by a DAPI counterstain and visualized under inverted fluorescence microscope (BH2-RFL-T3 model fluorescence attachment, Olympus).

#### Limbal stem cell isolation and characterization

Corneoscleral tissue from healthy male rabbits was obtained from the Stem Cell Research and Development

Center, at the Universitas Airlangga. Cell isolation was conducted according to protocol established by Komaratih *et al* (2019). In short, the tissues were incubated in 0.2mg/mL collagenase type I and á-MEM for 30 minutes at 37°C with gentle agitation. Cell suspensions were collected and then cultured in a complete medium containing α-MEM, 1% penicillin-streptomycin, 1% amphotericin B, 1% NEAA, 5ng/mL bFGF and 10% FBS). After 5 to 7 days of culture, colonies of LSCs were harvested and passaged. To confirm the LSC's phenotype, cells were observed at passage 3 for the expression of p63. $5\times10^3$ cells were then cultured in 96 multiwell plates until 80% confluence was reached. The cells were then fixated with 10% formaldehyde for 15 minutes and then each stained with FITC conjugated antibody and incubated for 60 minutes at 37°C. An overnight incubation at 4°C followed. Stained cells were washed 3 times using PBS tween 0.2%, followed by a DAPI counterstain and visualized under inverted fluorescence microscope (BH2-RFL-T3 model fluorescence attachment, Olympus).

**Secretome production**

$1\times10^6$ AD-MSCs and LSCs obtained at passage 4 were cultured in medium for 24 hours in 100mm diameter culture dishes to reach a confluence of 80%. One day later, the cells were treated using medium composed of α-MEM, 1% penicillin-streptomycin, 1% amphotericin B, 1% NEAA and 2% FBS. Cell secretome collected after 48 hours was then filtered using a 0.45 um Millipore and stored at -80°C until further use.

**Alkali-induced limbal niche damage**

$5\times10^3$ cells/well of LSCs were cultured in 96 multiwell plates for 24 hours in a complete culture medium until 80% confluence was reached. One day later, the cells were destroyed using 20ul of 6.25mM NaOH for 1 minute, followed by three repetitions of a PBS washing until greater than 50% damage to LSCs was achieved.

**Cell proliferation**

A 3-(4,5-dimethylthiazol-2-yl)-2,5-diphyniltetrazolium bromide assay (MTT assay) was conducted to determine the effect that AD-MSC and LSC secretome had on cell proliferation following alkali-induced LSC damage. This assay was performed according to methods put forth by Riss *et al* (2004), with eight replications for each group. Finally, after damaging LSCs using NaOH, the cells were treated for 48 hours in each of the following five culture media: complete, supplemented with 25% AD-MSC secretome, supplemented with 50% AD-MSC secretome, supplemented with 25% LSC secretome, and finally, supplemented with 50% LSC secretome. After 48 hours of incubation, 10 µl of 0.45mg/mL of tetrazolium solution was added to each well for a further incubation time of 4 hours at 37°C. Following incubation, 100 µl of solubilization solution was added to dissolve the formazan crystals. The plates were then immediately analyzed using a microplate reader at an absorbance of 570nm.

***In vitro* scratch assay**

$3\times10^5$ LSCs/well were seeded into six-well culture dishes, allowed to attach, and grown to confluence. The cells were then scratch wounded by yellow pipette tips and washed with medium to remove loose or dead cells. Cells were then treated with each of the five culture media (as discussed above) for 48 hours. Cell migration rates were analyzed according to Freshney (2000). Each scratch wound, marked by a reference line drawn on the outside of the dish, was photographed at varying time periods with a phase-contrast microscope equipped with a camera for 48 hours after treatment. Each scratch wound was photographed over two different fields for a total of four replications. The wound closure area denoted the cell migration rate, and was analyzed with Image J software. The data expressed reflected average percentages of wound closure areas at measured time points over the original width, starting from hour zero (Freshney, 2000).

**Growth factor measurement**

Growth factor measurement was conducted to determine concentrations of EGF and HGF contained in the secretome of AD-MSCs and LSCs. The concentration of VEGF supernatant after cell treatment was measured to determine the anti-angiogenic activity of secretome derived from AD-MSCs and LSCs in an alkali-induced limbal niche damage model. An enzyme-linked immunosorbent assay was performed according to Bioassay Technology Laboratory's protocol. In short, samples were collected and centrifuged at 3000rpm for 20 minutes. 40 µl of supernatant was placed into ELISA well plates. Biotinylated antibody and streptavidin HRP were added, then samples were incubated at 37°C for 60 minutes. Samples were then washed 3x with washing buffer, solutions A and B were added, followed by an incubation at 37°C for 10 minutes. Stop solution was then added and the plates were read immediately using a microplate reader at an absorbance of 450nm.

**Statistical analysis**

The data of cell proliferation and migration were presented as mean ± standard deviation. T cell proliferation and migration difference between groups was analyzed with ANOVA and Post-hoc Tukey using SPSS software, version 19.0, where $P< 0.05$ was considered to be statistically significant.

## RESULTS

### Cell characterization

AD-MSCs and LSCs were successfully isolated, with observations of fibroblast-like morphology appearances beginning at 72 hours. Cell characterization at the third passage indicated a AD-MSC population with positive stains for CD 105, CD 90, and CD 73 FITC conjugated antibody, but negative stain for CD 45 antibody. The population of LSCs stained positive for p63 antibody, as depicted in Fig. 1.

### Cell proliferation and migration

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphyniltetrazolium bromide assay displaying the highest cell proliferation rate were as follows: those treated with 50% LSC secretome (94.38 ± 2.51%), followed by 50% AD-MSC secretome (92.68 ± 2.01%), 25% AD-MSC secretome (90.96 ± 3.11%), 25% LSC secretome (88.85 ± 2.61%), and finally, complete culture medium, which served as a control (70.57 ± 2.57%) (Fig. 2). A significant difference was found between the control group and all of the other groups (ANOVA, post-hoc Tukey, $P$ 0.000), whereas differences between secretome groups of 50% and 25% concentrations were not significant (ANOVA, post-hoc Tukey, $P > 0.05$).

*In vitro* scratch assay showed that cells treated with 50% LSC secretome had the most rapid wound closure rate (68.52±3.51%), followed by 50% AD-MSC secretome (67.17±3.51%), 25% AD-MSC secretome (66.62 ± 3.11%), 25% LSC secretome (66.05 ± 2.01%), and finally, a control with complete culture medium (48.07 ± 1.57%) (Fig. 3). The wound closure area of the control group differed significantly compared to other groups, (ANOVA, post-hoc Tukey, $P > 0.000$). However, no differences were found among treatment groups, (ANOVA, post-hoc Tukey, $P > 0.05$).

### EGF, HGF and VEGF concentration

An enzyme-linked immunosorbent assay showed EGF concentrations of 183.33 pg/mL and 1216.67 pg/mL in secretome derived from AD-MSCs and LSCs, respectively. The concentration found in LSC secretome was 6.6x higher than in AD-MSC secretome. HGF concentration in AD-MSC secretome was 261.33 pg/mL, whereas HGF concentration in LSC secretome was 11.26x higher, at 2943.33 pg/mL. The secretome derived from AD-MSCs and LSCs at different concentrations was found to inhibit VEGF secretion at varying levels. In the 50% and 25% AD-MSC secretome treated group, concentrations of 65 pg/mL and 115 pg/mL, respectively, inhibited VEGF secretion. And in the 50% and 25% LSC secretome treated group , concentrations of 250 pg/mL and 265 pg/mL, respectively, inhibited VEGF secretion as compared to the control group, which had a concentration of 785 pg/mL (Fig. 4).

## DISCUSSION

This study found that stem cells of both ocular and non-ocular origin have a similar effect in regenerating LSC niche. However, the highest cell proliferation rate was obtained in the 50% LSC secretome group. In cell concentrations showing more than 50% alkali-induced damage, 50% LSC secretome was able to stimulate cell reconstitution at a rapid rate. The ability of secretome in promoting the proliferation rate was comparable to its migration rate induction, as shown in areas of wound closure. This is in line with a mouse model study conducted by Amirjamshidi *et al* (2011), which demonstrated that secretome derived from limbal fibroblasts had therapeutic benefits, such as the promotion of limbal stem cell proliferation and differentiation into corneal epithelial cells (Amirjamshidi *et al*, 2011). Conversely, secretome derived from skin fibroblasts failed to promote corneal epithelial growth in a mouse model of LSCD (Notara *et al*, 2011; Amirjamshidi *et al*, 2011).

In this study, concentrations of EGF and HGF in secretome generated from LSCs was much higher than that generated from AD-MSCs. Yet, cell proliferation and migration effects did not differ. Alkali injury is the highest cause of destruction in LSCs population, compromising not only the stem cell population, but also its microenvironment. Theoretically, it is possible to restore stem cell function, both by expanding its population through modulations in the microenvironment, and by inducing transient amplifying cell (TACs) mitosis with the use of appropriate growth factors (Solomon *et al*, 2002; Shimmura *et al*, 2008; Kolli *et al*, 2008; Basu *et al*, 2012). A variety of growth factors play key roles in limbal stem cell regeneration. Insulin like growth factor (IGF-1) that rapidly up-regulates following limbal stem cell damage, enhances the expression of IGF receptors and promotes the differentiation of LSCs into corneal epithelial cells. In contrast, EGF and basic fibroblast growth factor (bFGF), which are also produced by damage to corneal epithelium, support limbal cell proliferation without any effect on their differentiation (Trosan *et al*, 2012; Svobodova *et al*, 2012; Rama *et al*, 2017). In healthy limbal niche, HGF is produced by limbal fibroblasts, which cause LSC proliferation, migration, and differentiation into corneal epithelial cells (Ebrahimi *et al*, 2008). Studies have shown that the amniotic membrane contains high levels of EGF, KGF, HGF, TGF (tumor growth factor), and bFGF, which are all potentially involved in epithelial–

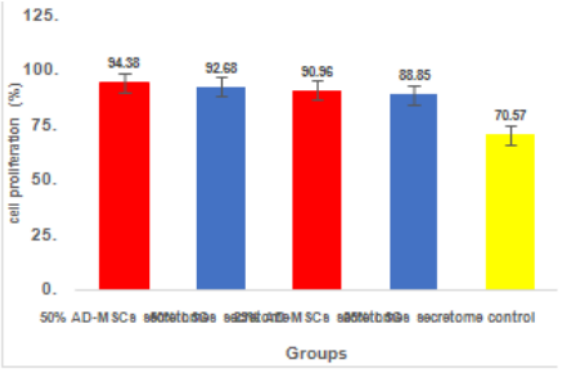


**Fig. 1 :** Cell proliferation rate among groups.

However, the availability of healthy donor limbal tissue remains a problem. Therefore, it is necessary to optimize the production of secretome from a non-ocular stem cell origin (Jeng *et al*, 2011; Notara *et al*, 2011). In this study, lower concentrations of EGF and HGF, as found in AD-MSC secretome, more effectively stimulated cell proliferation and migration compared to the lower concentrations of LSC secretome. However, the overall effect was similar. Adipose-derived MSCs proliferated faster than adult MSCs from other origins, and displayed later senescence compared to bone marrow MSCs. It was shown that AD-MSCs cultured in corneal epithelial

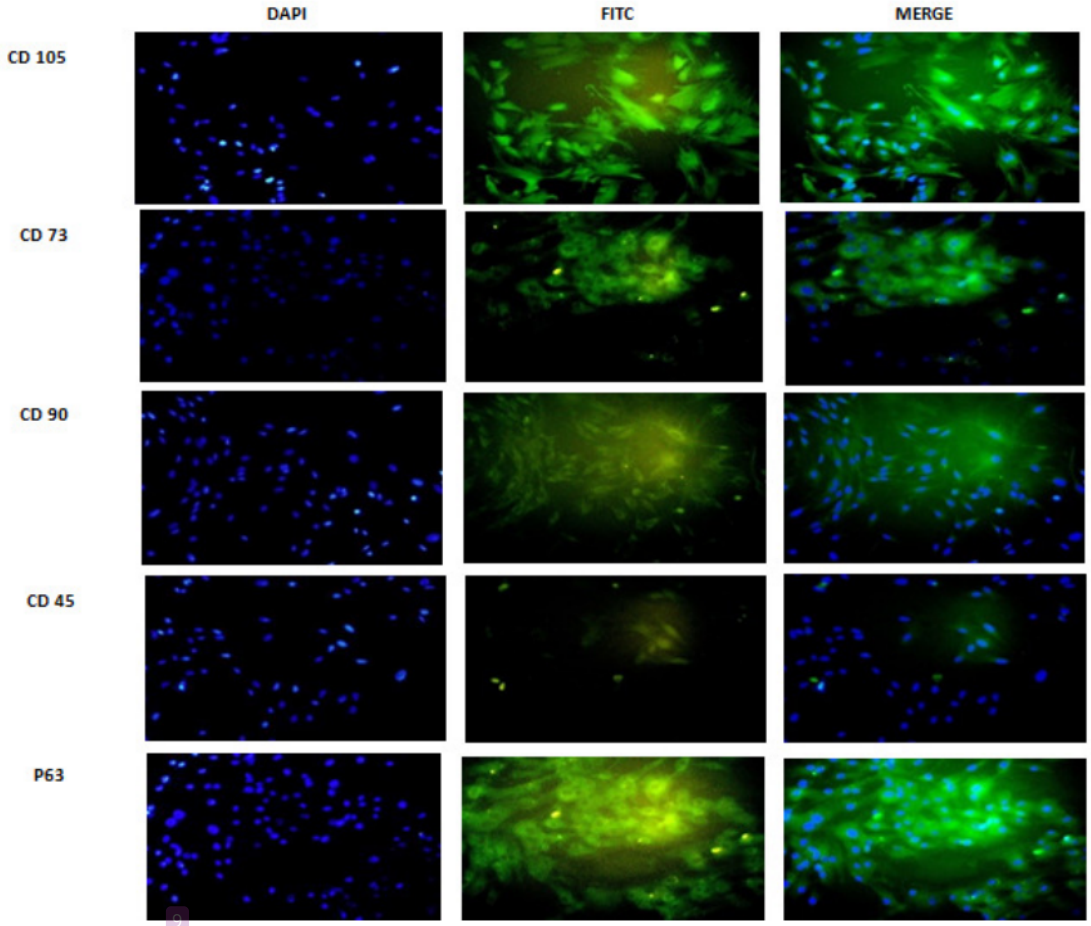


**Fig. 2 :** AD-MSCs P-3 were positive for CD 90, CD 105, CD 73 and negative for CD 45' LSCs were positive for p63. Left section: DAPI; middle section: FITC labelled antibody; right section: a merge of both previous sections (Inverted Fluorescence Microscope, at 200x magnification).

stromal interactions of the human ocular surface. These include epithelialization, modulation of proliferation, and differentiation of stromal fibroblasts (Anderson *et al*, 2001; Grueterich *et al*, 2002; Gomes *et al*, 2005; Sangwan *et al*, 2007).

cell conditioned medium up-regulated markers for corneal epithelial-like cells. Moreover, application of AD-MSCs to rabbit corneal surfaces injured by alkali-burn showed enhanced epithelial healing. Hence, AD-MSCs are a viable, alternative tissue-engineered cell source for

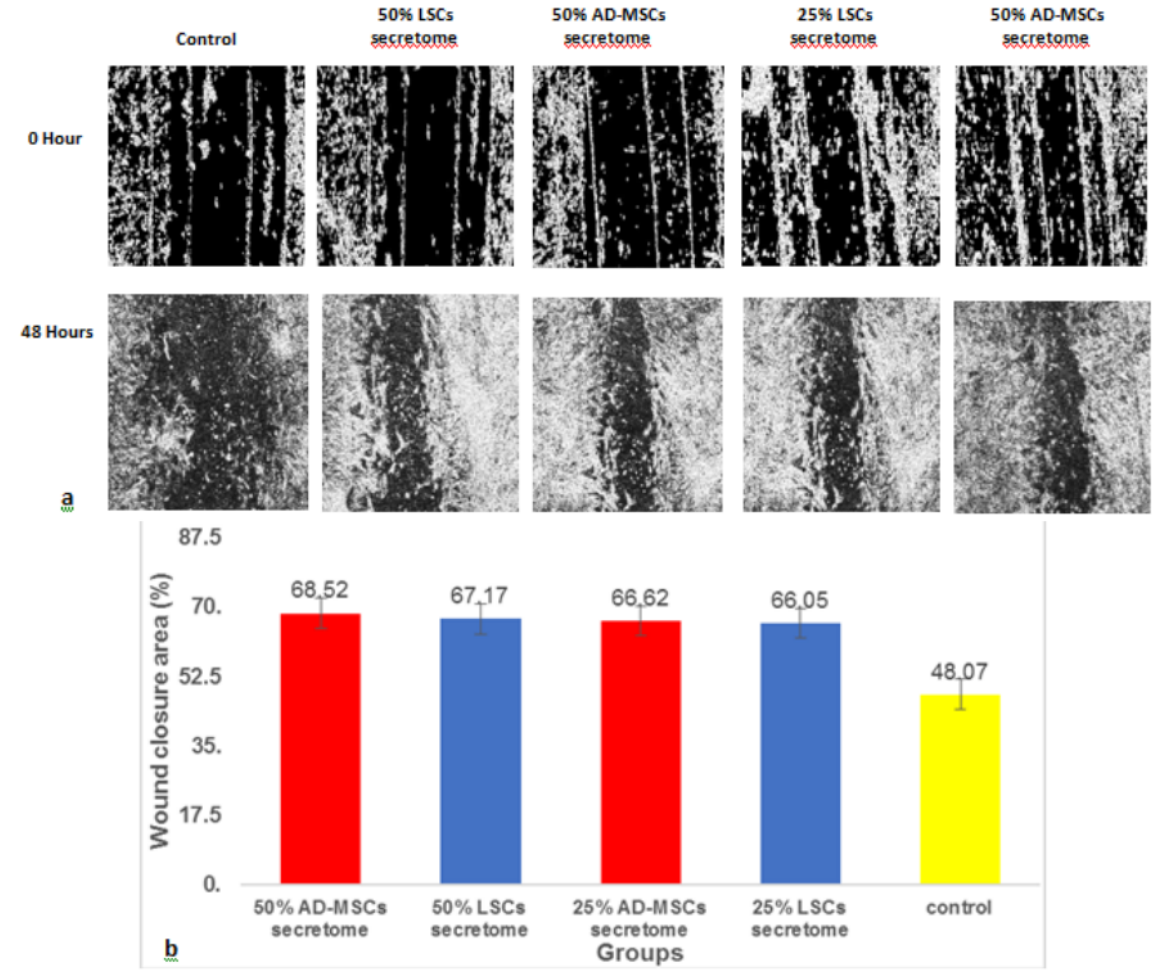


**Fig. 3 :** Cell migration analysis using *in vitro* scratch assay. (a) photograph of *in vitro* scratched cells at 0 hour (above image) and wound closure area at 48 hours (bottom image) (microscope inverted, 100x magnification, Image process by Image J). (b) graphic showing the percent of wound closure area at 48 hours.

treating corneal epithelial defects and reconstructing the ocular surface (Gu *et al*, 2009; Calonge *et al*, 2019; Kern *et al*, 2006; lan *et al*, 2012; Ma *et al*, 2013; Alio *et al*, 2017; Alio *et al*, 2019). Zeppieri *et al* (2017) reported that topical application of AD-MSCs promoted corneal epithelial wound healing and attenuated inflammation in laser-induced corneal wounds of mice (Zeppieri *et al*, 2017). Holan *et al* (2015) demonstrated that the therapeutic effect of BM-MSCs on the healing of alkali-injured corneal surfaces in rabbits was comparable to that of tissue-specific LSCs. Furthermore, they suggest that extra-ocular stem cells can be used for ocular surface regeneration in cases where autologous LSCs are difficult to obtain (Holan *et al*, 2015).

MSCs are known to have a therapeutic potential for corneal disease treatment because of their potent anti-inflammatory and angiogenic-regulatory properties. Topical and/or sub-conjunctival administration of bone marrow MSCs reduced corneal inflammation and angiogenesis following chemical injuries in murine models as seen through the suppression of inflammatory cell and CD68+ macrophage infiltration into corneas, down-regulation of pro-inflammatory cytokines and pro-angiogenic factors, including VEGF and bFGF (Ye *et al*, 2006; Jiang *et al*, 2010; Roddy *et al*, 2011). For the purposes of this study, AD-MSC secretome proved to be more effective in inhibiting VEGF secretion as compared to LSC secretome, suggesting a more potent anti-angiogenic effect. Angiogenesis is regulated by a variety of pro-angiogenic (VEGF, bFGF) and anti-angiogenic factors (including TSP-1, pigment epithelium-derived factor PEDF). Depending on the tissue microenvironment, MSCs exert pro- and anti-angiogenic functions. However,

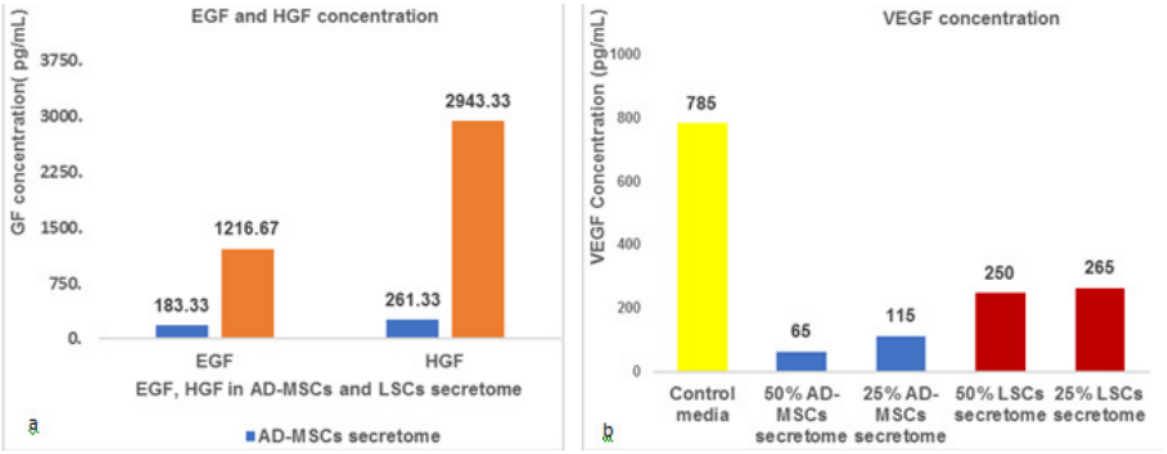


**Fig. 4 :** Growth factor concentration. (a) concentration of HGF and EGF in secretome derived from AD-MSCs and LSCs. (b) concentration of VEGF in LSCs supernatant after 48 hours of secretome treatment at various concentrations.

MSCs up-regulate TSP-1 to inhibit angiogenesis by disrupting CD47 and VEGF receptor-2 signaling, thereby suppressing the VEGF–Akt–eNOS pathway (Jackson *et al*, 2012; Kaur *et al*, 2010; Bazzazi *et al*, 2018). In a study by Zhang *et al* (2019) intravitreal injection of human umbilical cord MSC-Exo improved hyperglycemia-induced retinal inflammation in diabetic rats. This was achieved by a transference of miR-126, which suppressed HMGB1 (high-mobility group box 1) signaling. In another rat model of blue-light induced retinal damage, intravitreal umbilical cord MSC-Exo injection showed a dose-dependent suppression of choroidal neovascularization by down-regulating VEGFA and inhibiting the NFκB pathway (Zhang *et al*, 2019). In this study, it was shown that AD-MSCs of non-ocular origin could provide an adequate amount of growth factors for limbal niche reconstitution following alkali-induced damage.

## CONCLUSION

In summary, this study aimed to determine whether secreted factors from ocular and non-ocular stem cells have a therapeutic benefit in limbal niche damage. Given the relative absence of non-surgical treatments for partial limbal stem cell deficiency, such an approach has a high potential for clinical application. Cell-free based therapy with MSCs or LSCs may translate into relatively safer products due to the low risk of toxicity and immunological rejection. This scope of this study was limited, however, as in vitro examinations lack a full analysis of the cellular and extracellular factors present outside of a LSC niche damage model. Consequently, *in vivo* studies are necessary to further investigate the therapeutic effect that topical application of stem cell secretome has.

## ACKNOWLEDGEMENT

We are thankful to "Mandat" research grant from Universitas Airlangga for their assistance in this study.

## REFERENCES

Alio Del Barrio J L, El Zarif M, Azaar A, Makdissy, Khalil C, Harb W, ElAchkar I, Jawad ZA and de Miguel M P (2019) Regenerative surgery of the corneal stroma for advanced keratoconus: One year outcomes. *Am. J. Ophthalmol.* **203**, 53–68.

Ebrahimi M, Taghi-Abadi E and Bahaevand H (2008) Limbal stem cells in review. *J Ophthalmic Vis Res.* **4**(1), 40-58.

Alio Del Barrio J L, El Zarif, De Miguel M P, Azaar A, Makdissy, Harb W, ElAchkar I and Arnalich-Montiel F (2017) Cellular therapy with human autologous adipose-derived adult stem cells for advanced keratoconus. *Cornea* **36**, 952–960.

Anderson D F, Ellies P, Pires R T and Tseng S C (2001) Amniotic membrane transplantation for partial limbal stem cell deficiency. *Br J Ophthalmol*. **85**, 567-575.

Aslan R F, Akata H, Holme T and Vulliamy Dokal (2012) Limbalstem cell deficiency in patients with inherited stemcell disorder of dyskeratosiscongenita. *International Ophthalmology* **I**(32), 615–622.

Basu S, Ali H and Sangwan V S (2012) Clinical outcomes of repeat autologous cultivated limbal epithelial transplantation for ocular surface burns. *Am. J. Ophthalmol.* **153**, 643–650.

Bazzazi H, Zhang Y, Jafarnejad M, Isenberg J S, Annex B H and Popel A S (2018) Computer simulation of TSP1 inhibition of VEGF-Akt-eNOS: An angiogenesis triple threat. *Front. Physiol*. **9**, 644.

Calonge M, Perez I, Galindo S, Nieto-Miguel T, Lopez-Paniagua M, Fernandez I and Alberca M (2019) A proof-of-concept clinical trial using mesenchymal stem cells for the treatment of corneal epithelial stem cell deficiency. *Transl. Res.* **206**, 18–40.

Chuang K C, Lin C C, Chio C C, Wang C P and Chang J R Kuo (2012) Effects of secretome obtained from normoxiapreconditioned human mesenchymal stem cells in traumatic brain injury rats. *Journal of Trauma and Acute Care Surgery* **73**(5), 1161–1167.

Freshney R I (2000) Cytotoxicity, *Culture of animal cell: a manual of*

*basic technique*. 4th ed. New York: Wiley-Liss, 329-44.

Gomes J A, Romano A, Santos M S and Dua H S (2005) Amniotic membrane use in ophthalmology. *Curr Opin Ophthalmol*. **16**, 233-240.

Grueterich M and Tseng S C (2002) Human limbal progenitor cells expanded on intact amniotic membrane *ex vivo*. *Arch Ophthalmol*. **120**, 783-790.

Gu S, Xing C, Han J, Tso M O and Hong J (2009) Differentiation of rabbit bone marrow mesenchymal stem cells into corneal epithelial cells in vivo and ex vivo. *Mol. Vis* **15**, 99–107.

Haagdorens M, Acker S I V, Gerwen V V, Dhubhghail S N, Koppen C, Tassignon M and Zakaria N (2016) Limbal stem cell deficiency: current treatment options and emerging therapies. *Stem cells International* **9798374**, 1-22.

Holan V, Trosan P, Javorkova E, Zajicova A, Hermankova B, Chudickova M and 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 translational Medicine* **4**, 1052-1063.

Jackson W M, Nesti L J and Tuan R S (2012) Mesenchymal stem cell therapy for attenuation of scar formation during wound healing. *Stem Cell Res*. **3**, 20.

Jeng B H, Halfpenny C P, Meisler D M and Stock E L (2011) Management of focal limbal stem cell deficiency associated with soft contact lens wear. *Cornea* **30**, 18-23.

Jiang T S, Cai L, Ji W Y, Hui Y N, Wang Y S, Hu D and Zhu J (2010) Reconstruction of the corneal epithelium with induced marrow mesenchymal stem cells in rats. *Mol. Vis.* **16**, 1304–1316.

Kaur S, Martin-Manso G, Pendrak M L, Isenberg J S and Roberts D D (2010) Thrombospondin-1 inhibits VEGF receptor-2 signaling by disrupting its association with CD47. *J. Biol. Chem.* **285**, 38923–38932.

Kern S, Eichler H, Stoeve J, Kluter H and Bieback K (2006) Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue. *Stem Cells* **24**, 1294–1301.

Kolli S, Lako M, Figueiredo F, Mudhar H and Ahmad S (2008) Loss of corneal epithelial stem cell properties inoutgrowths from human limbal explants cultured on intact amniotic membrane. *Regen Med*. **3**, 329-342.

Komaratih E, Rindiastuti Y, Susilowati H, Wijayanti N, Dranindi S and Primitasari Y (2019) Antifibrotic Effects of LimbalMesenchymal Stem Cells-Conditioned Media (LMSCs-CM,) on Human Tenon's Fibroblasts (HTFs) in Glaucomatous Eyes: Comparison with Mitomycin C. *Scholars Academic Journal of Bioscience* **7**(1), 1-9.

Lan Y, Kodati S, Lee H S, Omoto M, Jin Y and Chauhan S K (2012) Kinetics and function of mesenchymal stem cells in corneal injury. *Investig.Ophthalmol. Vis. Sci.* **53**, 3638–3644.

Ma Y, Xu Y and Xiao (2006) Reconstruction of chemically burned rat corneal surface by bone marrow-derived human mesenchymal stem cells. *Stem Cells* **24**(2), 315–321.

Ma X Y, Bao H J, Cui L and Zou J (2013) The graft of autologous adipose-derived stem cells in the corneal stromal after mechanic damage. *PLoS ONE* **8**, e76103.

Notara M, Schrader S and Daniels J T (2011) The porcine limbal epithelial stem cell niche as a new model for the study of transplanted tissue engineered human limbal epithelial cells. *Tissue Eng Part A* **17**, 741-750.

Qihua L, Jianjiang X and Sophie X D (2018) Review, The Diagnosis of limbal stem cell deficiency. *Ocul Surf*. **16**(1), 58-69.

Rama P, Ferrari G and Pellegrini G (2017) Cultivated limbal epithelial transplantation. *Curr.Opin.Ophthalmol*. **28**, 387–389.

Riss T L, Moravec R A, Niles A L, Duellman S and Benink H (2013) *Cell viability assays*. Assay Guidance Manual. Bethesda, United States, 1-25.

Roddy G W, Oh J Y, Lee R H, Bartosh T J, Ylostalo J, Coble K and Rosa R H (2011) Action at a distance: Systemically administered adult stem/progenitor cells (MSCs) reduce inflammatory damage to the cornea without engraftment and primarily by secretion of TNF-alpha stimulated gene/protein 6. *Stem Cells* **29**, 1572–1579.

Sagaradze G, Grigorieva O, Nimiritsky P, Basalova N, Kalinina N, Akopyan Z and Efimenko A (2019) Conditioned medium from human mesenchymal stromal cells: towards the clinical translation. *International Journal of Molecular Science* **21**(1656), 1-16.

Sangwan V S (2007) Amniotic membrane transplantation: a review of current indications in the management of ophthalmic disorders. *Indian J Ophthalmol*. **55**, 251-260.

Sejpal K, Bakhtiari P and Deng S X (2013) Presentation, diagnosis and management of limbal stem cell deficiency. *Middle East Afr J Ophthalmol*. **20**, 5–10.

Shimmura S and Tsubota K (2008) Surgical treatment of limbal stem cell deficiency: are we really transplanting stem cells? *Am J Ophthalmol*. **146**, 154-155.

Solomon A, Ellies P, Anderson D F, Touhami A, Grueterich M and Espana E M (2002) Long-term outcome of keratolimbal allograft with or without penetrating keratoplasty for total limbal stem cell deficiency. *Ophthalmology* **109**, 1159-1166.

Svobodova E, Krulova M, Zajicova A, Prochazkova J, Trosan P and Holan V (2012) The role of mouse mesenchymal stem cells in differentiation of naive T cells into anti-inflammatory regulatory T cell and proinflammatory helper T-cell 17 population. *Stem Cells Dev*. **21**, 901–910.

Trosan P, Svobodova E, Chudickova M, Krulova M, Zajicova A and Holan V (2012) The key role of insulin-like growth factor I in the limbal stem cell differentiation and corneal wound healing process. *Stem cells and development* **21**(18), 3341-3351.

Wuchter K, Bieback H and Schrezenmeier (2014) Standardization of Good Manufacturing Practice–compliant production of bone marrow–derived human mesenchymal stromal cells for immunotherapeutic applications. *Cytotherapy* 1-14.

Ye J, Yao K and Kim J C (2006) Mesenchymal stem cell transplantation in a rabbit corneal alkali burn model: Engraftment and involvement in wound healing. *Eye* **20**, 482–490

Zeppieri M, Salvetat M L, Beltrami A, Cesselli D, Russo R and Alcalde I (2017) Adipose derived stem cells for corneal wound healing after laser induced corneal lesions in mice. *Journal of Clinical Medicine* **6**(115), 1-16.

# A COMPARATIVE STUDY ON THE THERAPEUTIC POTENTIAL

ORIGINALITY REPORT

| 19% | 12% | 16% | 0% |
|---|---|---|---|
| SIMILARITY INDEX | INTERNET SOURCES | PUBLICATIONS | STUDENT PAPERS |

PRIMARY SOURCES

| | | |
|---|---|---|
| 1 | edoc.ub.uni-muenchen.de<br>Internet Source | 1% |
| 2 | stemcellsjournals.onlinelibrary.wiley.com<br>Internet Source | 1% |
| 3 | synapse.koreamed.org<br>Internet Source | 1% |
| 4 | Trosan, Peter, Eliska Svobodova, Milada Chudickova, Magdalena Krulova, Alena Zajicova, and Vladimir Holan. "The Key Role of Insulin-Like Growth Factor I (IGF-I) in Limbal Stem Cell Differentiation and the Corneal Wound Healing Process", Stem Cells and Development, 2012.<br>Publication | 1% |
| 5 | arrow.tudublin.ie<br>Internet Source | 1% |
| 6 | oro.open.ac.uk<br>Internet Source | 1% |
| 7 | gala.gre.ac.uk<br>Internet Source | 1% |

| | | |
|---|---|---|
| 8 | worldwidescience.org<br>Internet Source | 1 % |
| 9 | Vellore Kannan Gopinath, S. Soumya, Manju Nidagodu Jayakumar. "Osteogenic and odontogenic differentiation potential of dental pulp stem cells isolated from inflamed dental pulp tissues (I-DPSCs) by two different methods", Acta Odontologica Scandinavica, 2019<br>Publication | 1 % |
| 10 | Cejkova, Jitka, Peter Trosan, Cestmir Cejka, Anna Lencova, Alena Zajicova, Eliska Javorkova, Sarka Kubinova, Eva Sykova, and Vladimir Holan. "Suppression of alkali-induced oxidative injury in the cornea by mesenchymal stem cells growing on nanofiber scaffolds and transferred onto the damaged corneal surface", Experimental Eye Research, 2013.<br>Publication | <1 % |
| 11 | "Tissue-Specific Stem Cell Niche", Springer Science and Business Media LLC, 2015<br>Publication | <1 % |
| 12 | Laurence Hoareau, Florian Fouchet, Cynthia Planesse, Sophie Mirbeau et al. "Combined therapy for critical limb ischaemia: Biomimetic PLGA microcarriers potentiates the pro-angiogenic effect of adipose tissue stromal | <1 % |

vascular fraction cells", Journal of Tissue Engineering and Regenerative Medicine, 2018
Publication

| | | |
|---|---|---|
| 13 | www.spandidos-publications.com<br>Internet Source | <1 % |
| 14 | Romesh I. Angunawela, Jodhbir S. Mehta, Julie T. Daniels. "Ex-vivo ocular surface stem cell therapies: current techniques, applications, hurdles and future directions", Expert Reviews in Molecular Medicine, 2013<br>Publication | <1 % |
| 15 | eprints.qut.edu.au<br>Internet Source | <1 % |
| 16 | www.jbc.org<br>Internet Source | <1 % |
| 17 | doaj.org<br>Internet Source | <1 % |
| 18 | www.gubbagroup.com<br>Internet Source | <1 % |
| 19 | "Corneal Regeneration", Springer Nature, 2019<br>Publication | <1 % |
| 20 | www.ijarbs.com<br>Internet Source | <1 % |
| 21 | Gökhan Karaca, Faruk Pehlivanli, Oktay Aydin, Canan Altunkaya, Hafize Uzun, Mehmet Niyaz, | <1 % |

Hüseyin Özden, Huri Bulut. "The effect of mesenchymal stem cell use on intra-abdominal adhesions in a rat model", Annals of Surgical Treatment and Research, 2018
Publication

| | | |
|---|---|---|
| 22 | Tumor-Associated Fibroblasts and their Matrix, 2011.<br>Publication | <1 % |
| 23 | "Stem Cells in Toxicology and Medicine", Wiley, 2016<br>Publication | <1 % |
| 24 | Bauyrzhan Umbayev, Abdul-Razak Masoud, Andrey Tsoy, Dauren Alimbetov et al. "Elevated levels of the small GTPase Cdc42 induces senescence in male rat mesenchymal stem cells", Biogerontology, 2018<br>Publication | <1 % |
| 25 | Raquel Hernáez-Moya, Sheyla González, Arantza Urkaregi, Jose Ignacio Pijoan, Sophie X. Deng, Noelia Andollo. "Expansion of Human Limbal Epithelial Stem/Progenitor Cells Using Different Human Sera: A Multivariate Statistical Analysis", International Journal of Molecular Sciences, 2020<br>Publication | <1 % |
| 26 | link.springer.com<br>Internet Source | <1 % |

| | | |
|---|---|---|
| 27 | Clémence Bonnet, JoAnn S. Roberts, Sophie X. Deng. "Limbal stem cell diseases", Experimental Eye Research, 2021<br>Publication | <1 % |
| 28 | Jasmin S. Nurković, Radiša Vojinović, Zana Dolićanin. "Corneal Stem Cells as a Source of Regenerative Cell-Based Therapy", Stem Cells International, 2020<br>Publication | <1 % |
| 29 | Seyed Mahmoud Hashemi, Zuhair Mohammad Hassan, Ali Akbar Pourfathollah, Sara Soudi, Abbas Shafiee, Masoud Soleimani. "Comparative immunomodulatory properties of adipose-derived mesenchymal stem cells conditioned media from BALB/c, C57BL/6, and DBA mouse strains", Journal of Cellular Biochemistry, 2013<br>Publication | <1 % |
| 30 | Shimazaki, J.. "Factors Influencing Outcomes in Cultivated Limbal Epithelial Transplantation for Chronic Cicatricial Ocular Surface Disorders", American Journal of Ophthalmology, 200706<br>Publication | <1 % |
| 31 | Translational Medicine Research, 2015.<br>Publication | <1 % |

| | | |
|---|---|---|
| 32 | Trosan, Peter, Eliska Javorkova, Alena Zajicova, Michaela Hajkova, Barbora Hermankova, Jan Kossl, Magdalena Krulova, and Vladimir Holan. "The Supportive Role of Insulin-like Growth Factor-I in the Differentiation of Murine Mesenchymal Stem Cells into Corneal-like Cells", Stem Cells and Development, 2016.<br>Publication | <1 % |
| 33 | citeseerx.ist.psu.edu<br>Internet Source | <1 % |
| 34 | dspace.cuni.cz<br>Internet Source | <1 % |
| 35 | www.hindawi.com<br>Internet Source | <1 % |
| 36 | "Microporation Is a Valuable Transfection Method for Gene Expression in Human Adipose Tissue–derived Stem Cells", Molecular Therapy, 02/2009<br>Publication | <1 % |
| 37 | Akpinar, Kahraman, Yaman. "Ochratoxin A Sequentially Activates Autophagy and the Ubiquitin-Proteasome System", Toxins, 2019<br>Publication | <1 % |
| 38 | Aylin Kara, Semra Koçtürk, Gokcen Bilici, Hasan Havitcioglu. "Development of biological meniscus scaffold: Decellularization method | <1 % |

and recellularization with meniscal cell population derived from mesenchymal stem cells", Journal of Biomaterials Applications, 2021
Publication

---

39 Balu Venugopal, Bernadette K. Madathil, Anil Kumar P.R.. "Stem cell–based therapeutic approaches toward corneal regeneration", Elsevier BV, 2020 <1%
Publication

---

40 Balu Venugopal, Sachin J. Shenoy, Sumitha Mohan, P. R. Anil Kumar, T. V. Kumary. "Bioengineered corneal epithelial cell sheet from mesenchymal stem cells—A functional alternative to limbal stem cells for ocular surface reconstruction", Journal of Biomedical Materials Research Part B: Applied Biomaterials, 2019 <1%
Publication

---

41 Diamantis Almaliotis, Georgios Koliakos, Eleni Papakonstantinou, Anastasia Komnenou et al. "Mesenchymal stem cells improve healing of the cornea after alkali injury", Graefe's Archive for Clinical and Experimental Ophthalmology, 2015 <1%
Publication

---

42 Francesca Lombardi, Paola Palumbo, Francesca Rosaria Augello, Maria Grazia <1%

Cifone, Benedetta Cinque, Maurizio Giuliani. "Secretome of Adipose Tissue-Derived Stem Cells (ASCs) as a Novel Trend in Chronic Non-Healing Wounds: An Overview of Experimental In Vitro and In Vivo Studies and Methodological Variables", International Journal of Molecular Sciences, 2019
Publication

| | | |
|---|---|---|
| 43 | Regenerative Medicine - from Protocol to Patient, 2016.<br>Publication | <1 % |
| 44 | Robert M. Lavker, Nihal Kaplan, Junyi Wang, Han Peng. "Corneal epithelial biology: Lessons stemming from old to new", Experimental Eye Research, 2020<br>Publication | <1 % |
| 45 | Ye Shen, He Shen, Dongyu Guo, Xinghuai Sun et al. "Recent developments in regenerative ophthalmology", Science China Life Sciences, 2020<br>Publication | <1 % |
| 46 | kanazawa-u.repo.nii.ac.jp<br>Internet Source | <1 % |
| 47 | nrronline.org<br>Internet Source | <1 % |
| 48 | onlinelibrary.wiley.com<br>Internet Source | <1 % |

| | | |
|---|---|---|
| 49 | repositorio.unicamp.br<br>Internet Source | <1 % |
| 50 | stemcellstm.alphamedpress.org<br>Internet Source | <1 % |
| 51 | trepo.tuni.fi<br>Internet Source | <1 % |
| 52 | www.jannyorganically.com<br>Internet Source | <1 % |
| 53 | Hamed Nosrati, Zohreh Alizadeh, Ali Nosrati, Korosh Ashrafi-Dehkordi et al. "Stem cell-based therapeutic strategies for corneal epithelium regeneration", Tissue and Cell, 2021<br>Publication | <1 % |
| 54 | Michele d'Angelo, Annamaria Cimini, Vanessa Castelli. "Insights into the Effects of Mesenchymal Stem Cell-Derived Secretome in Parkinson's Disease", International Journal of Molecular Sciences, 2020<br>Publication | <1 % |
| 55 | Submitted to Universitas Airlangga<br>Student Paper | <1 % |
| 56 | "Perinatal Tissue-Derived Stem Cells", Springer Science and Business Media LLC, 2016<br>Publication | <1 % |

| | | |
|---|---|---|
| 57 | Agnese Fiori, Vincenzo Terlizzi, Heiner Kremer, Julian Gebauer, Hans-Peter Hammes, Martin C. Harmsen, Karen Bieback. "Mesenchymal stromal/stem cells as potential therapy in diabetic retinopathy", Immunobiology, 2018<br>Publication | <1 % |
| 58 | Camilla Machado, Adriana Kakehasi, Felipe Dias, Gustavo Resende, Patrícia Oliveira, Ricardo Xavier, Marcus Andrade. "Comparative morphofunctional analysis of fibroblast-like synoviocytes in human rheumatoid arthritis and mouse collagen-induced arthritis", Research Square, 2020<br>Publication | <1 % |
| 59 | F.C. Figueiredo, J.M. Glanville, M. Arber, E. Carr et al. "A systematic review of cellular therapies for the treatment of limbal stem cell deficiency affecting one or both eyes", The Ocular Surface, 2021<br>Publication | <1 % |
| 60 | Ljubimov, Alexander V., and Mehrnoosh Saghizadeh. "Progress in corneal wound healing", Progress in Retinal and Eye Research, 2015.<br>Publication | <1 % |

| | | | |
|---|---|---|---|
| Exclude quotes | On | Exclude matches | Off |
| Exclude bibliography | On | | |

# A COMPARATIVE STUDY ON THE THERAPEUTIC POTENTIAL

GRADEMARK REPORT

FINAL GRADE

/100

GENERAL COMMENTS

**Instructor**

PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5

PAGE 6

PAGE 7

PAGE 8