

# Antifibrotic Effects of Limbal Mesenchymal Stem Cells- Conditioned Media

*by Evelyn Komaratih*

---

**Submission date:** 03-Mar-2021 11:58AM (UTC+0800)

**Submission ID:** 1522884015

**File name:** c\_Effects\_of\_Limbal\_Mesenchymal\_Stem\_Cells-Conditioned\_Media.pdf (508.22K)

**Word count:** 4732

**Character count:** 26601

## Antifibrotic Effects of Limbal Mesenchymal Stem Cells-Conditioned Media (LMSCs-CM) on Human Tenon's Fibroblasts (HTFs) in Glaucomatous Eyes: Comparison with Mitomycin C

Evelyn Komaratih<sup>1</sup>, Yuyun Rindiastuti<sup>1\*</sup>, Helen Susilowati<sup>2</sup>, Nurita T. Wijayanti<sup>2</sup>, Sylva Dranindi<sup>1</sup>, Yulia Primitasari<sup>3</sup>, Nurwasih<sup>1</sup>, Widya Artini<sup>3</sup>, Fedik A. Rantam<sup>2</sup>, Cita R Prakoeswa<sup>4</sup>

<sup>1</sup>Department of Ophthalmology, Universitas Airlangga, Jl. Prof. dr.Moestopo No 4-6, Surabaya, Indonesia

<sup>2</sup>Stem Cell Research and Development Center, Universitas Airlangga, Kampus C Mulyrejo, Surabaya, Indonesia

<sup>3</sup>Department of Ophthalmology, Universitas Indonesia, Jl. Salemba Raya, Jakarta, Indonesia

<sup>4</sup>Department of Dermatovenereology, Universitas Airlangga, Jl. Prof. dr.Moestopo No 4-6, Surabaya, Indonesia

\*Corresponding author: Yuyun Rindiastuti

Received: 01.01.2019 | Accepted: 10.01.2019 | Published: 20.01.2019

DOI: [10.21276/sajb.2019.7.1.2](https://doi.org/10.21276/sajb.2019.7.1.2)

### Abstract

### Original Research Article

Human tenon's fibroblasts (HTFs) is the key effector of bleb fibrosis after trabeculectomy. Excessive fibroblast proliferation and differentiation into myofibroblast contribute to disturbance of extracellular matrix remodeling, result in irreversible fibrosis. In this study we investigated the effect of LMSCs-CM on cell viability, cell migration, cell contractility, collagen synthesis and degradation in HTFs. HTFs from glaucomatous eye were divided into 3 groups consist of FBS 2% control group, MMC, and LMSCs-CM for 7 days. The cell viability was determined by MTT-assay while collagen synthesis and degradation were determined by Sirius red binding assay. Cell migration was determined by in vitro scratch assay and cell contractility was analyzed by fibroblast populated-collagen gel assay. The differences of cell viability, cell contractility, collagen synthesis and degradation among groups were analyzed using Oneway ANOVA or Kruskal Wallis test followed by post-hoc test with 95% confidence interval ( $p < 0.05$ ). The results showed that LMSCs-CM inhibited collagen synthesis and induced collagen degradation in HTFs. LMSCs-CM inhibit cell contraction, while maintain cell viability in HTFs. However, MMC provides better antifibrotic effect on HTFs. In conclusion, LMSCs-CM might have antifibrotic effect on HTFs.

**Keywords:** Tenon's fibroblast, limbal stem cell, fibrosis, collagen, MMC.

**Copyright © 2019:** This is an open-access article distributed under the terms of the Creative Commons Attribution license which permits unrestricted use, distribution, and reproduction in any medium for non-commercial use (NonCommercial, or CC-BY-NC) provided the original author and source are credited.

### INTRODUCTION

It was estimated that more than 60 million people worldwide suffer from glaucoma in 2010, and this population will increase to approximately 80 million by 2020 [1]. Trabeculectomy is still regarded as the "gold standard" for glaucoma filtration surgery (GFS). Trabeculectomy differs from most surgical procedures in that inhibition of wound healing is desirable to achieve surgical success. Formation of scar tissue in the bleb is the main complication which obstructs the outflow of the aqueous humor and consequently leads to the failure of the surgery [1,2]. Therefore, wound healing modulation in the bleb is mandatory to improve the outcome of the surgery. Mitomycin C (MMC) improved success rate of trabeculectomy, yet it is still far from ideal. Nonspecific cytotoxic effects of MMC is associated with sight-threatening side effects and a pathology characterized by extensive cell death. Hence, the search for alternate, safer and more targeted anti-fibrotic agents and

strategies is important to increase surgical success and the lifespan of GFS in patients [2-4].

The human Tenon's fibroblasts (HTFs) are considered the major cell type contributing to the fibrotic response after trabeculectomy. Upon TGF- $\beta$  stimulation, fibroblasts are activated and undergo phenotypic transition into myofibroblasts, the key effector cells in fibrotic states. In addition to its role in myofibroblast differentiation, TGF- $\beta$  promotes matrix preservation and deposition by enhancing matrix protein synthesis and by altering the balance between matrix-preserving and matrix-degrading signals [5,6]. Excessive expression of collagen type I is the major hallmark of bleb fibrosis, therefore collagen deposition is commonly used to evaluate surgical failure in trabeculectomy [5].

Mesenchymal stem cells (MSCs) from different origins and limbal MSCs (LMSCs) share

comparable immunoregulatory properties in vitro. However, LMSCs showed higher anti-inflammatory properties for ocular surface wound healing compared to adipose derived-mesenchymal stem cells (ADSC) [7]. Given the immunomodulatory and trophic properties of MSCs, they have become attractive candidates for the treatment of fibrosis in a variety of models. Various studies on stem cell conditioned media (CM) showed that the secreted factor within its conditioned media alone without the stem cell itself may cause tissue repair in various conditions that involved tissue/organ damage [8,9]. Secretome containing CM are easily manufactured and no need to match the donor and the recipient to avoid rejection problems as it is devoid of cells. Therefore, stem cell-derived conditioned media have a promising prospect to be produced as pharmaceuticals for regenerative medicine [10,11].

3D culture was reported to enhance the anti-fibrotic potentials of MSCs. Hence, we conducted in vitro study to investigate anti-fibrotic effect of 3D LMSCs-CM in HTFs of glaucomatous eye and examined parameters important for wound repair and fibrosis. In this study, we describe the properties of these LMSCs-CM treated HTFs in assays for proliferation, cell migration, collagen deposition and degradation, and collagen gel contraction. We compared these effects against MMC-treated counterparts as the 'gold standard'.

## MATERIALS AND METHODS

### Materials

The main reagents included Type I collagenase (Roche USA), alpha modified eagle medium ( $\alpha$ -MEM, Gibco-Life Technologies, USA), non-essential amino acid (NEAA, Sigma, USA), Trypsin (Gibco - Life Technologies, USA), fetal bovine serum (FBS, Biowest, USA), basic fibroblast growth factor (bFGF, Gibco-Life Technologies, USA), Insulin-transferin-selenium (ITS, Gibco- Life Technologies, USA), rat tail collagen type I (Gibco- Life Technologies, USA), anticoagulant citrate phosphate acid dextrose,  $\text{CaCl}_2$  (sigma). Primary FITC antibodies for immunochemistry to CD73, CD90, CD105, CD45, vimentin, and secondary antibodies were purchased from BIOCSS (USA).

### Methods

#### Study Design

This is an in vitro study in HTFs of glaucomatous eye, conducted in Stem Cell research and development center, Universitas Airlangga. All experiments conformed to local ethics review board, dr. Soetomo Hospital. HTFs culture were divided into 3 groups consist of control group treated with 2% FBS in culture media, MMC treated group, and LMSCs-CM treated group. Cells viability was evaluated at 3<sup>rd</sup> day after treatment. Wound scratch assay was performed for each group, cell migration rate, collagen deposition and

degradation assays, and cell contractility were measured in 7<sup>th</sup> day after treatment.

### LMSCs Isolation and Characterization

Rabbit corneoscleral tissues of healthy male rabbit was obtained from Stem cell research and development center, Universitas Airlangga. The experimental protocol was evaluated and exempted by the Local Review Board, Universitas Airlangga. Cells isolation was conducted according to protocol established by Chen *et al.* with our modification. In brief, the tissues were incubated in 0.2mg/mL of collagenase a type I in  $\alpha$ -MEM for 30 minutes at 37°C with gentle agitation. Cells suspension were collected and cultured in complete media containing  $\alpha$ -MEM, 1% penicillin-streptomycin, 1% amphotericin B, 1% NEAA, 5ng/mL bFGF, and 10% FBS). After 5 to 7 days of culture, colonies of LMCs were harvested and passaged. To confirm the mesenchymal origin phenotype, LMSCs were characterized at passage 3 for the expression of the mesenchymal markers to CD90, CD73, CD105, and CD45 FITC antibody (BIOCSS, USA). Stained cells were visualized under the fluorescence microscope (BH2-RFL-T3 model fluorescence attachment, Olympus) [12].

### Generation of LMSCs-CM

LMSCs of P4 with good growth status were collected by trypsin digestion, and 3D spheroids were generated according to the previous report protocol established by Rangarunlert *et al.* Briefly, MSCs were prepared as  $7.5 \times 10^5$ /mL cell suspensions. 30  $\mu$ L of cell solution per drop (containing about 25,000 cells) was prepared onto the culture plate. Cell drops were cultured inversely for 24 hours in an incubator at 37°C with 5% CO<sub>2</sub>. To obtain LMSCs-CM the plate were re-inversed and floating colonies of 3D spheroid were cultured in complete culture media with 2% FBS for 24 hours. Conditioned media were collected and filtered using Millipore filter 0.45um [13].

### Isolation of HTFs

The Tenon's biopsies were collected from 2 female patients (n = 2) aged 25–40 years who had been diagnosed with advanced open-angle glaucoma, had no previous surgical therapy, and were undergoing trabeculectomy. Cells isolation was conducted according to protocol established by Przekora *et al.* with our modification. The tissues were washed twice with PBS and cut into 2 pieces using a sterile scalpel. These were placed in separate wells of a 12-well plate using light pressure and left to air dry for up to 1 min to attach to the well bottom. Then the tissues were cultured in media containing  $\alpha$ -MEM, 1% penicillin-streptomycin, 1% amphotericin B, 1% NEAA, 5 % FBS, 5ug/mL ITS, and 5ng/mL bFGF. After 5 to 7 days of culture, cells were harvested and passaged. To confirm the fibroblast phenotype, cells were characterized at passage 3 for the expression of vimentin FITC antibody. Stained cells were visualized

under the fluorescence microscope (BH2-RFL-T3 model fluorescence attachment, Olympus) [14].

#### Cell viability, collagen degradation, and collagen deposition

HTFs of P4 were seeded in 96 multiwell plates with  $2.5 \times 10^4$  cells/well with complete culture media for 24 hours. Treatment with 2% FBS as a control group, MMC, LMSCs-CM were given in each group for 7 days. Cell viability was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) assay according to method used by Freshney. Collagen degradation and deposition were determined using Sirius red binding assay according to method used by Heng *et al.* and Taskiran *et al.* respectively. Concentration of type I collagen degradation and deposition were determined according to standard curve established from rat tail collagen type I [15-17].

#### In vitro scratch assay

HTFs were seeded into wells of six-well culture dishes and allowed to attach and grow to confluence. The cells were then scratched wounded by a pipette tip and washed with medium to remove loose or dead cells. Treatment with 2% FBS as a control group, MMC, and LMSCs-CM were given in each group for 7 days. The wound at the same spot, marked by a reference line drawn on the outside of the dish, was photographed at different time points for a period of 3<sup>rd</sup> and 7<sup>th</sup> day after treatment with a phase-contrast microscope equipped with a camera. Two different fields of each scratch wound were photographed. Each condition was performed in triplicate. The wound widths were analyzed as cell migration rate with Image J software. The data were expressed as averages of the percentage width of the wounds at the measured time point over the original width at time 0 hr

#### Cell contraction assay

To assess the influence of LMSCs-CM on HTFs contraction, we measured the contraction of

HTFs-seeded collagen gels. First, HTFs were resuspended at a density of  $3 \times 10^5$  cells/mL with complete culture media. Each gel was made from dialyzed collagen 0.1% acetic acid and mixed gently. Sodium hydroxide (0.1 M) was then added to the gel to return the solution to physiological pH and to precipitate the collagen. Thirty microliters of HTFs (at  $3 \times 10^5$  cells/mL) were then seeded into the neutralized gel and resuspended briefly. This was added to a well of a 12-well plate and incubated at 37°C for 24 hours. Each treatment was then given into each collagen seeded HTFs. The fibroblast-populated collagen gels were incubated for 7 days. The images of contractile cells grown over collagen coated dish were taken with a digital camera. The images were then measured and assessed using ImageJ software by comparing cell-gel contractile area with total area [17].

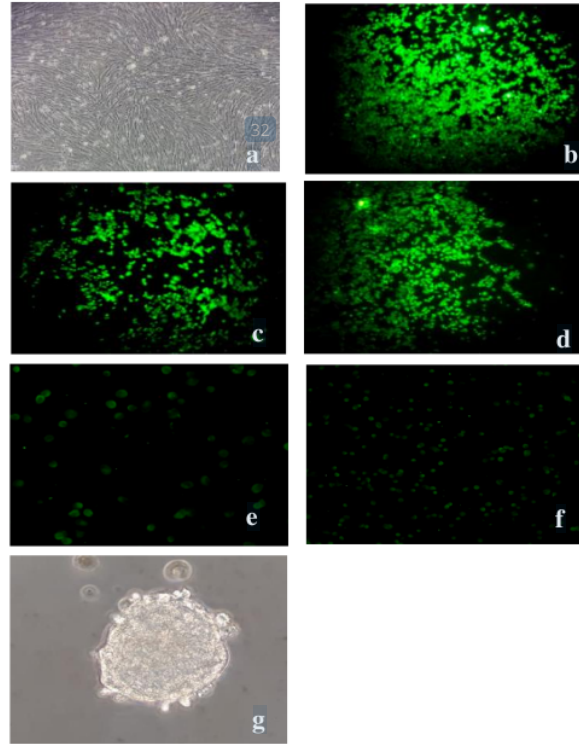
#### Statistical analysis

Statistical analysis was performed using SPSS version 19.0 software. One way ANOVA or Kruskal wallis test and post-hoc test with 95% confidence interval ( $p < 0.05$ ) was used to determine the statistical significance of differences of cell viability, collagen degradation and deposition, and cell contraction among HTFs after treatment.

## RESULTS

#### Identification of LMSCs and Generation of LMSCs-CM

LMSCs exhibited fibroblast morphology and expanded easily when cultured in regular medium in vitro. They were confirmed positive for CD73, CD90, and CD105 and negative for CD45 and P63 according to immunocytochemistry analysis of stem cell-related surface markers. Hanging drop technique successfully generated 3D spheroid colonies for producing conditioned media (Figure 1).



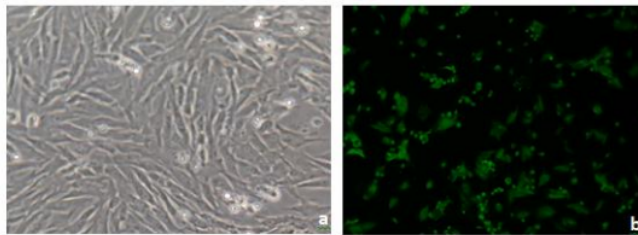
**Fig-1: Limbal mesenchymal stem cells, inverted microscope, objective 100x, (a) fibroblast like morphology (phase-contrast), immunocytochemistry positive to (b) CD105 +, (c) CD73 +, (d) CD90 +, negative to (e) CD45, (f) P63 FITC antibody, (g) 3D spheroid formation.**

**HTFs, Cell viability and migration**

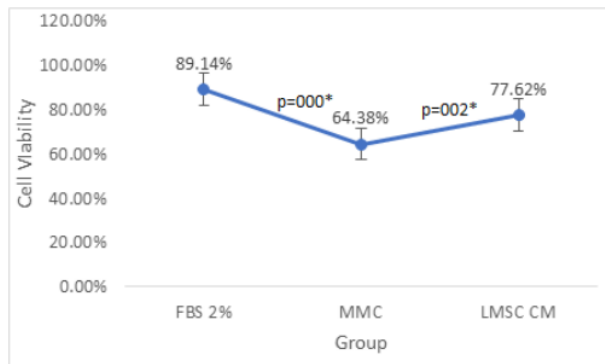
This study successfully established rapid cultivation of HTFs in 14<sup>35</sup> days with good cell performance as seen in figure 2.

The effect of LMSCs-CM on cell viability of HTFs culture is presented in figure 3. Cell viability of HTFs decreased after MMC treatment ( $64.38\% \pm 3.12\%$ )

compared to those with FBS 2% control group ( $89.14\% \pm 8.69\%$ ,  $p=0.000$ ) and LMSCs-CM ( $77.62\% \pm 6.52\%$ ,  $p=0.002$ ). However, there was significant decreased of cell viability in MMC ( $p=0.001$ ) and LMSCs-CM ( $p=0.021$ ) compared to control group.



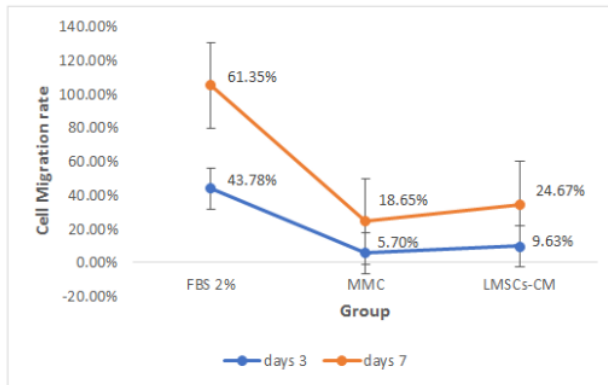
**Fig-2: HTFs on passage 4. (a) Fibroblast morphology in phase contrast, 200x, (b) vimentin + of HTFs on passage 3**



**Fig-3: Effect of LMSCs-CM on cell viability of HTFs compared to FBS 2% control group and MMC group (Mann-Whitney, \*statistically significant)**

MMC decreased cell migration rate on days 3 and 7 compared to FBS 2% control group and LMSCs-CM group (Figure 4). Cell migration rate on days 3 in LMSCs-CM and MMC group were 4.5 fold

and 8.5 fold lower than FBS 2% control group, respectively. Cell migration rate tend to increase on days 7 from 1.5 to 3 fold in all groups.

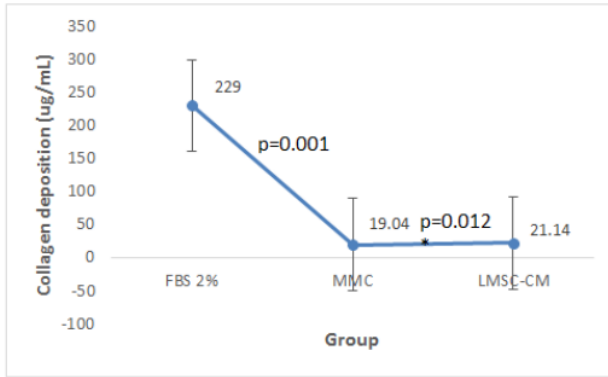


**Fig-4: Cell migration rate on days 3 and 7**

**Collagen Deposition, Degradation, and cell contractility**

MMC (19.04±0.54ug/mL) and LMSCs-CM (24.14±3.19ug/mL) were significantly decreased

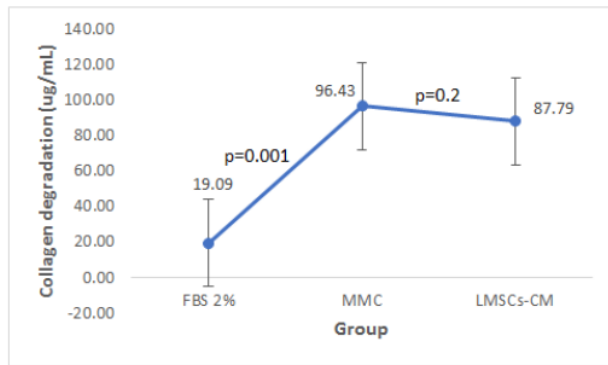
collagen deposition compared to FBS 2% control group (229.0±1.24ug/mL, p=0.001). Yet, collagen deposition in MMC group was significantly lower compared to LMSCs-CM group (p=0.01) (Figure 5).



**Fig-5: Collagen deposition (Mann-Whitney, \* statistically significant).**

MMC (96.43±1.04ug/mL) and LMSCs-CM (87.79±1.05ug/mL) were significantly increased collagen degradation compared to FBS 2% control

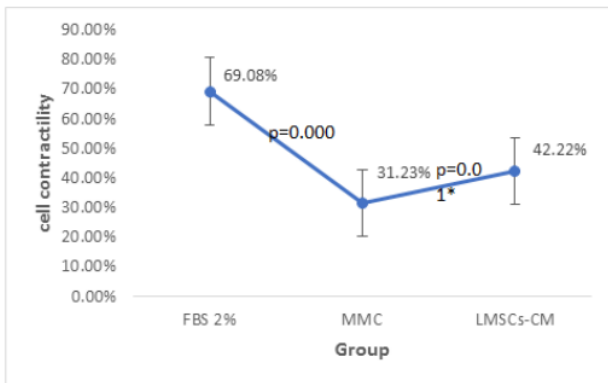
group (19.09±3.1ug/mL, p=0.001). However, collagen degradation in MMC and LMSCs-CM group were not different statistically (p=0.2) (Figure 6).



**Fig-6: Collagen degradation (Mann-Whitney, \* statistically significant)**

MMC (31.23±6.96ug/mL) and LMSCs-CM (42.22±7.05ug/mL) were significantly decreased cell contractility compared to FBS 2% control group

(69.08±5.23ug/mL, p=0.000). Cell contraction in MMC was significantly lower than LMSCs-CM group (p=0.01) (Figure 7).



**Fig-7: Cell contractility (Tukey HSD, \* statistically significant)**

## DISCUSSION

Fibroblast is the main effector in bleb fibrosis after trabeculectomy. Fibroblast stimulated by macrophages, neutrophils, and inflammatory cytokines are involved in complex processes, leading to further extracellular matrix (ECM) secretion and remodeling. Any overwhelming activity within this process can cause overstimulation of fibroblasts, formation and accumulation of extraneous ECM. Matrix metalloproteinases (MMPs) contribute in ECM remodeling and the subsequent matrix contraction in scar formation. The unrestrained proliferation of fibroblasts induces a strong inflammatory reaction with abundant ECM, mainly type I collagen formation [14, 18]. MMC still proven as gold standard in trabeculectomy to halt bleb fibrosis.

This study indicates that MMC leads the antifibrosis effect in HTFs since that agent successfully decrease cell viability and inhibit cell migration in wound scratch assay. Seet *et al.* showed that MMC was far more effective in effecting this inhibition compared to secreted protein acid rich in cysteine (SPARC) silencing HTFs model. MMC affects HTFs by inhibiting cell proliferation, inducing widespread apoptosis, increasing susceptibility to T cell-mediated lysis, reducing the production of pro-fibrotic genes and proteins and reducing cell migration. MMC treated HTFs inhibit type I collagen deposition in accordance with induction of collagen degradation as the main fibrosis related ECM compared to FBS 2% control group and LMSCs-CM group. Moreover, MMC successfully inhibited type I collagen deposition compared to LMSCs-CM group. MMC-treated HTFs were severely limited in the ability to contract the collagen gel compared to FBS 2% control group and LMSCs-CM group. It has been proposed that MMC improves surgical outcome at least in part through the induction of apoptosis, which leads to early termination of the wound healing response [1,2,4,19].

We generated LMSCs-CM from cultured of 3D spheroid colonies to improve antifibrosis secreted factor. Three-dimensional (3D) cell culture has been reported to increase the therapeutic potentials of MSCs. MSCs have been reported to have antifibrotic properties by paracrine secretions of cytokines, such as insulin growth factor 1 (IGF-1), interleukin-6 (IL-6), and hepatocyte growth factor (HGF) [20,21]. Aggregation of MSCs into 3D spheroids increased the expression of the anti-inflammatory protein TNF $\alpha$  stimulated gene/protein 6 (TSG-6), as well as their paracrine secretion of angiogenic factors, including vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), and angiogenin [13,22]. *In vitro* studies indicated conditioned medium of 3D cultured MSCs protected hepatocytes from cell injury and apoptosis more effectively than 2D cultured cells [22]. CM provides key advantages over stem-cell based applications related to safety of immune compatibility,

tumorigenicity, emboli formation and the transmission of infection, stable in preservation, economical, and more practical in clinic [22,23].

This study indicates that LMSCs-CM was able to maintain HTFs cell viability compared to MMC group, while LMSCs-CM proven its antifibrosis effect on HTFs. LMSCs-CM decrease cell migration compared to FBS 2% control, yet MMC has better inhibitory effect on cell migration. Usunier *et al.* demonstrated that systemic murine MSC administration attenuated fibrosis in a bleomycin-induced lung injury model. In that study, MSCs synergistically reduced TGF $\beta$ -induced fibroblast proliferation and myofibroblast differentiation [11,19].

LMSCs-CM significantly inhibit collagen synthesis in line with induction of collagen degradation in HTFs. This study was consistent with Arti *et al.* which reported that less ECM was produced by fibroblasts after exposure to MSC-conditioned medium, and transplantation of rat MSCs into infarcted rat myocardium attenuated the fibrotic response as assessed by Sirius red staining. Several factors could be responsible for these anti-fibrotic effects, like HGF, bFGF, IGF-1, and adrenomedullin (ADM) [24]. Li *et al.* showed that ADM secretion by MSCs was associated with inhibition of cardiac fibroblast proliferation and collagen I synthesis [25]. Conversely, hepatocyte growth factor (HGF) has been shown to be antifibrotic. Exogenous HGF enhanced histological and functional healing in both acute and more chronic vocal fold fibrosis in canine model [26]. Recently, Qi *et al.* highlighted the importance of TSG-6 (TNF-stimulated gene 6) in the antifibrotic effect of MSCs. In addition to suppressing the secretion of TNF- $\alpha$  by activated macrophages, this protein induces a change in the TGF- $\beta$ 1/TGF- $\beta$ 3 balance, from a profibrotic high ratio to an antifibrotic low ratio [27-29]. These results are confirmed in a coculture model in which recombinant TSG-6 partially reproduced the effects of MSCs [10,29,30]. In different fibrosis models, a decreased expression and concentration of collagen, the main component of the ECM, were found after MSC transplantation. This effect is also obtained after transplanting microvesicles or exosomes secreted into an MSC culture, suggesting a paracrine control of MSCs on ECM degradation [31,32].

Inhibition of collagen deposition and collagen degradation stimulation by treated HTFs with LMSCs-CM indicates that those secreted factors might have role in balance of ECM reconstitution through proteolytic and anti-proteolytic enzyme activity. This study is in accordance with Ishikane *et al.* they showed that after MSC transplantation, the increased expression of MMP-2, MMP-9, MMP-13, and MMP-14 has been observed in several fibrosis models. Following the addition of MSC-CM to a culture of heart fibroblasts, an increase in the activity of MMP-2 and MMP-9 was



found [33]. MSCs seem to have a repressive effect on the expression of TIMPs such as TIMP-1. A reduction in the concentration of TIMP 1 to 4 was shown after MSC transplantation. In an *in vitro* model, a decrease in the expression of TIMP-2 was observed, suggesting that MSCs have a paracrine effect [32-34].

As limitation, this study did not conduct proteomic analysis of LMSCs-CM to determine secreted antifibrosis factor. Moreover, the expression of pro-fibrosis cytokines within HTFs were not observed, hence we could not convince the mechanism governing the antifibrosis effects LMSCs-CM.

## CONCLUSION

3D spheroid generated LMSCs-CM exerts antifibrosis effect on HTFs of glaucomatous eye. Yet, MMC provides better inhibitory effect on fibrosis in HTFs. This study might provide further insight for application of LMSC as antifibrosis in trabeculectomy.

## Acknowledgement

Airlangga Health Science Institute research grant.

## REFERENCES

1. Yamanaka O, Kitano-Izutani A, Tomoyose K, Reinach PS. Pathobiology of wound healing after glaucoma filtration surgery. In *BMC ophthalmology* 2015 Nov (Vol. 15, No. 1, p. 157). BioMed Central.
2. Seet LF, Su R, Toh LZ, Wong TT. In vitro analyses of the anti-fibrotic effect of SPARC silencing in human Tenon's fibroblasts: comparisons with mitomycin C. *Journal of cellular and molecular medicine*. 2012 Jun;16(6):1245-59.
3. Radcliffe NM. Trabeculectomy revision as a treatment for failed trabeculectomy. *Glaucoma Today*. 2010;25-8.
4. Masoumpour MB, Nowroozzadeh MH, Razeghinejad MR. Suppl 1: M5: Current and Future Techniques in Wound Healing Modulation after Glaucoma Filtering Surgeries. *The open ophthalmology journal*. 2016;10:68.
5. Wang L, Wang DB, Liu MY, Yao RY. Correlation between tissue characterization and dynamic expression of matrix Metalloproteinase-2 and its tissue inhibitor in conjunctival filtering bleb of rats. *BioMed research international*. 2017;2017.
6. McKleroy W, Lee TH, Atabai K. Always cleave up your mess: targeting collagen degradation to treat tissue fibrosis. *American Journal of Physiology-Lung Cellular and Molecular Physiology*. 2013 Apr 5;304(11):L709-21.
7. Holan V, Trosan P, Cejka C, Javorkova E, Zajicova A, Hermankova B, Chudickova M, Cejkova J. A comparative study of the therapeutic potential of mesenchymal stem cells and limbal epithelial stem cells for ocular surface reconstruction. *Stem cells translational medicine*. 2015 Sep 1;4(9):1052-63.
8. Li GG, Zhu YT, Xie HT, Chen SY, Tseng SC. Mesenchymal stem cells derived from human limbal niche cells. *Investigative ophthalmology & visual science*. 2012 Aug 1;53(9):5686-97.
9. Tabélé C, Montana M, Curti C, Terme T, Rathelot P, Gensollen S, Vanelle P. Organic glues or fibrin glues from pooled plasma: efficacy, safety and potential as scaffold delivery systems. *Journal of Pharmacy and Pharmaceutical Sciences*. 2012;15(1):124-40.
10. Zhang X, Hu MG, Pan K, Li CH, Liu R. 3D spheroid culture enhances the expression of antifibrotic factors in human adipose-derived mscs and improves their therapeutic effects on hepatic fibrosis. *Stem cells international*. 2016;2016.
11. Usunier B, Benderitter M, Tamarat R, Chapel A. Management of fibrosis: the mesenchymal stromal cells breakthrough. *Stem cells international*. 2014;2014.
12. Chen SY, Hayashida Y, Chen MY, Xie HT, Tseng SC. A new isolation method of human limbal progenitor cells by maintaining close association with their niche cells. *Tissue Engineering Part C: Methods*. 2011 Feb 9;17(5):537-48.
13. Rungarunlert S, Techakumphu M, Purity MK, Dinnyes A. Embryoid body formation from embryonic and induced pluripotent stem cells: Benefits of bioreactors. *World journal of stem cells*. 2009 Dec 31;1(1):11.
14. Przekora A, Zarnowski T, Ginalska G. A simple and effective protocol for fast isolation of human Tenon's fibroblasts from a single trabeculectomy biopsy—a comparison of cell behaviour in different culture media. *Cellular & molecular biology letters*. 2017 Dec;22(1):5.
15. Heng E, Huang Y, Black J.r. CCN2. Connective tissue growth factor stimulates deposition by gingival fibroblast via module 3 and alpha6 and alpha1 integrins. *J Cell Biochem*. 2006; 89 (2): 490-20.
16. YERCAN H, KUTAY FZ. Quantification of total collagen in rabbit tendon by the Sirius red method. *Turkish Journal of Medical Sciences*. 1999;29:7-9.
17. Freshney RI. Cytotoxicity. *Culture of animal cell: a manual of basic technique*. 4th ed. New York: Wiley-Liss. 2000; 2000; 329-44.
18. Kundrotas G. Surface markers distinguishing mesenchymal stem cells from fibroblast. *Acta Medica Lituanica*. 2012;19(2): 75-79.
19. Nakatsu MN, González S, Mei H, Deng SX. Human limbal mesenchymal cells support the growth of human corneal epithelial stem/progenitor cells. *Investigative ophthalmology & visual science*. 2014 Oct 1;55(10):6953-9.
20. Sarvar DP, Shamsasenjan K, Akbarzadehlaleh P. Mesenchymal stem cell-derived exosomes: new opportunity in cell-free therapy. *Advanced pharmaceutical bulletin*. 2016 Sep;6(3):293.

21. Spees JL, Lee RH, Gregory CA. Mechanism of mesenchymal stem/stromal cell function. *Stem Cell Research & Therapy*. 2016; 7(125): 1-13.
22. Maguire G. Stem cell therapy without the cells. *Commun. Integr. Biol.* 2013;6: e26631.
23. Madrigal M, Rao KS, Riordan NH. A review of therapeutic effects of mesenchymal stem cell secretions and induction of secretory modification by different culture methods. *J. Transl. Med.* 2014;12: 260.
24. Ramkisoensing AA, de Vries AA, Atsma DE, Schalij MJ, Pijnappels DA. Interaction between myofibroblasts and stem cells in the fibrotic heart: balancing between deterioration and regeneration. *Cardiovascular research*. 2014 Feb 27;102(2):224-31.
25. Li L, Zhang S, Zhang Y, Yu B, Xu Y, Guan Z. Paracrine action mediate the antifibrotic effect of transplanted mesenchymal stem cells in a rat model of global heart failure. *Molecular biology reports*. 2009 Apr 1;36(4):725-31.
26. Xu W, Hu R, Fan E, Han D. Adipose-derived mesenchymal stem cells in collagen—hyaluronic acid gel composite scaffolds for vocal fold regeneration. *Annals of Otology, Rhinology & Laryngology*. 2011 Feb;120(2):123-30.
27. Qi Y, Jiang D, Sindrilaru A, Stegemann A, Schatz S, Treiber N, Rojewski M, Schrezenmeier H, Vander Beken S, Wlaschek M, Böhm M. TSG-6 released from intradermally injected mesenchymal stem cells accelerates wound healing and reduces tissue fibrosis in murine full-thickness skin wounds. *Journal of Investigative Dermatology*. 2014 Feb 1;134(2):526-37.
28. Kotani T, Masutani R, Suzuka T, Oda K, Makino S, Ii M. Anti-inflammatory and anti-fibrotic effects of intravenous adipose-derived stem cell transplantation in a mouse model of bleomycin-induced interstitial pneumonia. *Scientific reports*. 2017 Nov 6;7(1):14608.
29. Wu HJ, Yiu WH, Li RX, Wong DW, Leung JC, Chan LY, Zhang Y, Lian Q, Lin M, Tse HF, Lai KN. Mesenchymal stem cells modulate albumin-induced renal tubular inflammation and fibrosis. *PLoS One*. 2014 Mar 19;9(3):e90883.
30. Choi M, Ban T, Rhim T. Therapeutic use of stem cell transplantation for cell replacement or cytoprotective effect of microvesicle released from mesenchymal stem cell. *Molecules and cells*. 2014 Feb 28;37(2):133.
31. Linard C, Busson E, Holler V, Strup-Perrot C, Lacave-Lapalun JV, Lhomme B, Prat M, Devauchelle P, Sabourin JC, Simon JM, Bonneau M. Repeated autologous bone marrow-derived mesenchymal stem cell injections improve radiation-induced proctitis in pigs. *Stem cells translational medicine*. 2013 Nov 1;2(11):916-27.
32. Ishikane S, Hosoda H, Yamahara K, Akitake Y, Kyoungsook J, Mishima K, Iwasaki K, Fujiwara M, Miyazato M, Kangawa K, Ikeda T. Allogeneic Transplantation of Fetal Membrane-Derived Mesenchymal Stem Cell Sheets Increases Neovascularization and Improves Cardiac Function after Myocardial Infarction in Rats. *Transplantation*. 2013 Oct 27;96(8):697-706.
33. Hiwatashi N, Bing R, Kraja I, Branski RC. Mesenchymal stem cells have antifibrotic effects on transforming growth factor- $\beta$ 1-stimulated vocal fold fibroblasts. *The Laryngoscope*. 2017 Jan;127(1):E35-41.

# Antifibrotic Effects of Limbal Mesenchymal Stem Cells-Conditioned Media

---

## ORIGINALITY REPORT

---

18%

SIMILARITY INDEX

10%

INTERNET SOURCES

16%

PUBLICATIONS

0%

STUDENT PAPERS

---

## PRIMARY SOURCES

---

- 1 A. A. Ramkisoensing, A. A. F. de Vries, D. E. Atsma, M. J. Schaliij, D. A. Pijnappels. "Interaction between myofibroblasts and stem cells in the fibrotic heart: balancing between deterioration and regeneration", *Cardiovascular Research*, 2014  
Publication 2%
- 2 Qiankun Li, Cuiping Zhang, Xiaobing Fu. "Will stem cells bring hope to pathological skin scar treatment?", *Cytotherapy*, 2016  
Publication 1%
- 3 Lukas Widhiyanto, Dwikora Novembri Utomo, Adrianto Prasetyo Perbowo, Kukuh Dwiputra Hernugrahanto. "Macroscopic and histologic evaluation of cartilage regeneration treated using xenogenic biodegradable porous sponge cartilage scaffold composite supplemented with allogenic adipose derived mesenchymal stem cells (ASCs) and secretome: An in vivo experimental study", *Journal of Biomaterials Applications*, 2020 1%

---

4	Jeanne Adiwinata Pawitan. "Prospect of Stem Cell Conditioned Medium in Regenerative Medicine", BioMed Research International, 2014 Publication	1 %
5	ijriar.com Internet Source	1 %
6	www.mdpi.com Internet Source	1 %
7	stemcellres.biomedcentral.com Internet Source	1 %
8	www.freepatentsonline.com Internet Source	1 %
9	mdpi.com Internet Source	<1 %
10	repository.maranatha.edu Internet Source	<1 %
11	worldwidescience.org Internet Source	<1 %
12	docplayer.net Internet Source	<1 %
13	pubs.rsc.org Internet Source	<1 %

---

14

Internet Source

<1 %

---

15

Arti V. Shinde, Nikolaos G. Frangogiannis. "Chapter 24 Aging-Associated Alterations in Myocardial Inflammation and Fibrosis: Pathophysiological Perspectives and Clinical Implications", Springer Science and Business Media LLC, 2014

Publication

<1 %

---

16

Chenxia Hu, Lingfei Zhao, Jinfeng Duan, Lanjuan Li. "Strategies to improve the efficiency of mesenchymal stem cell transplantation for reversal of liver fibrosis", Journal of Cellular and Molecular Medicine, 2019

Publication

<1 %

---

17

[citeseerx.ist.psu.edu](http://citeseerx.ist.psu.edu)

Internet Source

<1 %

---

18

[www.derm.med.ed.ac.uk](http://www.derm.med.ed.ac.uk)

Internet Source

<1 %

---

19

"Stem Cells and Cancer Stem Cells, Volume 3", Springer Science and Business Media LLC, 2012

Publication

<1 %

---

20

[www.inabj.org](http://www.inabj.org)

Internet Source

<1 %

---

21 Adriana Torres Crigna, Cristina Daniele, Carolina Gamez, Sara Medina Balbuena et al. "Stem/Stromal Cells for Treatment of Kidney Injuries With Focus on Preclinical Models", Frontiers in Medicine, 2018  
Publication <1 %

---

22 H Tsuboi. "Tartrate resistant acid phosphatase (TRAP) positive cells in rheumatoid synovium may induce the destruction of articular cartilage", Annals of the Rheumatic Diseases, 2003  
Publication <1 %

---

23 A.A. Temnov, A.V. Vagabov, A.N. Sklifas, V.I. Novoselov, Y.A. Belyi. "The role of mesenchymal stromal cells in the repair of acute organ injury", Wiley, 2016  
Publication <1 %

---

24 Mark Zada, Ushasree Pattamatta, Andrew White. "Modulation of Fibroblasts in Conjunctival Wound Healing", Ophthalmology, 2018  
Publication <1 %

---

25 [www.i-scholar.in](http://www.i-scholar.in)  
Internet Source <1 %

---

26 Li-Fong Seet, Li Zhen Toh, Stephanie W. L. Chu, Sharon N. Finger, Jocelyn L. L. Chua, Tina T. Wong. "Upregulation of distinct collagen

transcripts in post-surgery scar tissue: a study of conjunctival fibrosis", Disease Models & Mechanisms, 2017

Publication

---

27

Rohit Shetty, Nimisha Rajiv Kumar, Murali Subramani, Lekshmi Krishna et al. "Safety and efficacy of combination of suberoylamilide hydroxyamic acid and mitomycin C in reducing pro-fibrotic changes in human corneal epithelial cells", Scientific Reports, 2021

Publication

---

<1 %

28

Stephana Carelli, Mattia Colli, Valeriano Vinci, Fabio Caviggioli, Marco Klinger, Alfredo Gorio. "Mechanical Activation of Adipose Tissue and Derived Mesenchymal Stem Cells: Novel Anti-Inflammatory Properties", International Journal of Molecular Sciences, 2018

Publication

---

<1 %

29

Ying-Wei Lan, Si-Min Theng, Tsung-Teng Huang, Kong-Bung Choo, Chuan-Mu Chen, Han-Pin Kuo, Kowit-Yu Chong. "Oncostatin M-Preconditioned Mesenchymal Stem Cells Alleviate Bleomycin-Induced Pulmonary Fibrosis Through Paracrine Effects of the Hepatocyte Growth Factor", STEM CELLS Translational Medicine, 2017

Publication

---

<1 %

- |    |  |      |
|----|--|------|
| 30 | <a href="https://escholarship.org">escholarship.org</a><br>Internet Source   | <1 % |
| 31 | <a href="https://hdl.handle.net">hdl.handle.net</a><br>Internet Source   | <1 % |
| 32 | <a href="http://www.jpatholtm.org">www.jpatholtm.org</a><br>Internet Source  | <1 % |
| 33 | Claudia Caroline Bosio Meneses. "Papel de Endocanabinoides na modulação de células-tronco de Papila Apical in vitro", Universidade de Sao Paulo, Agencia USP de Gestao da Informacao Academica (AGUIA), 2020<br>Publication  | <1 % |
| 34 | Guru-Dutt Sharma, Jiucheng He, Haydee E.P. Bazan. "p38 and ERK1/2 Coordinate Cellular Migration and Proliferation in Epithelial Wound Healing", Journal of Biological Chemistry, 2003<br>Publication   | <1 % |
| 35 | Lourdes Cortes-Dericks, Laurene Froment, Gregor Kocher, Ralph A. Schmid. "Human lung-derived mesenchymal stem cell-conditioned medium exerts in vitro antitumor effects in malignant pleural mesothelioma cell lines", Stem Cell Research & Therapy, 2016<br>Publication | <1 % |
| 36 | Yohanes Widodo Wirohadidjojo, Arief Budiyanto, Hardyanto Soebono. "Platelet-Rich   | <1 % |



# Fibrin Lysate Can Ameliorate Dysfunction of Chronically UVA-Irradiated Human Dermal Fibroblasts", Yonsei Medical Journal, 2016

Publication

37

[journals.lww.com](http://journals.lww.com)

Internet Source

<1 %

38

[www.hindawi.com](http://www.hindawi.com)

Internet Source

<1 %

39

Joni H. Ylöstalo, Thomas J. Bartosh, Katie Coble, Darwin J. Prockop. "Human Mesenchymal Stem/Stromal Cells Cultured as Spheroids are Self-activated to Produce Prostaglandin E2 that Directs Stimulated Macrophages into an Anti-inflammatory Phenotype", STEM CELLS, 2012

Publication

<1 %

40

Airan Liu, Xiwen Zhang, Hongli He, Li Zhou, Yoshifumi Naito, Shinji Sugita, Jae-Woo Lee. "Therapeutic potential of mesenchymal stem/stromal cell-derived secretome and vesicles for lung injury and disease", Expert Opinion on Biological Therapy, 2019

Publication

<1 %

Exclude quotes  On

Exclude matches  Off

Exclude bibliography  On

# Antifibrotic Effects of Limbal Mesenchymal Stem Cells-Conditioned Media

---

GRADEMARK REPORT

---

FINAL GRADE

**/100**

GENERAL COMMENTS

**Instructor**

---

PAGE 1

---

PAGE 2

---

PAGE 3

---

PAGE 4

---

PAGE 5

---

PAGE 6

---

PAGE 7

---

PAGE 8

---

PAGE 9

---