

SUMMARY

Effect of Fibrin Glue and Conditioned Media of Limbal Mesenchymal Stem Cells On The Expression of Fibrotic Factors Transforming Growth Factor- β dan α -Smooth Muscle Actin In Human Tenon's Fibroblasts As Wound Healing Model After Trabeculectomy In Vitro

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Glaucoma is one of leading causes of blindness in the world. People with glaucoma are increasing in number and estimated become 76 millions in 2020. Trabeculectomy is one of treatment modalities to decrease intraocular pressure. But the results are not satisfied yet and fibrosis after trabeculectomy still become the main causes of failure. Fibrosis begins with the expression of fibrotic factors, such as Transforming Growth Factor- β (TGF- β) that induces fibroblasts transdifferentiation into myofibroblasts, the main cells that produce extracellular matrix in fibrosis. Adjuvant therapy has been used to increasing the possibility of successful trabeculectomy. One of the well-known adjuvant is Mitomycin C. Although it can successfully increasing trabeculectomy result, MMC causes severe complications, such as severe hipotonia, bleb leakage, and endophthalmitis. Because of that, scientists still researching other choices of adjuvant therapy in trabeculectomy. Fibrin glue and limbal mesenchymal stem cell conditioned media are known to have antifibrotic effects. But until now FG and LMSCs-CM effects on TGF- β and α -SMA expression in human tenon fibroblasts are still unknown.

This study aimed to investigate the capability of fibrin glue (FG) and limbal mesenchymal cell's conditioned media (LMSCs-CM) in reducing profibrotic factors (TGF- β and α -SMA) expression in human tenon fibroblasts (HTFs) of glaucomatous eye. This study use Human Tenon Fibroblasts (HTFs), as key effector of bleb fibrosis after trabeculectomy, that were isolated from tenon's tissue in eye with glaucoma. HTFs were cultured and divided into 4 groups consist of FBS 2% control group, MMC group, FG group, and LMSCs-CM group. After 7 days, TGF- β and α -SMA expression were assessed by immunofluorescence staining and its intensity was measured using ImageJ software. The results between groups were analyzed using Kruskal-Wallis or oneway ANOVA test followed by post-hoc test with 95% confidence ($p < 0.05$).

This study result showed that fibrin glue successfully decreases TGF- β and α -SMA expressions as key role in fibrosis with mean expression 93.68 ± 3.96 pixels for TGF- β and 142.97 ± 11.03 pixels for α -SMA. LMSCs-CM also successfully decreases TGF- β (95.77 ± 8.27 pixels) and α -SMA (143.44 ± 15.84 pixels) expressions. These are considered significantly lower than control group ($p < 0.05$). MMC showed the lowest expression of TGF- β (77.13 ± 15.79 pixels, $p < 0.05$) dan α -SMA (102.63 ± 6.62 pixels, $p < 0.05$) among groups. This results showed that FG and LMSCs-CM has a role in fibrosis inhibition since its early beginning phase by decreasing profibrotic factors. However, MMC has the best antifibrotic effect among groups. FG and LMSCs-CM may have a potential effect as antifibrotic agent in trabeculectomy.

ABSTRACT**ANTIFIBROSIS EFFECT OF FIBRIN GLUE AND LIMBAL MESENCHYMAL STEM CELL CONDITIONED MEDIA ON TGF- β AND α -SMA EXPRESSION IN HUMAN TENON FIBROBLAST AS WOUND HEALING MODEL AFTER TRABECULECTOMY IN VITRO**

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Purpose: To investigate the capability of fibrin glue (FG) and limbal mesenchymal cell's conditioned media (LMSCs-CM) in reducing profibrotic factors (TGF- β and α -SMA) expression in human tenon fibroblasts (HTFs) of glaucomatous eye.

Material and Methods : This study using Human Tenon Fibroblasts (HTFs), as key effector of bleb fibrosis after trabeculectomy, that were isolated from tenon's tissue in eye with glaucoma. HTFs were cultured and divided into 4 groups consist of FBS 2% control group, MMC group, FG group, and LMSCs-CM group. After 7 days, TGF- β and α -SMA expression were assessed by immunofluorescence staining and its intensity was measured using ImageJ software. The results between groups were analyzed using Kruskal-Wallis or oneway ANOVA test followed by post-hoc test with 95% confidence ($p < 0.05$).

Result and Discussion: FG successfully decreases TGF- β and α -SMA expressions as key role in fibrosis with mean expression 93.68 ± 3.96 pixels for TGF- β and 142.97 ± 11.03 pixels for α -SMA. LMSCs-CM also successfully decreases TGF- β (95.77 ± 8.27 pixels) and α -SMA (143.44 ± 15.84 pixels) expressions. These are considered significantly lower than control group ($p < 0.05$). This results showed that FG and LMSCs-CM has a role in fibrosis inhibition since its early beginning phase by decreasing profibrotic factors. However, MMC has the best antifibrotic effect among groups.

Conclusion : FG and LMSCs-CM has a role as antifibrotic agent in HTFs by reducing profibrotic factor.

Keywords: fibrin glue, limbal mesenchymal stem cell conditioned media, human tenon fibroblast, fibrosis, mitomycin C.