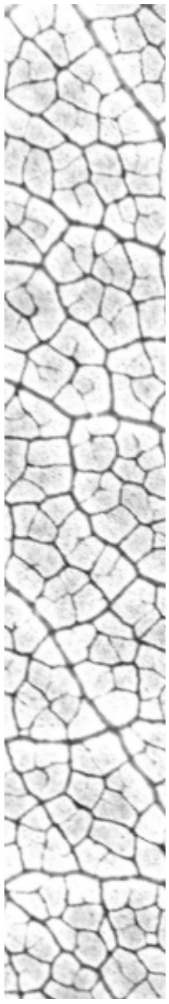

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Effect of Mitomycin C on human tenon fibroblast proliferation in contracted socket tissue

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Abstract
Background: This study aimed to evaluate the effects of mitomycin C on human tenon fibroblast proliferation in contracted socket tissue.
Material and Methods: Human tenon fibroblasts from a patient who underwent socket reconstruction surgery were cultured in vitro. Cell cultures were exposed to 0.2, 0.4, and 1 mg/ml of mitomycin C solution for 5 min, and serum-free culture medium was used as a control. Proliferation was observed with the MTT cell proliferation assay 24 h after treatment.
Results: A 5 min exposure to mitomycin C at 0.2, 0.4, and 1 mg/ml caused inhibition of fibroblast proliferation ($p < 0.05$). The dose of mitomycin C was not significantly related to the rate of fibroblast proliferation inhibition. Mitomycin C 0.4 mg/ml caused the greatest inhibition of fibroblast proliferation compared with doses of 0.2 and 1 mg/ml.
Conclusion: Mitomycin C can inhibit tenon fibroblast proliferation of contracted socket tissue. Dose escalation of mitomycin C is not related to the rate of fibroblast proliferation inhibition.

Keywords: contracted socket, mitomycin c, tenon fibroblast proliferation

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BACKGROUND

Anophthalmos sockets that are unable to resist a prosthesis are called contracted sockets. Of 1739 anophthalmos sockets, 7.7% (137) were found to be contracted sockets (Adhikari et al. 2007, Jordan and Klapper 2012). Contracted sockets have a characteristic loss of fornix with the formation of scar tissue and conjunctival granulation tissue. Scar tissue formation mainly occurs due to the wound healing process, which is influenced by the proliferation of fibroblasts, collagen biosynthesis, and other extracellular materials (Grierson et al. 1988). Many antiproliferative agents have been applied to suppress of fibroblast cellular function, such as mitomycin C (MMC). MMC is an antineoplastic drug that can cause cross-linking of DNA, inhibiting RNA and protein synthesis. Decreased fibroblast proliferation is thought to be due to the inhibitory effect of MMC on DNA replication in the G1 phase and the resulting cell death (Singhs et al. 2013). In this study we determinated the effect of MMC on the proliferation of fibroblast cells from socket tissue contractures related to the prevention of excessive scarring.

MATERIALS AND METHODS

This was an experimental in vitro study conducted at the Faculty of Medicine Laboratory of Universitas Brawijaya Malang in April 2015. Fibroblast cell cultures were collected from patients with contracted sockets through surgical socket reconstruction at RSSA Malang. Cell cultures were divided into the following four experimental groups.

I Control group: no exposure
II Treatment group 1: exposure to MMC 0.2 mg/ml diluted with phosphate buffered saline (PBS) for 5 min
III Treatment group 2: exposure to MMC 0.4 mg/ml (diluted with PBS) for 5 min
IV Treatment group 3: exposure to MMC 1 mg/ml (diluted with PBS) for 5 min

Culture of Fibroblasts from Tenon Tissue

Tenon tissue that met the inclusion criteria was obtained from patients with permission from the patient or a relative. The tenon tissue was stored in a sterile Eppendorf containing HBSS solution at 50 °C for less than 3 h after excision.Tenon tissue was washed with HBSS containing penicillin (200 µg/ml), streptomycin (100 µg/ml), neomycin sulfate (100 µg/ml), and amphotericin B (5 µg/ml) (PNSA) then placed in a 60-mm culture plate. Tenon tissue was culture in Dulbecco's Modified Eagle's Medium (DMEM) containing 15% fetal bovine serum (FBS) and PSNA for

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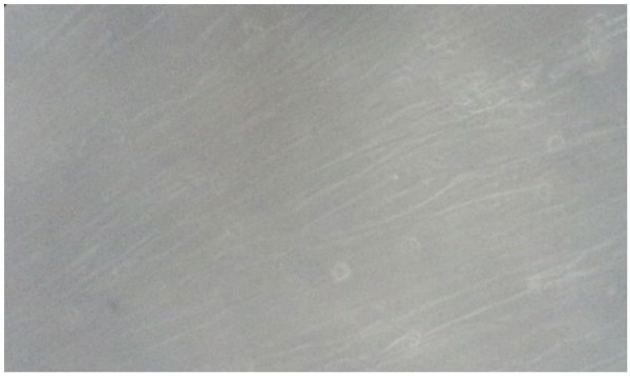

Fig. 1. Cell culture before treatment (confluence)

24 h, 37 °C 5% CO_2. Subculture was performed 3 times before treatment in cell culture.

Fibroblasts Experiments with Mitomycin C

Cells were cultured in 24-well plates at a density of 3–5 x 10^3 cells per well and incubated in 1 ml DMEM with 15% FBS at 37 °C and 5% CO_2 for 24 h. The culture medium was removed. To each well was added 1 ml of MMC at 0, 0.2, 0.4, or 1 mg/ml and incubated at 37 °C and 5% CO_2 for 24 h.

MTT Pro Cell Proliferation Assay Procedure

After the incubation period was complete, 100 µl MTT 0.5 mg/ml was added to each well. Cells were incubated for 2–4 h until purple formazan crystals appeared. Once formazan was clearly formed, 10% SDS in 0.1 NHCL was added to stop the reaction. The plate was wrapped in paper or aluminum foil and incubated in the dark (at room temperature) overnight. The plate was homogenized (shaken) for 10 min to dissolve the formazan. Absorbance was read using an ELISA plate reader at 550–600 nm (595 nm). Living cells appeared purple, and dead cells appeared appeared yellow.

RESULTS

Fig. 1 illustrates a culture of confluenced fibroblast cells showing the spindle shape, the oval nuclei, and the branched cytoplasm. After treatment with Mytomycin C and incubation overnight, fibroblasts appeared as presented in **Fig. 2**.

Table 1 shows the MTT assay results for the control and treated groups of fibroblast cells after treatment. The mean percentage of proliferation of fibroblasts in the control and treatment groups is presented in **Table 2**.

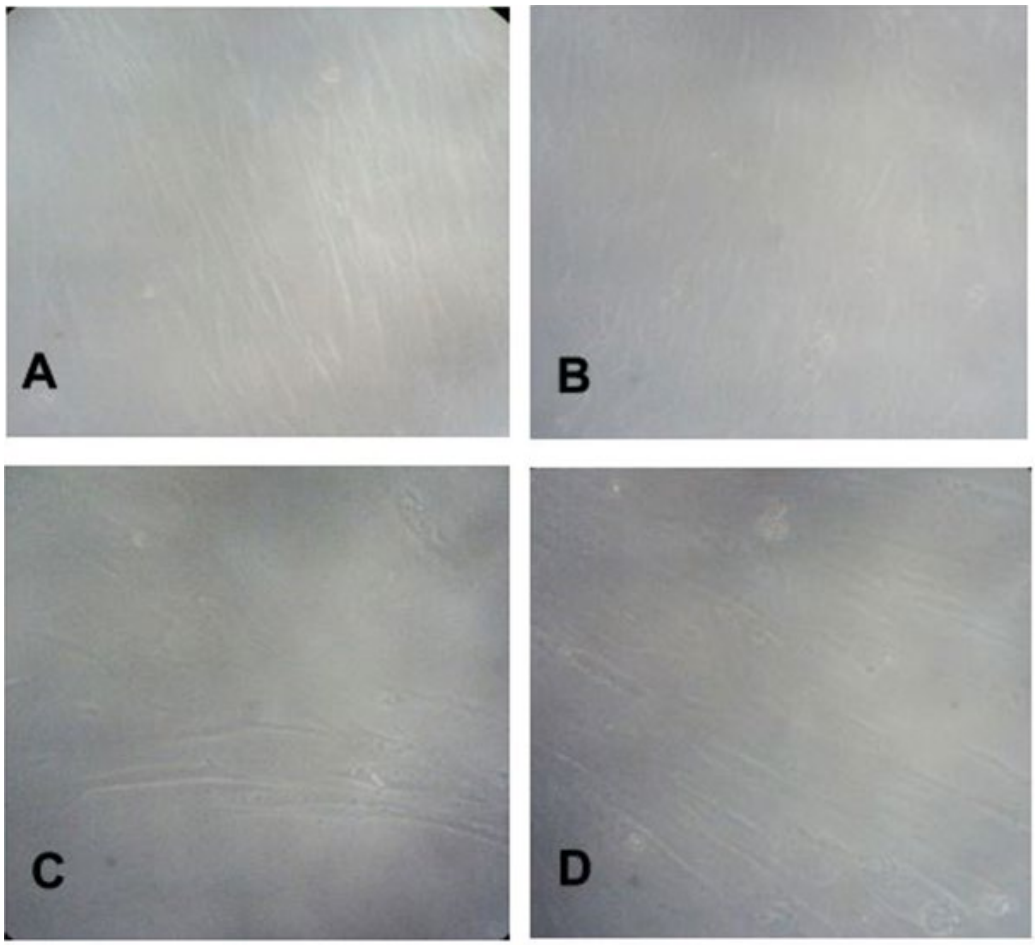

Fig. 2. Cell proliferation in the Control Group (a), Group I (b), Group II (c), and Group III (d)

Table 1. Absorbance results in the MTT assay for control and fibroblast culture cells after treatment

	Control media	Cell control	MMC 0.2 mg/ml	MMC 0.4 mg/ml	MMC 1 mg/ml
Average	0.420	0.559	0.524	0.493	0.512

Table 2. Percent proliferation of fibroblasts in control and treated groups

Treatment	N	Mean percentage	Standard deviation
Control (Group I)	6	99.88	12.36
0.2 mg (Group II)	6	74.82	17.08
0.4 mg (Group III)	6	52.39	29.82
1 mg (Group IV)	6	65.83	26.09

Based on ANOVA and Tukey's post-test, there was a significant difference in the average percent fibroblast proliferation between the Control Group and Group II ($p = 0.008$). However, there was no significant difference between the Control Group and Group I, neither between Control Group and Group III. Based on Pearson's correlation test, there was no significant correlation between the Mytomycin C dose and percent fibroblast proliferation ($\alpha = 0.05$).

DISCUSSION

Fibroblast proliferation in Group III was higher than that in Group II. Bailey (2007) and Gupta (2011) reported that replication of MMC application 1 mg/ml 3 weeks after the first application for 5 min prevented a keloid recurrence (Bailey et al. 2007, Gupta and Narang 2011). Li et al. (2014) reported that MMC application at doses of 0.2, 0.5, and 1 mg/ml for 2 min on vocal fold fibroblasts decreased fibroblast cell viability significantly on the third and fifth days. The dose-response effects were statistically insignificant in the MMC dose range tested in this study.

Velpandian (2008) mentioned that several factors influence the antifibroblast effect of MMC, such as the concentration used, the area of application, the duration of the application, and the material on which it is applied. The optimal concentration, duration, area, and number of applications are so far unknown. In this study, among the three doses studied, the dose of 0.4 mg/ml had the highest anti-proliferative effect on fibroblast cells of the contracted socket tenon.

Based on Pearson's test, it can be concluded that there was no significant correlation between the dose of MMC and the decrease in fibroblast proliferation ($p > 0.05$). The highest dose in this study (1 mg/ml) did not obtain the highest antiproliferative effect. Chen (2013) mentioned that the efficacy and effective concentration of MMC are clasically debated. The ideal dose is unknown, and there is no literature mentioning the optimal concentration of topical MMC.

In this study, MMC 0.4 mg/ml was the dose with the largest anti-proliferative effect. However, this dose can not be claimed as optimal, since there was no statistically significant result. It is known that the wound healing process is a dynamic and complex process that is influenced by various cellular and humoral reactions. In fibroblasts, the integrity of the structure and function of epithelial and cellular components is also important in the wound healing process (Takahashi et al. 1998). The application of MMC at 1 mg/ml was tested in previous clinical studies where many factors and wound healing phases were involved in the wound healing process in addition to fibroblast proliferation (Grierson et al. 1988). In contrast, this study was an in vitro study where the observations focused solely on the proliferation of fibroblast cells.

CONCLUSION

Mitomycin C can decrease the proliferation of fibroblast cells cultured from the contracted socket. There was no statistical correlation between increased dosage of MMC and decreased fibroblast cell proliferation in contracted socket tissue culture.

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