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Fibrin glue as a novel therapy for contracted socket in comparison to mitomycin-c and triamcinolone acetonide at Dr. Soetomo General Academic Hospital, Surabaya, Indonesia



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ABSTRACT

Background: Contracted socket is still a major problem for patients with anophthalmia. The main factor for this condition is inflammation and fibrosis, which result in conjunctival shortening. Myofibroblasts that express α -SMA are the primary mediators of anophthalmic socket contraction.

Methods: One healthy eye of a New Zealand white rabbit were randomly selected for evisceration and divided into four treatment groups, each with five rabbits. Each rabbit in the group received a single subconjunctival injection of a different agent. Group I was the control group that received no injection, Group II received MMC 0.4 mg/ml, Group III received TCA 40 mg/ml, and Group 4 received fibrin glue. After 14 days, the animals were euthanized, and conjunctival samples were submitted for histopathological analysis. A monoclonal α -SMA antibody was applied, and pathologists provided the IRS score for each sample. The differences in α -SMA expression were statistically analyzed with a significant level of $p < 0.05$.

Result: All groups displayed a statistically significant decrease in α -SMA expression compared to the control group ($p < 0.05$) as sorting as MMC group with $p = 0.007$, TCA group with $p = 0.007$, and fibrin glue with $p = 0.009$. MMC was the most effective at reducing α -SMA ($p = 0.004$). Surprisingly, there were no statistically significant differences between MMC, TCA, and fibrin glue when they were evaluated independently.

Conclusion: A single subconjunctival injection of fibrin glue may be used as a novel treatment to prevent socket contracture in actively healing sockets.

Keywords: α -SMA, contracted socket, fibrin glue, fibrosis, MMC, TCA.

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INTRODUCTION

Anophthalmia is the absence of the eyeball and its surrounding tissue from the orbital cavity.^{1,2} The majority of cases are caused by destructive eye surgery, such as evisceration.² Inadequate wound healing (following evisceration) might result in conjunctival and subconjunctival scarring that leads to socket contractures.³ Contracted socket is still a major problem for anophthalmic patients because the prosthetic eye cannot be properly attached, resulting in cosmetic eyelid issues such as entropion, ectropion, infection, and ptosis.⁴ A prospective study showed that contracted sockets occurred in 7.7% of 1739 anophthalmic sockets⁵ and currently, there is no specific treatment

available to prevent socket contractures.

Myofibroblasts are uniquely differentiated fibroblasts and become an essential component in forming scar tissue. Persisting myofibroblasts were found in a contracted socket, and they express alpha-smooth muscle actin (α -SMA).⁶ Mitomycin-c (MMC) is commonly used for reducing the degree of postoperative scarring by suppressing fibroblast proliferation. However, MMC's efficacy is very dose-dependent, and it has side effects such as chemosis, inhibition of conjunctival healing, conjunctival granulomas, and thinning of the sclera leading to scleral melting.^{7,8} Triamcinolone acetonide (TCA) is a glucocorticoid agent which acts to inhibit fibroblast proliferation by preventing migration

and activation of inflammatory cells and myofibroblasts.^{9,10} Subconjunctival injection of TCA has potential side effects such as delayed wound healing.¹⁰

Fibrin glue (FG) is another treatment to reduce scar tissue formation by inhibiting inflammation, reducing excessive inflammatory reactions, reducing fibroblast proliferation and differentiation, and reducing myofibroblast cellular responses to deposited collagen.^{11,12} FG provides the advantages of the fact that it is biocompatible, biodegradable, non-toxic on the surface of the eyeball, simple to produce, and can be isolated autologously.¹³⁻¹⁵ FG has been widely used in the practice of ophthalmological surgery.¹⁶ However, there is still no scientific literature that mentions the

efficacy of FG in anophthalmic surgery or socket surgery. The aim of this study was to determine the fibrin glue as a novel therapy for contracted sockets in comparison to mitomycin-c and triamcinolone acetonide at Dr. Soetomo General Academic Hospital, Surabaya, Indonesia.

METHODS

Fibrin Glue Preparation

A sterile syringe with CPDA in a 9:1 ratio was used to puncture the rabbit's ear vein, and 40 ml of peripheral blood total was obtained. In order to concentrate the plasma, the blood was placed in a sterile 10 mL centrifuge tube and centrifuged for 15 minutes at 3000 rpm. The plasma portion was stored in a sterile centrifuge tube at -20°C for 24 hours. Then the second centrifugation was performed at 4°C, 3000 rpm for 15 minutes. After the second centrifugation, the upper 2/3 of the plasma was stored for as much as 10 ml to prepare the fibrinogen component. The lower 1/3 (PRP) was stored in sterile micro-tubes to be prepared as a material for thrombin production. The upper 2/3 of the plasma was added with 95% ethanol, as much as 1 ml and then incubated at 4°C for 30 minutes. Then third centrifugation was carried out at 4°C at 3000 rpm for 15 minutes. The sediment was used as a fibrinogen component, while the supernatant was discarded. To create thrombin, 0.05ml of 10% CaCl₂ was added to the PRP component. FG was made by mixing fibrinogen and thrombin in a ratio of 1:1.^{17,18}

Study design and subject recruitment

This true experimental study was done in February-July 2022 at the Universitas Airlangga Faculty of Veterinary Medicine. A healthy New Zealand white rabbit (*Oryctolagus cuniculus*) with a body weight of 2.5–3.5 kg and an age range of 4–6 months served as the sample. In this study, there were four treatment groups, each with five samples, for a total of 20 eyes from 20 rabbits. Animals diagnosed by a veterinarian as having a disease or the potential to transmit disease were excluded from this study. The dropout criteria are when the rabbits become ill, die, and have complications such as scleral perforation, infection, and bleeding during and after

surgery.

One eye of each rabbit was selected randomly. A combination of 5 mg/kg xylazine (Xylazine 20, Pantex, Holland) and 30 mg/kg to 50 mg/kg ketamine-HCL (Keta-A-100, Agrovvet Market S.A., Peru) was used to anesthetize the animals; booster doses were administered as needed. Evisceration surgery was carried out in a sterile condition. The procedure involved a 360° peritomy, excision of the corneal button, scooping out the intraocular tissues, and suturing the sclera and the conjunctiva using 6.0 polyglactin (Vicryl™, Ethicon, US). The sort of injection administered was not masked by the operating surgeon. Each group of four rabbits underwent a single subconjunctival injection of a different substance. The injection was performed at the mid-superior bulbar conjunctiva close to the vertical meridian at the inferior fornix. Group I did not receive injection as a control group, Group II received 0.1 ml of mitomycin-C (Mito*10, NEON, India) 0.4 mg/ml, Group III received 0.1 ml of Triamcinolone Acetonide (Flamicort, DexaMedica, Indonesia) 40 mg/ml, and Group 4 received 0.1ml of fibrin. Chloramphenicol eye ointment (Erlamycetin, Erela, Indonesia) was applied three times daily, and Tylenol solution was mixed with their drinking water to alleviate the pain. On day 14, after surgery, all the rabbits were euthanized. Conjunctiva and Tenon capsule samples were carefully harvested and cut uniformly approximately 10mmx10mm as near as feasible to the original site injection. The samples were carefully transferred to a vial containing freshly made fixative solution for further processing after being placed epithelial side up on a thin sheet of cardboard (surgical suture packaging) to flatten it and prevent wrinkling. The study pathologist was present during sample collection, rapidly processing the samples. H&E staining was done on each serial section sample.⁶

Immunohistochemistry

Conjunctival samples that had been formalin-fixed and paraffin-embedded were cut into four-micrometer slices. Alpha Smooth Muscle Actin antibody [1A4] (FITC) (GeneTex, Inc., North

America) was prediluted to 1:1000 dilution² for immunohistochemical staining, and a Leica Bond Max - Fully Automated IHC/ ISH Staining equipment was used. The expression of α-SMA was located in the cytoplasm.

Quantification of cell

α-SMA+ cells were stained as light brownish to chocolate brown under a light microscope. A semi-quantitative method using an immunoreactivity score (IRS) was used to analyze the α-SMA+ cells. IRS is a product of multiplication between positive cells proportion score and staining intensity score. The intensity of the immunoreactive products was scored under a light microscope as follows: no expression= 0; minimal intensity= 1; moderate reaction= 2; and strong reaction= 3. The positive ratio was scored as follows: no positive cells= 0; positive cells <10%= 1; positive cells 11–50%= 2; positive cells 51–80%= 3; positive cells >80%= 4.¹⁹

The pathologist conducted this at ×400 magnification. There is only one pathologist to analyze the α-SMA+ cells. In order to reduce bias, this technique was carried out by a pathologist on serially numbered slides in a blinded manner using an Olympus microscope (Cx51) equipped with an Olympus camera using SIS software (Japan, Tokyo).

Statistical Analysis

The pathologist provided the data in an Excel spreadsheet (Excel 2010, Microsoft Corporation, Redmond, WA, United States), and statistical analysis was carried out using SPSS software version 26 for Windows (IBM Corporation, New York, NY, U.S.A.). Statistical comparison was performed using Kruskal-Wallis test and Mann-Whitney test.

RESULT

Postoperative clinical evaluations in all groups revealed a deep fornix, both superior and inferior. A qualitative analysis of the H&E sections showed that the conjunctival epithelium was intact in all groups. In the control groups, there was an incredibly dense inflammatory cell, but there was a decrease in other groups, particularly in the MMC group. The

Table 1. IRS score of α -SMA between group.

Group	Total (N=20)	IRS Score of α -SMA					p Kruskal-Wallis
		Mean	Median	IQD	Minimum	Maximum	
Control*	5	11.4	12	2	9	12	0.005
MMC*	5	3.6	4	3	2	6	
TCA	5	4.6	4	3	3	6	
FG	5	5.8	6	4	4	9	

*The same superscript letter in one column indicates that the statistics are the most significantly different using Post-Hoc Dun

Table 2. IRS score of α -SMA between injected group.

Group		p Mann-Whitney
MMC	TCA	0.324
MMC	FG	0.100
TCA	FG	0.316

detection of α -SMA expression was done by immunohistochemical staining, and the IRS score was employed as a method for quantification.

The control group had the highest median myofibroblast count (12), followed by FG (6), TCA (4), and MMC, with the lowest median value of 4. All groups displayed a statistically significant decrease in α -SMA expression compared to the control group ($p < 0.05$) as sorting as MMC group with $p = 0.007$, TCA group with $p = 0.007$,⁵ and FG with $p = 0.009$. Surprisingly, there were no statistically significant differences between MMC, TCA, and FG when they were evaluated independently ($p > 0.005$) (Tables 1 and 2).

DISCUSSION

The contracted socket is still a major problem for anophthalmic patients because it causes cosmetic eyelid issues and psychological problems in the patient.⁴ Mostly, it needs more than one surgery to fix the socket, so oculoplastic surgeons have continuously investigated ways to improve the conjunctival wound healing process. In this study, the Inferior conjunctival fornix was chosen as the site for injection because fornix shortening first occurred in the inferior area. This is related to the inflammatory process caused by friction of the eye prosthesis, which is mainly supported by inferior fornix.²⁰

All groups displayed a statistically significant decrease in α -SMA expression compared to the control group, and MMC had the lowest score of α -SMA. This result was in line with Tawfik *et al.*, who revealed

that a single injection of MMC 0.4mg/ml got the lowest count of myofibroblast compared to 5-FU, TCA, and Bevacizumab. The same dose of MMC was also used to reduce the TGF- β and α -SMA expression in human tenon fibroblast of glaucomatous patient²¹ and suppresses the rate of fibroblast migration.¹⁸ As in this study, a single subconjunctival injection of 40mg/ml of TCA has proven to reduce the myofibroblast count.⁶ TCA can also be adjuvant therapy for patients with non-inflammatory glaucoma. Sub tenon injection in the bleb area after trabeculectomy can improve the final outcome of trabeculectomy surgery.²² The administration of TCA can reduce inflammation, hence decreasing collagen and glycosaminoglycan synthesis, fibroblast proliferation, and increasing hypoxia.²³ Sakarya *et al.* hypothesized that the application of fibrin glue to the subconjunctival tenon area could reduce the formation of post-trabeculectomy scar tissue.¹³ Komaratih *et al.* reported that the application of subconjunctival fibrin glue after trabeculectomy in rabbits was able to reduce the area of bleb fibrosis by modulating the remodeling phase.¹⁴ Self-made FG in this study successfully suppresses α SMA expression. As mentioned in other studies, FG added to Tenon's fibroblast cell culture will express lower TGF- β and -SMA.²¹

Fibroblasts have a crucial role in wound healing prior to socket contractures.⁶ The profibrotic cytokine TGF- β will stimulate fibroblasts to differentiate into myofibroblasts. The differentiation is complete only when α -SMA is acquired.

Thus α -SMA is the reliable marker for myofibroblast. Myofibroblasts are essential to tissue repair because they provide extracellular matrix material and mechanical strength for wound closure.^{6,24-25} MMC, TCA, and FG are agents that can modify the wound healing process to reduce scar tissue formation. MMC affects almost all profibrotic processes in conjunctival fibroblasts, as indicated by decreased fibroblast proliferation and collagen secretion, and induces apoptosis through a signaling-dependent mechanism of c-Jun N-terminal kinase 1 (JNK1).²⁶ Administration of TCA is used to prevent fibrosis by slowing the initial phase of wound healing and decreasing the production of proinflammatory cytokines (VEGF, TGF- β , and IL-1).²³ FG is a biological tissue adhesive that mimics the final stages of the coagulation cascade by activating human fibrinogen with thrombin. FG releases a variety of growth factors IGF-1, HGF, and HGF- that play important in the proliferation phase by activating tenon fibroblast proliferation and migration. FG provides the advantages of the fact that it is biocompatible, biodegradable, and non-toxic. The biodegradability takes 14-21 days from subconjunctival bleb space.¹³⁻¹⁵

Currently, commercial fibrin glue preparations are seemed to be used more frequently. Moreover, fibrin glue can be prepared at a blood transfusion center or from a patient's own blood. The remarkable benefit of self-made FG, compared to synthetic glues and other biomaterials, is to be made autologous with low cost and ease of preparation. Autologous FG eliminates the possible risks of foreign body reactions and viral infection. Although MMC is still superior in reducing the fibrosis process, the findings of this study suggest that FG could be used as an adjuvant or treatment to reduce the risk of socket contracture through its safety ness, promoting

better wound healing and reducing the inflammatory response. The primary limitation of this study is the relatively brief duration of the observations, which only evaluated the α -SMA expression at one time (day 14) and did not evaluate the other profibrotic marker such as TGF- β , MMP, and collagen. It is hoped that longer-term research can be conducted so that the entire wound-healing process can be completed (day 7, day 14, and day 21). Further studies must figure out which agent can maintain the fornix depth by evaluating the fornix depth preoperatively and postoperatively. Based on the findings of this study, a clinical trial of using fibrin glue in human socket surgery or anophthalmia surgery to prevent a contracted socket could be conducted.

CONCLUSION

A single dose of autologous FG, mitomycin-C, and triamcinolone acetonide can significantly reduce the number of α -SMA expressions in actively healing sockets. Most importantly, FG may be used as adjuvant therapy and a novel treatment to prevent socket contracture. However, MMC remains the best agent to inhibit fibrosis in this study.

ETHICAL CLEARANCE

Ethics approval has been received prior to the study being conducted, and also already following cope and ICJME guidelines.

CONFLICT OF INTEREST

There is no conflict of interest regarding the manuscript.

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AUTHOR CONTRIBUTIONS

All authors equally contribute to the study from the conceptual framework, data acquisition, and data analysis until reporting the study results through publication.

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