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Role of neutrophil gelatinase-associated lipocalin (NGAL) as a acute prerenal kidney injury marker: Exploring factors associated with its postoperative levels in hypotension-controlled otorhinolaryngology surgery

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The effect of ethanol extract of green meniran leaves (*Phyllanthus niruri* Linn) in a mixture of calcium hydroxide and 2% chlorhexidine digluconate on matrix metalloproteinase-9, transforming growth factor-1 levels, osteoclast, and osteoblast cells in Wist

Ni Kadek Eka Widiadnyani, I Nyoman Mantik Astawa, I Wayan Putu Sutirta Yasa, I Dewa Made Sukrama, I Made Bakta, Ida Bagus Putra Manuaba, I Wayan Suardana, I Made Sudarmaja

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High maternal homocysteine (Hcy) levels as a risk factor of preterm labor: a case-control study

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Diagnostic test of Immature Granulocyte Ratio (IG Ratio) to detect Early-Onset Neonatal Sepsis (EONS)

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Treatment outcomes of severe acute malnutrition children aged 1 month - 5 years hospitalized at Mohammad Hoesin Hospital in Palembang, Indonesia

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Visual science mapping and future direction of pediatric acupuncture: a bibliometric analysis from Scopus database and VOSviewer

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The pattern of osteocyte in dental socket bone regenerative induced by hydroxyapatite bovine tooth graft

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The effectiveness of topical hyaluronic acid on decreasing Interleukin-6 and acceleration of wound healing (Push Score) in Wagner II-III diabetic foot ulcer in Dr. Soetomo Hospital Surabaya

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The effectiveness of the self-care management model in midwife care through android application as an effort to increase the ability of pregnant mothers in early detection of pregnancy at risk during the COVID-19 pandemic

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Symptoms identification of ICD-11 based on clinical NLP mobile apps for diagnosing the disease (ICD-11)

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Analysis of the effectiveness of sphenopalatine ganglion block on fentanyl needs in endoscopic endonasal surgery as measured by qNOX score

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Effect of aromatherapy blend essential oils (lemongrass and lemon) on sleep quality in pregnant women's third trimester

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Stress due to online learning during the COVID-19 pandemic affects the menstrual cycle in college students

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Association between serum TNF- α level with the incidence of metastases in women with breast cancer in Dr. Soetomo General Hospital, Indonesia

Rachman Efendi, Husnul Ghaib, Iskandar Ali

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Antioxidant potential effect combination of the bitter, red ginger, and turmeric extract with the DPPH method

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The effect of Quran recitation on t-cell lymphocyte activity in mice model of breast cancer

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Effect of secretome of adipose stem cell (ASC) in photoaging skin

Winawati Eka Putri, Anang Endaryanto, Damayanti Tinduh, Fedik Rantam, Hari Basuki Notobroto, Cita Rosita Sigit Prakoeswa

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A study of efficacy, safety, and acceptance of two combined oral contraceptive pills containing 150 mcg Levonorgestrel and 30 mcg Ethinyl Estradiol at Dr. Kariadi Hospital, Semarang, Indonesia

Syarief Thaufik Hidayat, Hary Tjahjanto, Robert Eria, Yenny Kartika Gozali

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Transformation of the learning system in nursing education after the COVID-19 pandemic

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The Effect of Beta vulgaris L. on the Malondialdehyde Levels in Male Wistar Rats Exposed to Cigarette Smoke

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Comparison of intestinal fatty acid binding protein (I-FABP) level between pre- and post-surgery and its associated determinants in patients with microscopic otorhinolaryngology surgeries

Yuda Atmajaya, Mohammad Satriyo Wibowo, Prananda Surya Airlangga, Maulydia, Prihatma Kriswidyatomo, Muhtarum Yusuf, Budi Utomo

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Application of DNA Barcoding for authentication of Balinese traditional medicinal plant Purnajiwa (Kopsia arborea Blume. and Euchresta horsfieldii) (Lesch.) Benn

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Robusta BP-42 coffee bean extract is a new anti-tyrosinase candidate to reduce melanogenesis activity

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Acute Sarcopenia in Elderly with COVID-19: An Overlooked Problem

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Comparison of sperm parameters and fertility rate in subclinical varicocele patients treated with varicocelectomy versus no varicocelectomy: a systematic review

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Benefits and challenges of peer learning methods in health professional students: A literature review

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Clostridium difficile virulence factors as the cause of antibiotic-associated diarrhea (AAD): a literature review

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Medicinal Plants - A Promising Breakthrough in the Management of Alzheimer's Disease Progression Compared to NSAID: A Systematic Review

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RESEARCH LETTER

The role of neuron-specific enolase (NSE) and S100B protein in the incidence of acute postoperative cognitive dysfunction (POCD) in geriatric patients receiving general anesthesia

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EDITORIAL

The 1st Airlangga International Endodontic Symposium 2022

Dian Agustin Wahjuningrum

Online First: Nov 8, 2022 |

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The effect of fibrin glue on suppressing Transforming Growth Factor- β (TGF- β) expression compared to Mitomycin-C (MMC) and Triamcinolone Acetonide (TCA) as antifibrotic agents in contracted socket prevention

Nike Aswati¹, Luki Indriaswati^{1*}, Sutjipto¹, Dyah Fauziah², Budi Utomo³

ABSTRACT

Background: Post-enucleation fibrosis remains the leading cause of contracted socket. The application of fibrin glue is considered an alternative adjuvant therapy in suppressing profibrotic factor (TGF- β) expression following enucleation surgery to prevent contracted socket. This study aimed to know the role of fibrin glue in suppressing the TGF- β expression following enucleation surgery.

Methods: An in vivo experimental study with a randomized post-test-only control group using twenty New Zealand rabbits were conducted. The sample was divided into four groups, each consisting of five rabbits, and one healthy eye was selected randomly for enucleation. Each rabbit in the study group was given a subconjunctival injection of agents corresponding to each study group, namely: Group I (control [no injection]), Group II (0.1 ml MMC 0.4 mg/ml), Group III (0.1 ml TCA 40 mg/ml), and Group 4 (0.1 ml fibrin glue) at the inferior fornix. Following a 14-day observation period, the animals were euthanized, and tenon conjunctiva in inferior fornix samples was collected for immunohistochemistry (IHC) staining examination. The pathologist calculates the immunoreactive scores (IRS) for each specimen. The differences in TGF- β expression were statistically analyzed with a significant $p < 0.05$. Data were analyzed using SPSS version 26 for Windows.

Result: The highest TGF- β expression was found in the control group (I), and the lowest was in the mitomycin-C group (III), followed by the triamcinolone group (IV) and fibrin glue group (II). There were significant differences in TGF- β expression among the four groups ($p = 0.004$). The difference between fibrin glue, MMC, and triamcinolone acetonide groups was insignificant ($p > 0.05$).

Conclusion: As an alternative to mitomycin-C and triamcinolone acetonide, fibrin glue can be used as adjuvant therapy to prevent constricted sockets after enucleation.

Keywords: contracted socket, fibrin glue, fibrosis, MMC, TCA, transforming growth factor.

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INTRODUCTION

A contracted socket refers to the dysfunction of conjunctival tissue due to inflammation and fibrosis following enucleation, which leads to shallowing or obliteration of the fornix.^{1,2} Fibrosis is caused by severe surgical dissection of orbital tissue, causing significant damage to the conjunctiva and Tenon's capsule.¹ The inability to retain an ocular prosthesis owing to fornix deformity impacts the patient's cosmetic impairment and psychosocial well-being.³ Acquired

anophthalmia due to surgical procedures is significantly more prevalent, with an incidence of 2.6-2.5 per 100,000 population.⁴ A study by Adhikari RK et al. found 137 (7.7%) of the 1739 anophthalmic patients, with a ratio of 1:12, were contracted sockets. About 5.9% were obtained from acquired cases. The demographic pattern of the study shows that there are twice as many males as females. Most contracted sockets are observed in males between the ages of 15 and 25, which is the active period of life, indicating that men are more susceptible

to trauma.⁵ The main factor determining the outcome of reconstructive socket surgery is the prevention of postoperative conjunctival fibrosis.⁶ Management of contracted sockets is quite challenging due to repeated contraction of the socket, despite meticulous surgery.^{1,2,6}

The transforming growth factor (TGF- β) is one of the growth factors produced by macrophages during the injury process. Overexpression of TGF- β is a major cause of extensive postoperative fibrosis. TGF- β induces the trans-differentiation of fibroblasts

into myofibroblasts and increases the expression of extracellular matrix proteins. TGF- β affects extracellular matrix degradation and deposition by increasing matrix protein synthesis and altering the balance between matrix deposition and degradation signals. TGF- β is the main profibrotic cytokine that regulates fibrogenesis, so modulating the expression of TGF- β which is one of the most potential targets in cicatricial tissue intervention; can prevent fibrosis.⁷⁻⁹

The effectiveness of mitomycin-C (MMC) as an antifibrotic agent has been investigated in a variety of research conducted in vivo and in vitro studies using both human and animal subjects. It has been proven that MMC decreases fibrosis.¹⁰ MMC is commonly used as an adjuvant therapy in contracted socket surgery, but it induces massive cell death, resulting in surgical failure.¹ Triamcinolone acetonide (TCA) is a moderately potent, long-acting synthetic corticosteroid with anti-inflammatory, anti-permeability, and antifibrotic effects by modulating fibroblast activity.¹¹ Using intraoperative TCA as an antifibrotic drug may result in delayed wound healing. Therefore, safer adjuvant therapies are still being developed. Autologous fibrin glue is an adhesive biomaterial, a mixture of fibrinogen and thrombin derived from blood plasma as its primary components. Fibrin glue is designed to mimic the final phase of the hemostasis cascade.¹² According to Abdurrauf M et al.¹³ and Taqryanka SD et al.¹⁴, fibrin glue is effective as an antifibrotic agent, as evidenced by decreased fibroblast proliferation in conjunctival human tenon fibroblasts. This finding demonstrates that fibrin glue is efficient as an antifibrotic agent. The application of biomaterial fibrin glue as adjuvant therapy for contracted socket prevention has not been studied.

Therefore, according to the explanation above, this study aimed to evaluate the role of fibrin glue in suppressing the TGF- β expression following evisceration surgery.

METHODS

Study design and sample collection

We conducted an in vivo experimental study with a randomized post-test-only control group design in New Zealand rabbits following evisceration. The research was conducted at the Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya. The inclusion criteria were adult New Zealand white rabbits aged 4 - 6 months, male, weighing 2.5 - 3.5 kg in healthy condition. Exclusion criteria are animals declared by a veterinarian to have a disease or have the potential to transmit the disease during evaluation. Drop-out criteria were rabbits that became sick, died, or were infected during and after surgery. Twenty rabbits met the inclusion criteria and were subjected to an acclimatization process for one week; then, one eye of each rabbit was selected randomly. The subjects were allocated into four different groups using a simple random sampling method (n=5), namely: control, fibrin glue, mitomycin-C (MMC), and triamcinolone acetonide (TCA) groups, respectively.

A portable slit lamp was used to examine the anterior segment. One eye from each rabbit was randomly selected, after which the evisceration procedure was performed under sterile conditions. Topical anesthesia was administered with 2% tetracaine hydrochloride (Pantocaine®) eye drops in all eyes. Animals were

anesthetized intramuscularly using a combination of 5 mg/kg xylazine (Xylazine 20, Pantex, Holland) and 30 mg/kg to 50 mg/kg ketamine-HCL (Keta-A-100, Agroviet Market S.A., Peru). The evisceration procedure was performed under sterile conditions. A 360° conjunctival peritomy was performed, followed by excision of the corneal button, then removing the contents of the eyeball with an evisceration spoon. The scleral and conjunctival wounds were sutured using Vicryl 6.0 (Vicryl™, Ethicon, USA).

Each rabbit within the study group received a single subconjunctival injection of a predetermined agent (Table 1) at the exact location in the inferior fornix close to the vertical meridian in the mid-inferior bulbar conjunctiva. Postoperatively, the subject was given a solution of Tylenol mixed with drinking water to relieve the pain. The eye ointment Chloramphenicol (Erlamycetin, Erela, Indonesia) was administered three times daily. The animals were euthanized on the 14th day. The conjunctival and Tenon tissue was harvested 10mm x 10mm in diameter in the area as close as possible to the injection site in the mid-inferior fornix. The samples were immediately placed epithelial side-up on a thin sheet of cardboard (surgical suture packing) to avoid wrinkling. They were then transferred gently to a vial containing freshly produced fixative solution for subsequent processing. The pathologist assisted with sample collection, and the collected samples were immediately processed. Histopathological examination was performed using Hematoxylin Eosin staining.

Table 1. Subconjunctival injection agents used in each study group.

Group I: Control group with no injection given.
Group II: 0.1 ml of fibrin glue (FG) injection
Group III: 0.1 ml of mitomycin-C 0.4 mg/ml (MMC) (Mito*10, NEON, India) injection
Group IV: 0.1 ml of triamcinolone 40 mg/ml (TCA) (Flamicort, DexaMedica, Indonesia) injection

Table 2. The median values of the TGF- β expression in the study groups.

Groups	Total (N=20)	IRS Score of TGF- β expression				p
		Median	IQD	Minimum	Maximum	
Control	5	12 ^a	2	9	12	0.004
Fibrin Glue	5	6 ^b	3	4	8	
Mitomycin-C	5	4 ^b	4	2	6	
Triamcinolone acetonide	5	6 ^b	2	4	6	

^{a,b} The same superscript letter in one column indicates that the statistics are not significantly different using Post-Hoc Mann-Whitney ($p > 0.05$).

Immunohistochemistry staining of TGF- β

Conjunctival specimens that had been formalin-fixed and paraffin-embedded blocks were cut into 4 μ m slices. The slices underwent xylene deparaffinization, ethanol dehydration, and methanol deoxidation. The samples were stained with a fully automated machine (Leica Bond MAX). The monoclonal TGF- β antibody kit (Genetex Inc, CA, USA) was used to perform immunohistochemical staining, and the antibody was prediluted to a 1:1000 dilution.

Fibrin glue preparation

Fibrin glue is prepared from 40 ml of peripheral blood drawn aseptically from a rabbit ear vein, added with citrate-phosphate-dextrose-adenine (CPDA) with a ratio of 9:1. The samples were well mixed by gently shaking it to ensure CPDA anticoagulant effects. The blood was centrifuged at 3000 rpm for 15 minutes to separate plasma from erythrocytes. The collected plasma was stored in a sterile tube at -20°C for 24 hours and thawed at room temperature before being further centrifuged at 4°C at 3000 rpm for 15 minutes to obtain the fibrinogen component. The top two-thirds of the plasma (platelet-poor plasma) were collected as fibrinogen, while the bottom one-third, PRP, was stored in sterile microtubes to generate thrombin. The top two-thirds of the plasma was added 1 ml of 95% ethanol, incubated at 4°C for 30 minutes, then centrifuged at 4°C at 3000 rpm for 15 minutes. The supernatant was removed, and the sediment was collected as a fibrinogen component. One-third of the PRP component was mixed with 0.1 ml of 10% calcium chloride (CaCl₂). Fibrin glue is made by mixing the components of fibrinogen and thrombin in a ratio of 1:1.^{13,15}

Cell Quantification

Light microscopy was utilized to perform a semi-quantitative examination of the stained sections using IRS. IRS values were calculated by multiplying the intensity of the staining (no staining, 0 points; weak, 1 point; moderate, 2 points; and strong, 3 points) and percentage groups of positive cells (no cells with reaction, 0 points; <25% with a positive reaction, 1 point;

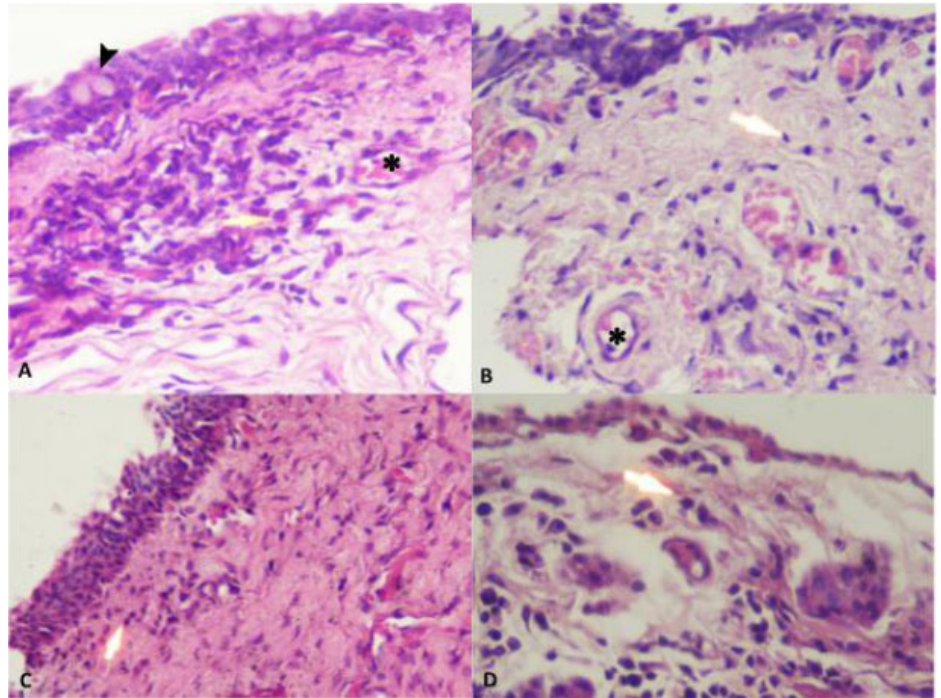


Figure 1. The conjunctival tissue. Yellow arrows represent inflammatory cells, black arrows represent fibroblasts, asterisks represent blood arteries, and arrowheads represent goblet cells. Control group (A); MMC group (B); TCA group (C), and fibrin glue group (D). Intact epithelial surfaces were seen in all groups. The control group showed densely, and numerous inflammatory cellular infiltrates (MNCs) (yellow arrows). The number of fibroblasts and MNCs decreases significantly in the MMC group (B) and the TCA group (C). (H&E stain, 400x magnification).

25%–50%, 2 points; 51%–80%, 3 points; >80%, 4 points). Based on the final score, the IRS was divided into three different groups: negative immunoreactivity was defined as a total score of 0; low expression was defined as a cumulative score of 1-4, while high expression was defined as a cumulative total score of >4. To minimize bias, this procedure was performed by a single pathologist in a blinded fashion on serially numbered slides using an Olympus microscope (Cx51) equipped with an Olympus camera and SIS software (Japan, Tokyo).

Statistical Analysis

All data were presented as median values. To compare the histology data, Kruskal-Wallis nonparametric tests were used. Mann-Whitney U tests were used for post hoc analyses. The significance level was set at $p < 0.05$ and was considered statistically significant. The collected data was analyzed using SPSS version 26 (IBM Corporation, New York, NY, USA).

RESULTS

All rabbits were monitored throughout the 14-day experimentation before harvesting the Tenon's conjunctiva. There was no complication found during the observational period. According to postoperative clinical examinations, all groups had a deep fornix, both superior and inferior. The median values of the IRS score in the study groups are presented in Table 2, respectively. It was found that the control group had the greatest median myofibroblast count (12), followed by fibrin glue (8), MMC and TCA with the lowest median values of 6. The Kruskal-Wallis test revealed a significant difference in TGF-B expression among four groups ($p=0.004$) are shown in Table 2. The Post-hoc Mann-Whitney test demonstrated a significant difference in TGF-B expression between the fibrin glue, MMC, and TCA groups compared to the control group ($p<0.05$). Surprisingly, there was no statistically significant difference between

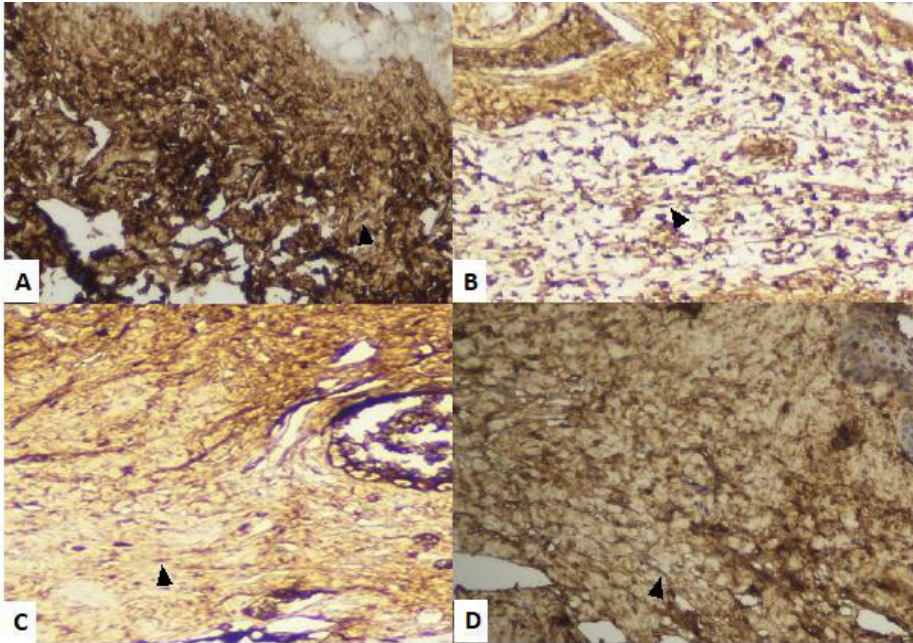


Figure 2. The immunohistochemistry staining of TGF- β expression in Tenon's conjunctiva. The arrowheads indicate fibroblasts. Fibroblasts are distinguished from lymphocytes by their longer and bigger nuclei. The cytoplasm of fibroblasts with a spindle form. Immunohistochemistry microphotographs show the intensities of the transforming growth factor (TGF)- β immunostaining of one subject from each study group. Control group (a), mitomycin-C (MMC) group (b), triamcinolone acetonide (TCA) group (c), fibrin glue group (d). TGF- β immunostaining is lower in the MMC group, the TCA group, and the FG group compared to the control group.

the FG, MMC, and TCA groups ($p > 0.05$).

Microphotographs illustrating the proliferation of fibroblasts and the infiltration of MNCs at the Tenon's conjunctiva tissue in animal model evisceration surgery from all the groups are displayed side by side in Figure 1. The microphotographs of immunostaining of TGF- β from each group are shown in Figure 2.

DISCUSSION

A contracted socket is one of the most dreaded complications of anophthalmos; it refers to the shrinkage of orbital tissue caused by fibrosis that is followed by a decrease in the orbital volume and shallowing of the forniceal depth.⁴ The management of a contracted socket necessitates reconstructive surgery to deepen the sockets, providing for the retention of a cosmetically acceptable prosthesis. Fibrosis may cause further contracted sockets in individuals who have undergone numerous prior reconstructive

procedures, ultimately leading to surgical failure.^{6,16} This study is concerned with the prevention of contracted sockets, with the expectation that intraoperative adjuvant therapy following evisceration helps prevent further fibrosis of Tenon's conjunctival tissue, which leads to surgical failure.

According to the findings of our study, the TGF- β expression of the MMC groups was significantly different compared to the control group ($p < 0.05$). MMC has been regarded as the gold standard of adjuvant therapy in ocular diseases over the past two decades by inhibiting fibroblast proliferation, reducing fibrovascular growth, and inhibiting fibrosis.⁸ Mitomycin-C 0.4 mg/ml resulting in significant inhibition of TGF- β expression postoperatively.² According to Lee et al., MMC reduced TGF- β expression in primary cultures of human tenon fibroblasts (HTF), and human pterygium fibroblasts (HPF) compared to the control group. Administration of a single MMC to HTF applied for 5 minutes at a

concentration of 0.4 mg/ml resulted in 56% cell death after 24 hours.¹⁷ MMC remains the most commonly used antifibrotic agent to inhibit postoperative scarring, yet it causes massive cell death and apoptosis, which leads to serious complications.

TCA 40 mg/ml injected subconjunctival in an animal model of evisceration found a significant decrease in TGF expression compared to the control ($p > 0.05$). This result is linear with a previous study by Tawfik et al.¹⁸ showed the number of myofibroblasts in the TCA group significantly decreased compared to the control group. In this investigation, MMC remained superior to TCA in lowering the number of myofibroblasts. The steroid provides anti-inflammatory effects through prostaglandin synthesis prevention by inhibiting the phospholipase A2 and lipo-oxygenase pathways, thereby reducing the inflammatory response and regulating wound healing. Suppression of the inflammatory response by TCA affects fibroblasts' proliferation and production capability, in which case TGF- β is a cytokine responsible for the process of fibroblast differentiation into myofibroblasts.^{9,11,19}

Our study showed the TGF- β expression after administration of FG was not significantly different than the MMC and TCA groups. These results demonstrate that fibrin glue is competitive with MMC and TCA in suppressing TGF- β expression. Biomaterial fibrin glue is non-toxic, biodegradable, and biocompatible without inducing excessive inflammation, no foreign body reactions, tissue necrosis, or extensive fibrosis. It positively affects the overall wound-healing process. Compared to commercial fibrin glue, autologous fibrin glue is cheaper and has no risk of getting a blood-borne virus or having an allergic reaction.²⁰⁻²² Fibrin glue can modulate the activity of monocytes and macrophages, thus playing a role in the resolution of inflammation and inhibiting the TGF- β expression. Fibrin glue forms a strong clot that seals the whole length of the lesion. Two weeks after application, the fibrin clot degrades physiologically after granulation tissue.²³

Komaratih E et al.²⁴ showed fibrin glue could suppress fibrosis by decreasing the expression of TGF- β . The studies

by Saed GM et al.²⁵ and Scapini F et al.²⁶ showed that FG modulated the wound healing process by inhibiting the release of TGF- β cytokines and reducing collagen contraction. Study Abdurrauf M et al.¹³ found fibrin glue significantly decreased TGF- β expression compared to the control group in human Tenon fibroblast cultures (HTFs). The lower expression of TGF- β indicates that fibrin glue can inhibit TGF- β . This study demonstrates that fibrin glue is an effective adjuvant therapy by lowering the proliferation of HPF fibroblasts to prevent fibrosis.

Our study is the first experimental study regarding the effects of biomaterial autologous fibrin glue on TGF- β expression as an adjuvant therapy for contracted socket prevention. Commercial fibrin glue is expensive, yet autologous fibrin glue is safe, easy to make, effective, and inexpensive. The feasibility of autologous fibrin glue as an adjuvant therapy is promising, and its efficacy is comparable with other antimetabolites.

The limitation of this study is that TGF- β expression was evaluated during the proliferative phase, so it did not represent the overall wound-healing phase. In future studies, it would be best to evaluate TGF- β expression in each wound healing phase, including the homeostasis, inflammatory, proliferative, and remodeling phases (on days 0, 7, 14, and 21) to provide a better understanding of fibrin glue as an antifibrotic agent. Further study is required to evaluate the effective dose of fibrin glue as an antifibrotic agent before clinical trials in humans.

CONCLUSION

In conclusion, fibrin glue was significantly decreasing the expression of TGF- β . The autologous fibrin glue can be used as an alternative to post-enucleation adjuvant therapy in preventing contracted sockets.

ETHICAL CLEARANCE

The Animal Ethics Committee has granted ethical approval at Universitas Airlangga Faculty of Veterinary Medicine, Surabaya, Indonesia.

CONFLICT OF INTEREST

The manuscript has no conflicts of interest.

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This study was self-funded without any third-party involvement.

AUTHOR CONTRIBUTIONS

All authors had made substantial contributions to the writing of the paper and approved the published version.

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