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Human-Derived Fibrin Glue: In Vitro Antibacterial Effects and Antibiotic Permeation

İnsan Kaynaklı Doku Yapıştırıcısı: İn Vitro Antibakteriyel Etkinliği ve Antibiyotik Geçirgenliği

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Summary

Objectives: This study investigated the in vitro antibacterial efficacy and antibiotic permeation of human-derived fibrin glue (FG).

Materials and Methods: FG was prepared under sterile conditions by the Blood Bank of Gazi University Faculty of Medicine. In this study, cultivations were performed in 5 main groups: Group 1 (control) - only FG; Group 2a (control) - pure Staphylococcus aureus (SA) and Group 2b (control) - pure Staphylococcus epidermidis (SE); Group 3a (control) - SA+antibiotic and Group 3b (control) - SE+antibiotic; Group 4a - FG+SA and Group 4b - FG+SE; Group 5a - FG+SA+antibiotic and Group 5b - FG+SA+antibiotic.

Results: Group 1 showed no bacterial growth, whereas Group 2a and Group 2b, Group 4a and Group 4b showed bacterial growth. Group 5a and Group 5b showed no growth.

Conclusion: Although FG has no antibacterial efficacy in vitro, it may be used safely due to antibiotic permeation in diseases with either infected or non-infected ocular surface that require suturing. (Turk J Ophthalmol 2014; 44: 347-50)

Key Words: Antibacterial activity, corneal perforation, fibrin glue, sutureless amniotic membrane transplantation

Özet

Amaç: İnsan kaynaklı doku yapıştırıcısının in vitro antibakteriyel etkinliğini ve antibiyotik geçirgenliğini araştırmak.

Gereç ve Yöntem: Çalışmada, kan bankasında steril koşullar altında hazırlanan doku yapıştırıcısı (DY) kullanıldı ve hazırlanan DY besiyerlerine ekildi. Çalışma 5 gruba ayrıldı. Grup 1 (kontrol), sadece DY, Grup 2a (kontrol); sadece Staphylococcus aureus (SA) and Group 2b (kontrol); sadece Staphylococcus epidermidis (SE), Group 3a (kontrol); SA+antibiyotik and Group 3b (kontrol); SE+antibiyotik, Group 4a; FG+SA and Group 4b; FG+SE, Group 5a; FG+SA+antibiyotik and Group 5b; FG+SA+antibiyotik.

Bulgular: Grup 1'de bakteriyel üreme görülmezken, Grup 2a ve Grup 2b, Grup 4a ve Grup 4b'de bakteriyel üreme görüldü. Grup 5a ve Grup 5b'de üreme saptanmadı.

Sonuç: DY, in vitro antibakteriyel etkinlik göstermezken, antibiyotik geçirgenliğinden dolayı, sütürasyon gerektiren enfekte veya enfekte olmayan oküler yüzeylerde güvenle kullanılabilir. (Turk J Ophthalmol 2014; 44: 347-50)

Anahtar Kelimeler: Antibakteriler etkinlik, korneal delinme, doku yapıştırıcısı, sütürsüz amniyotik membran transplantasyonu

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Introduction

Perforations due to corneal ulcer and abscess are ophthalmologic emergencies that require immediate treatment and there are various treatment methods for reparation.[1] Tissue sealers and amniotic membrane transplantation, two of these options, are used effectively and safely in cases of corneal perforation.[1,2] The main basis of all these methods are the presence of healthy basement membrane for normal proliferation and differentiation of corneal epithelial cells.[3,4]

In serious ocular surface disorders, sutured amniotic membrane transplantation is a common surgical technique which is known to restore the basement membrane functions and enables cellular adhesion and regular epithelial healing.[2] Despite the success of this surgical procedure, disadvantageous factors such as corneal irritation due to sutures, granulomatous foreign body reaction, peribulbar anesthesia need, re-damaging of the healed epithelium when removing sutures, gave rise to new surgical treatments with tissue glue rather than sutures.

Tissue adhesives are divided into two forms: Synthetic (cyanoacrylate) and biological (fibrin glue-FG).[1] Although some successful results were obtained with cyanoacrylate,[5] long-term follow-up showed inflammatory foreign body reaction, neovascularization, corneal toxicity, and increase in bacterial growth.[1,6]

Human-derived biologic tissue adhesive FG is biocompatible and may lead to full resolution, but it may cause minimal stromal inflammation, foreign body sensation, and neovascularization. Recent studies demonstrated that FG is useful by itself in corneal perforations[1,7] and sutureless amniotic membrane transplantation.[8] However, it is not known whether FG-applied surfaces are permeable to antibiotics. Therefore, in this study, we aimed to investigate in vitro the antibacterial efficacy and antibiotic permeability of FG.

Materials and Methods

Fibrin Glue

FG was prepared with CP-3 Plasma Processing Disposable System (CryoSeal® FS System) by the Blood Bank of Gazi University Faculty of Medicine under sterile conditions. It contains two different liquids, i.e. fibrinogen/aprotinin/Factor 13 and thrombin/calcium. These two liquids are conserved in two separate syringes with a common injecting tip (Duploject) and when injected, they mix with each other in equal volumes. In mixture, fibrinogen turns into fibrin in the presence of thrombin. Fibrin polymers cross-bind with the help of Factor 13 and turn into stout fibrin matrix. This fibrin matrix leads to tissue adhesion and is used as tissue glue.

For the study groups, FG discs (diameter: 10 mm, height: 1 mm) were prepared.

Study Groups

In this study, we used 5 main groups and sheep blood agar was used for all cultures: Group 1 (control) - only FG; Group 2a (control) - pure Staphylococcus aureus (SA); Group 2b (control) - pure Staphylococcus epidermidis (SE); Group 3a (control) - SA+antibiotic; Group 3b (control) - SE+antibiotic; Group 4a - FG+SA; Group 4b - FG+SE; Group 5a - FG+SA+antibiotic; Group 5b - FG+SA+antibiotic (Table 1).

Study Procedure

Human-derived (isolated from the axilla) methicillin-sensitive Staphylococcus aureus (reference isolate: 25923) and methicillin-sensitive Staphylococcus epidermidis (reference isolate: 29213) were grown in solid agar (Biomérieux) and were suspended in phosphate-buffered saline (GIBCO®). The bacterial suspensions were prepared according to 0.5 McFarland, and sheep blood agar (Biomérieux) medium was inoculate with a suspension containing about 10 microliter (105 bacteria/mL).

FG discs were inoculated into the pure blood agars (Biomérieux) (Group 1) and bacteria-cultured agars (Groups 4a, 4b, 5a, and 5b) (immediately after bacteria cultivation).

Vancomycin 500 mg and Ceftazidime 500 mg were 1/10 diluted and mixed in equal amounts. This mixture was added into the blood agars (Biomérieux) (Groups 3a, 3b, 5a, and 5b) at a concentration of 2.5 mg/mL as one drop (diameter: 10 mm) just after the bacteria cultivation and 5 minutes after FG application.

Each cultivation was repeated 3 times. All the media were evaluated after 24 hours of incubation at 37 °C by one masked observer. The antibacterial activities of FG, antibiotics, and FG+antibiotics were determined by a modification of the standard agar diffusion test. The mean inhibition diameter zone of each group was determined after 3 repeated cultivations.

Statistical Analysis

Statistical analysis was performed using SPSS® version 16.0 for Windows. The data obtained from the 5 groups were analyzed with the non-parametric Kruskal-Wallis test and Mann-Whitney U-test. A p-value of $p<0.05$ was considered to be statistically significant. All data were expressed as mean ± standard deviation.

Results

Groups 3a, 3b, 5a, and 5b showed a good antibacterial activity, whereas Groups 2a, 2b, 4a, and 4b showed bacterial growth. There was a statistically significant difference between Group 2 and Groups 4a/4b (respectively; p=0,002, p=0,002) and between Group 2 and Groups 5a/5b (respectively; p=0,002, p=0,002); however, there was no statistically significant difference between Group 2 and Groups 3a/3b (respectively; p=0,689, p=0,701). No bacterial growth was observed in Group 1 (Table 1).

Discussion

FG has been used in many surgical areas as topical hemostat, sealant, and tissue adhesive. FG is used in ophthalmology in vitreo-retinal surgery (full-thickness macular hole closure),[9] for conjunctival sealing in sutureless surgery,[10] closing incisions in cataract surgery,[11] and for bleb leakage in glaucoma surgery.[12] However, popularly, FG is used in pterygium surgery[13] and corneal surgery.[1,2,5,7,8,14,15] FG has several benefits such as shorter operation time than suturing, less corneal irritation, less

Table 1. Study groups and colonization of the groups in sheep blood agar

Group	Culture	Diameter of the Zone of Inhibition, cm
1	FG	No bacterial growth
2a	Pure SA	0 (None)
B	Pure SE	0 (None)
3a	SA+antibiotic	1.38±0.16*†
B	SE+antibiotic	1.40±0.12*†
4a	FG+SA	0.2±0.05#
B	FG+SE	0.1±0.05#
5a	FG+SA+antibiotic	1.39±0.13*†
B	FG+SE+antibiotic	1.42±0.15*†

FG: fibrin glue; SA: Staphylococcus aureus; SE: Staphylococcus epidermidis
Antibiotic: The mixture of 1/10 dilution of Vancomycin (500 mg) and Ceftazidime (500 mg).
p<0.05 when comparing Group 2 with Groups 3a/3b and Groups 5a/5b. #p>0.05 when comparing Groups 4a and 4b with Group 2. †p>0.05 when comparing Groups 3a and 3b with Groups 5a and 5b

granulomatous foreign body reaction and stromal inflammation. Therefore, it is used commonly in treatment of perforations and melting due to corneal ulcer and abscess.[1,2,7] It is also used in sutureless amniotic membrane transplantation[14] and limbal cell transplantation.[15]

Another important effect of FG is that it serves as a matrix for fibroblasts and keratocytes thus stimulates fibroblastic activity and prevents epithelial cell loss. Therefore, it has been considered that FG contributes to a fast closure of damaged epithelial layer and prevents colonization of microorganisms.[16] Furthermore, sutures may serve as a source for infection by mucus and debris collection. Some studies reported that FG reduced the risk of infection in the wound site after surgery.[17,18] In the literature, some studies have reported that FG may be used safely in infected wounds.[19] Kram et al.[20] studied in vitro the effects of non-autologous FG on brain-heart infusion media and found that it inhibited the growth of Staphylococcus aureus. Unlikely, Feinberg et al.[21] reported that fibrin matrix provided media for many bacteria and increased the bacterial growth. Chen et al.[22] demonstrated that FG had no bacteriostatic effect on Gram (+) and Gram (-) bacteria. In our study, FG showed no antibacterial effect in in vitro environment.

Although FG is successfully used alone or together with amniotic membrane in the treatment of perforations and melting due to corneal ulcer and abscess,[1,2,7] complete recovery depends on the control of infection. Kram et al.[20] and Marone et al.[23] showed antimicrobial efficacy of tissue adhesive when mixed with various antibiotics (gentamicin, teicoplanin, cephalothin, ciprofloxacin, cefoxitin, vancomycin). They also stated that this mixture can be used safely in infected and contaminated wounds.[20,23] On the other hand, there is no standard for mixing FG and antibiotics and there is no information on the effects of antibiotics on fibrin matrix formation and on the stretching forces of fibrin matrix. All these limit the use of these mixtures. Thus, when using FG in the treatment of perforations due to infection, an antimicrobial therapy is also required to prevent recurrence.

In the treatment of corneal perforation and melting, standard infection treatment is topical antibiotics after FG administration. At this point, we need to know the antibiotic permeability of FG. In our study, we determined the antibiotic permeability of FG in in vitro conditions. Topical antibiotic was administered 5 minutes after FG application in order to give sufficient time for the formation of fibrin matrix and this was the difference of our study from those by Kram et al.[20] and Marone et al.[23] who used fibrin-antibiotic mixture.

Besides the above-mentioned benefits of FG in eye surgery, it may lead to some undesirable complications such as contagious diseases because it is prepared from blood products. However, in the literature, there is no report of any disease, except for 1 case infected with parvovirus.[24] In the Blood Center of Gazi University Faculty of Medicine, FG is prepared from fresh frozen plasma of donors who have negative viral markers at least 6 months (because of window period). After preparation, it is sterilized by gamma radiation. We observed no cases of infection after FG use.

Another disadvantage of FG use is its cost - FG is 4-fold more expensive than vicryl suture. However, it can be used up to 7 patients in the same operation list. This may balance the cost.[25] In addition, it shortens the use of operation room by reducing operation time. Montgomery et al.[26] estimated the cost of an operation room use in US to be approximately $67.50/min. Based on this figure, the cost of FG treatment is quite lower than suturing.

In conclusion, although FG has no antibacterial efficacy in vitro, it may be used safely due to antibiotic permeation in diseases with either infected or non-infected ocular surface that require suturing.

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