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Evaluation of the Effect of Fibrin Glue Prepared from Single-Donor Plasma on Wound Healing in Rats

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Introduction

Fibrin sealant is the only material approved by the Food and Drug Administration (FDA) acting as hemostat, sealant and adhesive at the same time. Fibrin glue is a model of the final step of blood coagulation. The conversion of fibrinogen to fibrin is catalyzed by the action of thrombin and fibrin monomers are covalently cross-linked by factor XIIl in the presence of ionized calcium. Antifibrinolytic agents have been used for controlling fibrinolysis [1,2].

While preparing commercially available fibrin tissue adhesive and essential component of fibrin glue, fibrinogen is separated from plasma and pooled from different donors. However, the risk of transmitting virus infections cannot be eliminated completely [3,4]. The fibrinogen needed for the fibrin glue can be obtained from screened single-donor human plasma, which greatly reduces the risk of transmitting hepatitis, Human Immunodeficiency Virus (HIV) and other pathogens.

The use of fibrin glue, as in concentrated human fibrinogen in combination with thrombin for hemostasis and tissue adhesion has been an important advance in a variety of surgical applications [5-9]. Fibrin glue not only causes hemostatis, but also enhances healing [10-13]. The presence

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of stabilized fibrin structure stimulates the growth of fibroblasts. This combined effect on wound healing is essential for the fibroblastic action. The purpose of this study is to evaluate the effect of fibrin glue prepared from single-donor on wound healing in rats.

Materials and Methods

Fibrin glue preparation

Blood was collected from a healthy volunteer donor. It was screened for the presence of antibodies against hepatitis, acquired immune deficiency syndrome and syphilis. Blood was collected in a standard blood bag containing citrate-phosphate-dextrose-adenin (CPDA) standard anticoagulation solution. Plasma was separated from the blood cells by centrifugation at 4000 rpm for 10 minutes. Plasma was frozen and stored at -20 ^{0}C. Frozen plasma was thawed at 4 ^{0}C before processing, and then it was precipitated with ethanol by cooling down at 0 ^{o}C for 30 minutes. It was centrifuged at 4000 rpm and the supernatant was removed. Fibrinogen and factor XIII containing precipitate was dissolved in an aprotinin (Bayer) solution (3000 KIH/ml) (Component I). Component II was prepared by dissolving 1000 NIH/ml bovine thrombin (Sigma) in 1 ml 100 mM $CaCI_2$ solution. Approximately 1 ml fibrin glue was obtained from 20 ml plasma. Both of these components were warmed for 10 minutes at 37 ^{0}C. Fibrin glue applicator is a double-barreled syringe used for application of the material to the operation area.

Clinical application

Fifteen Wistar Albino rats were used in this study. The experimental protocol was subjected to DETAM (Istanbul University - Istanbul Faculty of Medicine). The dorsal hair of the animals was shaved by an electrical clipper and the back of each animal was cleaned before creating the incisions. Then, two full-thickness, 5 cm-long, paravertebral incisions were made through the skin on each side of the vertebral column. One of the incisional wounds of each animal was treated with fibrin glue. The other incisional wound was not treated and acted as a control for the evaluation of the effect of fibrin glue. Both of the incision sites were fixed with surgical sutures. This procedure was performed under ether anesthesia. At the 2nd (group I; n=5 rats), 7th (group II; n=5 rats) and 14th (group III; n=5 rats) days of the wound healing, rats were sacrificed by

decapitation under ether anesthesia. Dermal biopsies were obtained at 2nd, 7th and 14th days from the fibrin glue treated and control incisional areas. A segment of skin material was harvested from each rat, fixed with 10% formalin and embedded in paraffin; 5 mm thick sections were prepared, stained with Hematoxylin-eosin and examined under regular light microscope. Infiltration of neutrophils, eosinophils, plasma cells and lymphocytes, and fibroblast proliferation were scored semi-quantitatively as follows; (-): none; (±): minimal; (+): slight; (++): modarete; (+++): extensive. Chi-square test was used for statistical analysis.

Determination of fibrinogen concentration

Immune diffusion plates containing monospecific antiserum (Nor-Partigen®, Behring) were used for the quantitative determination of fibrinogen.

Factor XIII Determination

Presence of factor XIII was analyzed by testing the solubility of fibrin clots in 5M urea solution by means of Lorand's method [14]. 100 ml fibrinogen solution and 100 ml thrombin solution containing 0.1 M $CaCl_2$ were added and incubated for 30 minutes. 3 ml of 5M urea solution was added and thawed at 37 ^{0}C for 10 minutes. The presence of factor XIII was determined by whether or not the clot dissolved.

Results

Fibrin glue from individual units of human plasma was used in this study. The plasma was precipitated with ethanol to separate Component I. The mean fibrinogen concentration in plasma was found to be 3.62 mg/ml. Concentration of fibrinogen in Component I was found as 57.4 mg/ml. The total yield of plasma fibrinogen was 79 mg/ml. Presence of factor XIII was tested in 5M urea solution. The results of the histological examination were as presented in Table I. These findings show differences in the healing process throughout the 2nd, 7th and 14th days of observations for both the fibrin glue-treated and the control wounds. The extent of neovascularization and reepithelization, collagen deposition, inflammatory infiltrate and fibroblast proliferation that was observed at microscopic analysis of the sections from both incisional sites was recorded.

TABLE I

Presence of different cell types in the wound samples of the rats.

	DAY 2										DAY 7										DAY 14									
	Control					Fibrin					Control					Fibrin					Control					Fibrin				
Cells types	-	±	+	++	+++	-	±	+	+ +	+++	-	±	+	++	+++	-	±	+	++	+++	-	±	+	++	+++	-	±	+	++	+++
Fibroblasts	5						4	1				5							2	3			5							
Lymphocyts			5					4	1					5				2	3			5						4	1	
Neutrophils				4	1				2	3			5						3	2	5					3	2			
Eosinophils				5					2	3			5						2	3		5				2	3			
Plasma Cells			5					4	1					5				2	3			5						4	1	

(-): none; (±): minimal; (+): slight; (++): moderate; (+++): extensive; ($p<0.01$).

On the postoperative 2nd day, blood cells migrated from blood vessels into the extravascular space were observed for the control samples. Collagen fibers and neutrophilic granulocytes were found to increase slightly for the samples taken from the fibrin glue-treated sites.

Collagen fibers and inflammatory infiltrate were more visible in fibrin glue-treated incision area, than the control site on the postoperative 7th day. Complete reepithelization of the wound was observed in the fibrin glue-treated wounds, while only a minimal reepithelization was observed for the wounds in the control site. At the fibrin glue-treated wounds, neovascularization and amount of collagen fibers were also significantly higher than the control sites.

On the postoperative 14th day, the edge of the wound was covered with a layer of epithelial tissue in fibrin site. However, reepithelization had not yet been completed at the control site.

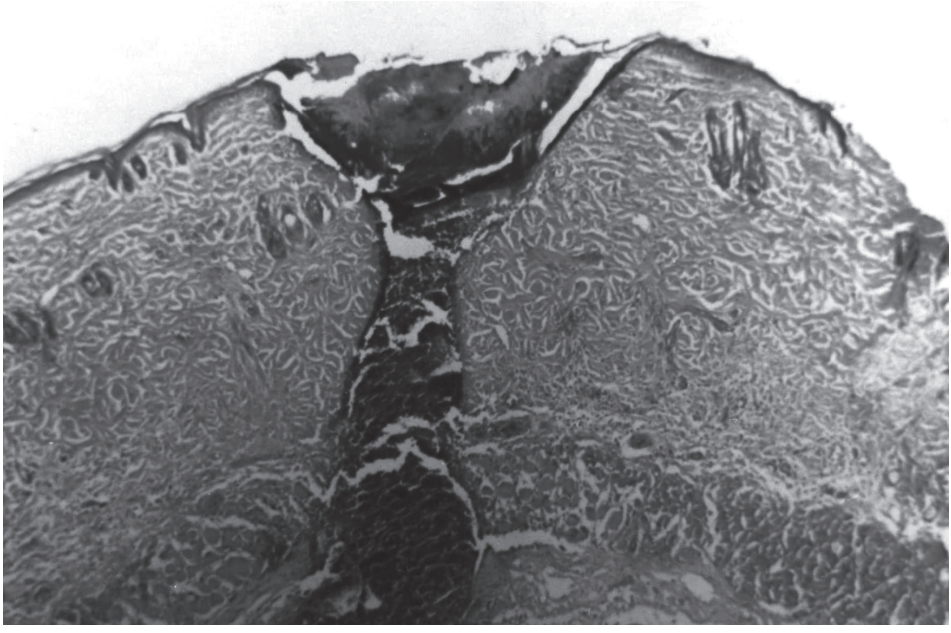

Figure 1
Histopathological appearance of the fibrin glue-treated wound site of the rats on the second day. Fibrin glue located in the incision line is seen. No epidermis is present on the surface, where exudates with erythrocytes, fibrin and cell debris is observed (H+E, x 40).

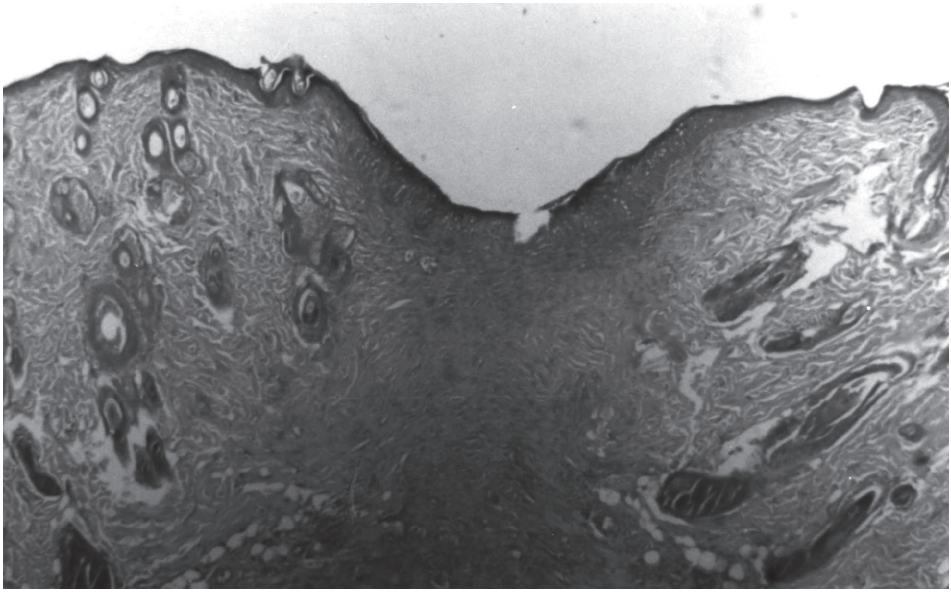

Figure 2
Histopathological view of the fibrin glue-treated wound site on the 7th day. Devascularization connective tissue with a few cells, rich in collagen fibers is seen under the regenerated epidermis (H+E, x 40).

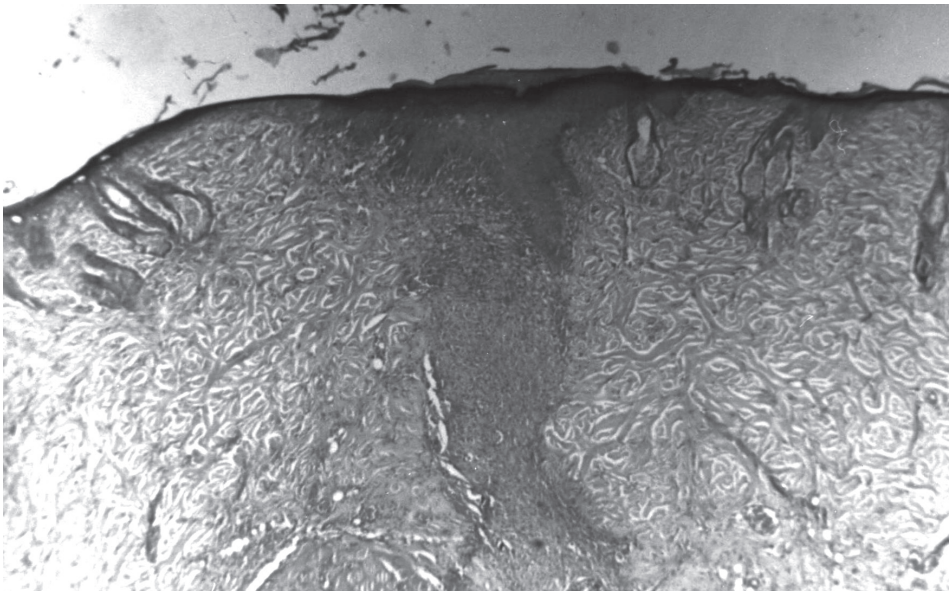

Figure 3
Histopathological appearance of the control wound site on the 14th day. Regenerated epithelium shows papillary proliferations towards the connective tissue. Large, hyperemic vessels are seen surrounding the fibrosis area (H+E, x 40).

Discussion

Currently, there are five U.S. Food and Drug Administration (FDA)-approved forms of fibrin-glue including products derived from pooled or autologous human plasma as well as bovine plasma.

Possibility of transmission of viral agents from pooled blood was reported in the literature [15,16]. The major difficulty in making autologous fibrin glue is the limited use of the product because of its small volume. It is not possible to obtain a sufficient quantity of concentrated fibrinogen by this technique. Amount of fibrin glue prepared is very low and the preparation procedure is not applicable in emergency conditions and trauma. This excludes the use of autologous fibrin glue, and blood donation is required in such cases. In these cases, the use of fibrin glue from single donor plasma could be favored [17-19]. The aim of the present study was to prepare fibrin glue from single-donor plasma, negative for AIDS, hepatitis B and syphilis antibodies and to evaluate its effect of cell proliferation and collagen formation resulting in wound healing.

Various methods for preparing fibrin glue with precipitation of fibrinogen, such as ammonium sulphate precipitation, polyethylen glycol precipitation, cryoprecipitation and ethanol precipitation are used [20-23]. The recoveries obtained by ammonium sulphate precipitation and cryoprecipitation were lower than those obtained by ethanol precipitation. In ammonium sulphate precipitation an amount of sulphate salt remnant remains in the precipitate. Polyethylen glycol precipitation requires long time and several reagents are necessary. Cryoprecipitation has a time-consuming preparation process and the blood must be taken from the donor at least one day before the procedure. Therefore, ethanol precipitation would possibly be the safest, fastest, the most profitable and simplest method for concentrating fibrinogen and factor XIII from single-donor plasma [24,25].

Additionally, the remained ethanol from the precipitation will disappear within a short time. Clots prepared from these concentrated solutions were all insoluble in 5M urea solution, indicating the presence of factor XIII. Ethanol precipitation of fibrinogen produced an average yield of 79%. The concentration of fibrinogen in the precipitate is high and it has increased effectiveness [25-27]. A high fibrinogen concentration is a criterion for the tensile and adhesive strength of fibrin glue which is

directly proportional to the concentration of fibrinogen. An increase in fibrinogen concentration correlates with an increase in adhesive strength.

The wound healing process is explained as a stimulation of fibroblastic cell proliferation and consecutive tissue synthesis. The migration of leucocytes to the inflammatory site is an immune response of the body in wound healing. Neutrophil migration to the inflammation site appears on the 2nd day of fibrin glue treatment. Lymphocytes and plasma cells appear on the 7th day and fibrin is completely absorbed on the 14th day. Brown et al. [28] reported that maximal fibroblast migration occurred in fibrin gels similar to healing wounds. Michel et al. [29] demonstrated that the effect of fibrin glue containing thrombin and Ca^{+2} stimulates fibroblast proliferation and collagen synthesis in vitro. Hashimoto et al. [30] obtained a much better result in comparison with the control group, by using fibrin glue experimentally in the incisional repair of mongrel dogs. Addition studies on fibrin glue including fibroblastic growth factor (bFGF) and endothelial cell growth factor (ECGF) revealed that wound healing in the presence of these materials were more successful than the controls [31-34].

In the present study no significant difference in terms of wound healing was observed between the two wound biopsies obtained at the postoperative 2nd day. However, in the fibrin glue-treated samples a slight increase in collagen fiber formation was observed. On the 7th day, capillary and endothelial as well as inflammatory proliferation was significantly higher at the fibrin glue-treated sites when compared with the controls. Marked fibroblastic proliferation, together with great amounts of collagen deposition occurred at the samples treated with fibrin glue. At the same time complete reepithelization was observed in the wound edge and maturing scar tissue was present on this site. On the postoperative 14th day, the samples from fibrin sites were covered with a layer of epithelial tissue. However, complete reepithelization width was not yet reached and an underlying scar tissue was present in the control sites. The results of this study were in accordance with the literature [10,15,19,29,31,35].

In conclusion, fibrin glue, prepared in short time from single donor plasma with a high concentration of fibrinogen is found to enhance an effective healing. It also has the advantages of safety from transmission of viral agents.

Summary

Commercial fibrin sealant products derived from pooled human plasma. Fibrin sealant has been approved for clinical use today. Fibrin glue prepared from single donor plasma has the advantage of safety from transmission of viral infections. Sufficient quantity of concentrated fibrinogen was produced by ethanol precipitation and obtained in a high percentage of recovery (79%). Additionally, presence of factor XlII was indicated in 5M urea solution. Two full-thickness, 5 cm-long, paravertebral incisions were made through the skin, on each side of the vertebral columns of the 15 Wistar Albino rats. One of the incisional wounds of each animal was treated with fibrin glue. The other incisional wound was not treated and acted as a control. The healing process was examined histologically at postoperative 2nd, 7th and 14th days. Cell proliferation and collagen formation were found to be significantly increased on the fibrin glue-treated sites of all groups. Fibrin glue revealed a positive effect on wound healing observed as enhanced granulation and reepithelization.

Keywords: Fibrin glue; wound healing; rats.

Özet

Tek-Donör Plazmasından Hazırlanan Fibrin Glue'nun Sıçanlarda Yara İyileşmesi Üzerine Etkisinin İncelenmesi

Ticari fibrin yapıştırıcılar, insan plazma havuzlarından elde edilmektedir, Fibrin yapıştırıcı günümüzde klinik kullanım için onaylanmıştır. Tek-donör plazmasından hazırlanan fibrin glue viral enfeksiyonların bulaşma riskini önlemeden dolayı önemli bir avantaja sahiptir. Bu çalışmada, etanol çöktürmesiyle yeterli miktarda konsantre fibrinojen hazırlanmış ve yüksek verimlilikte (79%)elde edilmiştir. Ayrıca, faktör XIII varlığı 5M üre çözeltisinde gösterilmiştir. Wistar Albino sıçanların (n=15) vertebralarının her iki yanına deriye 5'er cm uzunluğunda tam-kalınlıklı iki adet paralel paravertebral insizyon yapılmıştır. Her bir hayvanın insizyon yaralarından biri fibrin glue ile tedavi edilmiştir. İyileşme süreci postoperatif 2,7 ve 14. günlerde histolojik olarak incelenmiştir. Tüm grupların fibrin glue ile tedavi edilen

yaralarında hücre proliferasyon ve kolajen oluşumunun önemli derecede artmış olduğu gözlenmiştir. Fibrin glue, yara iyileşmesi üzerine artmış granülasyon ve reepitelizasyon şeklinde olumlu etki göstermiştir.

Anahtar Kelimeler: Fibrin glue, yara iyileşmesi, sıçanlar.

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