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Research Article

Improved Thrombin Hemostat Using the Cross-Linked Gelatin by Microbial Transglutaminase

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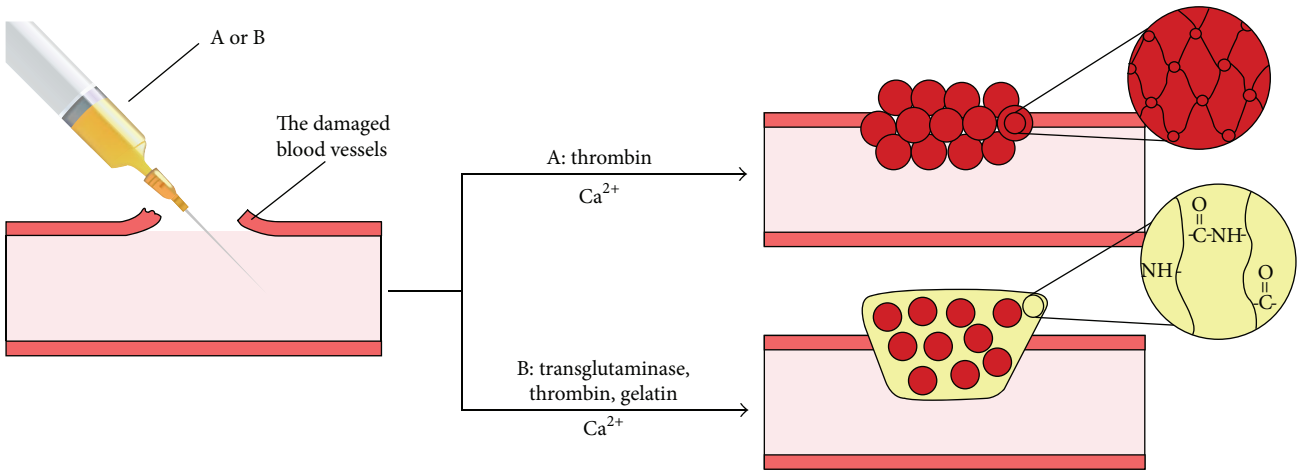
It is well known that the thrombin obtained from animal is available in clinical treatment, which plays an important role in hemostasis and the treatment of hemorrhagic diseases. However, how to achieve complete hemostasis in 2 min is still a challenge. In this report, the thrombin hemostatic has been improved using the cross-linked gelatin by microbial transglutaminase, and its efficacy was demonstrated by the vitro experiment. Compared with the traditional thrombin hemostatic the clotting time with the improved hemostat is significantly shorter. It may rapidly stop blood loss, which would provide a simple, safe, and cost-effective surgical sealant.

1. Introduction

Gelatin, a kind of biomaterials, is produced by the partial hydrolysis of native collagen. The essential constituent of gelatin, between 85 and 92%, is protein which exists as polymer chains of different lengths. Thus, colloidal solutions or sols are formed instead of real solutions. These sols convert to gels on cooling and revert to sols on warming. Because of its unique technological and biopharmaceutical properties, gelatin has been used in the manufacture of numerous medicine fields, for example, as injectable matrices for controlled drug delivery or injectable scaffolds for tissue engineering [1, 2]. To improve the mechanical properties of gelatin-based scaffolds, many cross-linking methods have been employed which include chemical methods and physical methods [3–8]. There are still some other advantages for gelatin as a functional material for medicine. (i) Gelatin is cheap and nontoxic. (ii) It is extracted from bones, cartilages, and skins. Therefore, it has a great biocompatibility. (iii) Gelatin, an environment-friendly material, can be easily decomposed in nature environment. Hemorrhage is the leading cause of preventable mortality after being injured. Uncontrollable bleeding accounts for approximately 50% of the total mortality in military trauma and 31% in civilian trauma cases [9, 10]. The profound importance of hemostasis has prompted a surge of research in recent years. More people begin to focus on hemostatic materials, especially the in situ gel-forming materials. This provides a new way to treat bleeding which is a life-threatening problem. In our previous work [11], the biomaterial gelatin was successfully used as a new nonpressure haemostasis within a short time in a large wound model by percutaneous injection under CEUS. But the hemostatic speed of the material is still not satisfying. In order to solve this problem the improved gelatin-based hemostatic using the microbial transglutaminase (mTG) was designed. The mechanism of this material is showed in Scheme 1.

Considering that calcium independent mTG/gelatin hemostat and thrombin/Ca^{2+} hemostat both have been reported as effective haemostasis methods [12–14], we are here trying to combine these two to create a novel approach. When the combined hemostat is injected in the wound, mTG will cross-link gelatin to form gel and thrombin/Ca^{2+} will theoretically cross-link both gelatin and blood. The gore containing gel forming here with double cross-linking will



Scheme 1: A sketch indicating the different designs for hemostatic of the thrombin and gelatin-based hemostatic.

have higher strength with more cross-linking point. And owing to the process described above, the speed of hemostasis can be increased obviously. In this report, we investigated the clot time and the biocompatibility of this double cross-linking system.

2. Materials and Methods

The animals were all kept in an Association for Assessment and Accreditation of Laboratory Animal Care International accredited climate-controlled facility. All animals were cared in strict compliance with the Guide for the Care and Use of Laboratory Animals as approved by our institutional animal care and use committee and according to the guidelines issued by the National Institutes of Health for the Care of Laboratory Animals (license number, SCX [Beijing] 2013–2011).

2.1. Haemostatic Reagents. The degradation product of collagen was derived from animal bovine collagen. Transglutaminase (TG) was provided by Beijing University of Chemical Technology. Lyophilizing thrombin solution (500 UI per bottle), saline, and calcium chloride for injection (0.5 g/10 mL) were provided by Chinese People's Liberation Army General Hospital.

2.2. Preparation of HCGT. Calcium chloride was added to a vial filled with thrombin, and the vial was gently swirled to obtain the thrombin solution. This thrombin solution was then added to the gelatin matrix sol with the mTG to prepare the HCGT. Here, each 1 mL of the HCGT contains 1000 UI of thrombin and 0.1 mL TG (1 g/mL).

2.3. Clotting Experiments. Ten adult male New Zealand rabbits (2.1–3.5 kg) were used in this study. The rabbits were generally anesthetized by injecting pentobarbital sodium (3%). Then the vena cava inferior blood was collected under the conditions of ultrasound-guided and poured into test tube for 1 mL each at 37–40°C.

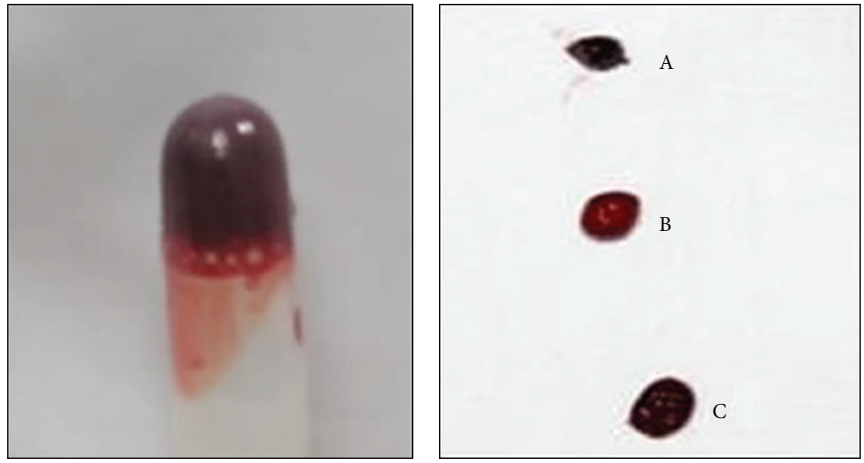
Randomize the blood into three groups: group A (adding saline), group B or LTP group (adding lyophilizing thrombin solution), and group C or HCGT group (adding HCGT). Add 0.1 mL designated reagent in each test tube and measure the clotting time (no blood flow after test tube inverted). Select 20 test tubes for each group randomly, remove the clots, draw the excess liquid with filter paper, and then weight the clots. The volume changes of the clots were also observed. After that fix the clots with formalin (4%) and embed and HE stain them, and the pathological sections were observed.

2.4. Statistical Analysis. Statistical analysis was performed using SPSS 10.0 software (SPSS/PC Inc., IL, USA). The success rate (sensitivity) of CEUS, the extent of peritoneal adhesions, and drug absorption were represented as percentages (%), while the remaining data were expressed as mean ± standard deviation (SD). The chi-square test was used for the analysis of the difference in haemostasis time between any two groups, while the other analyses were performed by analysis of variance (ANOVA). A P value <0.05 was considered statistically significant.

3. Results and Discussion

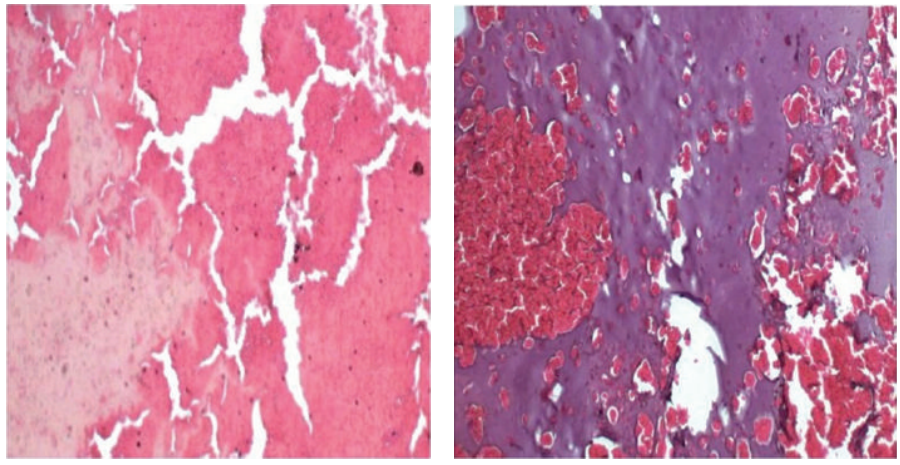
3.1. Measurement of the Clotting Time. The clotting time, which is calculated from designated reagents that were added in the test tube to no liquid flow out when the test tube was inverted, in group A was (715.67±290.11) seconds and in LTP group B was obviously decreased to (13.7±4.71) seconds. The time to clot was also significantly shorter in HCGT group C ((7.55 ± 4.00) seconds, $P < 0.01$) compared to the group B (Table 1).

3.2. Weight of the Clot. The weight of the clot denoted the difference between the total weight of the clot and the weight of the reagents added. The mean weight of the clots in HCGT group C was (0.5 ± 0.07) grams, much the same as group A ((0.42 ± 0.14) grams) lighter than LTP group B ((0.68 ± 0.22) grams) obviously (Table 1).



(a) (b)

Figure 1: (a) The photo of the clot using HCGT in the test tube; (b) the photo of clots: (A) normal, (B) LTP, and (C) HIGM.



(a) (b)

Figure 2: Images (10x) of panels (a) and (b) are taken from slides prepared from two bloods (a) normal, (b) HIGM, the red sections are the red blood cells, and the purple sections are the HIGM.

Table 1: Comparison of clotting time and weight of the clots between groups A, B, and C.

Group	Clotting time (s)	Weight of clot (g)
A	715.67 ± 290.11	0.42 ± 0.14
B (LTP)	13.7 ± 4.71	0.68 ± 0.22
C (HCGT)	7.55 ± 4.00[a]	0.50 ± 0.07[b]

[a]Comparison with group A ($P < 0.01$).
[b]Comparison with LTP group B ($P < 0.01$).

3.3. Shape and Volume of the Clot. It was found that adding 0.1 mL HCGT in the test tube, no bleeding flow out after the test tube inverted at once (see Figure 1(a)). The volumes of the clots that become larger gradually from group A to group C (HCGT) can be seen from Figure 1(b). And by the touch with a finger, we found that the clot of the HCGT group C is much more elastic with a high strength (Figure 1(b)). The pathological sections show that in the clot of HCGT group C the hemoglobin gels are embedded in the gelatin gel which is staining into purple (Figure 2).

3.4. Discussion. With the development of science and technology, people nowadays live a much better life than before. However, due to the security incidents, traffic accidents, and natural disasters, the improvement of trauma care is still a great challenge for people to concern about. The study done before shows that about 2/3 of the injured died in the trauma scene or on the way to the hospital [15]. The main reason of this phenomenon is the organ failure led by uncontrolled bleeding. Therefore, to stop bleeding quickly and effectively is the key to save life.

In previous research, our group used Wister rat open liver trauma model and observation of haemostatic agents of gelatin matrix (GTC, Gelatin/thrombin/calcium) in contrast-enhanced ultrasound guided to observe the effect of

interventional therapy in the treatment of trauma [16]. The results display, GTC, delivered by percutaneous injection under CEUS, may achieve haemostasis, especially in the case of no pressure. In order to further accelerate hemostasis speed, improved cross-linking hemostatic was designed. And the advantage was investigated through the test tube experiments. (i) High speed of hemostasis is achieved; by this way the clot time can be limited in about 10 seconds. Moreover the clot time can be also controlled by regulating the reaction rate of thrombin. (ii) The elasticity and the strength of the clot are improved owing to the reticular structure in the gel formed by the improved cross-link. Since gelatin is a dispersant with good biocompatibility, the gels of hemoglobin can be dispersed in the gelatin system uniformly. It also makes the clot become more elastic. (iii) Because gelatin has the ability of gel forming, it can increase the volume of the clot through cross-link and make the process of hemostasis much easier. (iv) Gelatin is a kind of macromolecule produced by the partial hydrolysis of native collagen. When added in the bleeding wound, gelatin is conducive to gathering the blood coagulation factor so that the hemostatic speed can be enhanced [17]. What else, gelatin would be able to be biodegradable and could promote the repair and regeneration process of the damaged tissue [16]. So the improved cross-link hemostatic described in this report is a very promising product in hemositasis.

4. Conclusion

What we reported in this paper offers a simple, safe, and cost-effective surgical sealant, which may stop blood loss rapidly. It was provided that cross-linking gelation by microbial transglutaminase can enhance the thrombin hemostatic obviously. And the clotting time with the improved hemostat, measured by vitro experiment, is significantly shorter compared with traditional thrombin hemostatic.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Tengfei Yu and Yuepeng Guan contributed equally to this work and should be considered co-first authors.

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