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Experimental Study on the Viscosity and Adhesive Performance of Exogenous Liquid Fibrin Glue

Takuro HAYASHI,[1,2] Mitsuhiro HASEGAWA,[1] Joji INAMASU,[1] Kazuhide ADACHI,[1] Shinya NAGAHISA,[1] and Yuichi HIROSE[1]

[1]*Department of Neurosurgery, Fujita Health University, School of Medicine, Toyoake, Aichi;*
[2]*Department of Neurosurgery, National Hospital Organization Tokyo Medical Center, Tokyo*

Abstract

Exogenous fibrin glue (FG) is highly suitable for neurosurgical procedures, because of its viscosity and adhesive properties. Several FGs are commercially available, but only few reports detail their differences. In the present study, we investigated the viscosity and adhesive performance of two types of FG: one is derived from blood donated in Europe and the United States (CSL Behring's Beriplast®, BP) and the other is derived from blood donated in Japan (the Chemo-Sero-Therapeutic Research Institute's Bolheal®, BH). The viscosity test that measured fibrinogen viscosity revealed that BP had significantly higher viscosity than BH. Similarly, the dripping test showed that BP traveled a significantly shorter drip distance in the vertical direction than BH, although the transverse diameter of the coagulated FG did not differ statistically significantly. In the tensile strength test, BP showed superior adhesion performance over BH. The histological study of the hematoxylin-eosin-stained specimens in both groups showed favorable adhesion. Although further studies are required on its manufacturing and usage methods, FG shows differences in viscosity and adhesive performance according to the blood from which it is derived. We conclude that it is desirable to select the type and usage method of FG according to the characteristics of the surgical operation in question. Our findings suggest that FG produced from the blood donated in Europe and the United States might be more suitable for use in surgical procedures that demand an especially high degree of viscosity and rapid adhesive performance.

Key words: adhesibility, fibrin glue, tensile strength, viscosity

Introduction

Exogenous fibrin glue (FG) is used as an effective biobinding agent in neurosurgical procedures.[1–3] However, the operative field in neurosurgery is cramped and often sloped owing to the skull structure. This carries a risk of the glue dripping and unintended adhesion to other important structures in the surrounding area. The viscosity and adhesive performance of FG therefore appears to influence surgical precision. Two types of FGs are currently available in Japan: one made from the blood donated in Europe and the United States, and the other made from the blood donated in Japan. However, no studies have been conducted to compare their viscosity and adhesive performance. In the present study, we used two types of exogenous liquid FG and measured their physical properties via viscosity, dripping, tensile strength tests, and histological examination.

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Materials and Methods

The study protocol was approved by our institutional review board. Each of the experiments was repeated six times.

I. Fibrin glue

For the purpose of this study, we purchased and used FGs derived from blood donated in Europe and the United States [Beriplast®, (BP), CSL Behring, King of Prussia, Pennsylvania, USA], and in Japan [Bolheal®, (BH), The Chemo-Sero-Therapeutic Research Institute, Kumamoto]. To formulate both FGs, we used solution A, which comprised fibrinogen and aprotinin, and solution B, which comprised thrombin and calcium chloride. Aprotinin impedes plasmin, suppressing fibrinolysis, is mixed with fibrinogen

before use. Thrombin and calcium chloride act enzymatically on fibrinogen, converting it to a fibrin polymer.[4] In short, the two FGs differ in that BP is made from blood donated via plasmapheresis in Germany, Austria, and United States, and can be stored for up to 60 days; any viruses are deactivated by liquiform heating at 60°C for 10 min. By contrast, BH is made from whole and component blood donated in Japan and has a storage period of 6 months; viruses are inactivated by drying and heating at 65°C for 144 min for fibrinogen and 96 h for thrombin. Both types of FG contain 40 mg of fibrinogen per 0.5 mL of the preparation, whereas 150 units and 125 units of thrombin are present in BP and BH, respectively.[5,6]

II. Viscosity test

To examine the viscosity of the fibrin monomer transformed from fibrinogen, which had not been subject to any enzymatic action, we measured the viscosity of solution A for both FGs using a rotational viscometer (Toki Sangyo Co. Ltd., Tokyo). Solution A (1.1 mL) was allowed to stand for 5 min at 23°C and then mixed for 3 min. Its rotational viscosity was then measured.

III. Dripping test

Since operative fields in actual neurosurgery are often cramped and sloped, the FG is at risk of dripping on and adhering to blood vessels, brain tissue, and nerves, as well as onto the surgical cotton that protects them. To evaluate viscosity in a way that more closely approximates surgical conditions, we used a nebulizer (Beriplast Spray Set, Nipro Co., Osaka) and horizontally sprayed FG onto a vertically installed polyethylene terephthalate (PET) board at assuming 23°C to be the optimal temperature of the operation room. Separate solutions of A and B were mixed at the nozzle before spraying. The spray volume was set as 0.5 mL; and the distance between the spray tip and the PET board, as 5 cm. A microsyringe pump (Fusion 100, Chemyx Inc., Stafford, Texas, USA) was inserted to spray the FG, using spray pressures that delivered 7.5 mL/min and 15 mL/min, respectively. We measured the distance from the center of the coagulated FG on the board to the tip of the FG dripped vertically and the transverse diameter of the coagulated FG on the board (Fig. 1).

IV. Tensile strength test

To measure the adhesive performance of FG vis-à-vis tissue slices, the tensile strength test was performed. Solutions A and B (0.1 mL each) were applied to the bonding surface (20 × 5 mm) of two slices of pig skin (10 × 20 × 40 mm; Fig. 2A). After bonding, the slices were allowed to stand for 30 min at 23°C; then, using a tensile tester (Instron, 5582 Universal Testing Machine, Kawasaki), we pulled them vertically at 10 mm/min (Fig. 2B). The tensile strength at which the bond separated was divided by the adhesion area of 100 mm^2 to measure adhesion strength per 1 mm^2 (gf/mm^2).

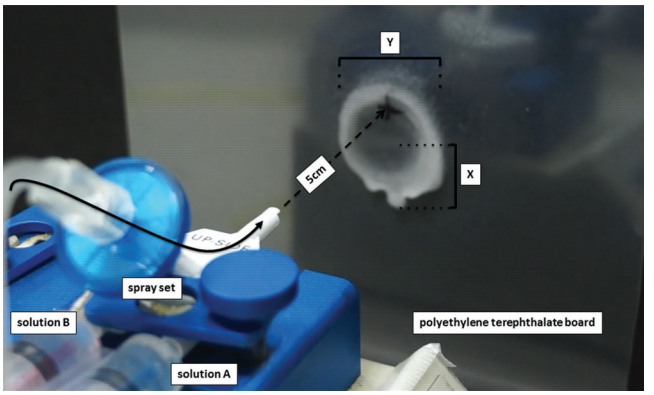
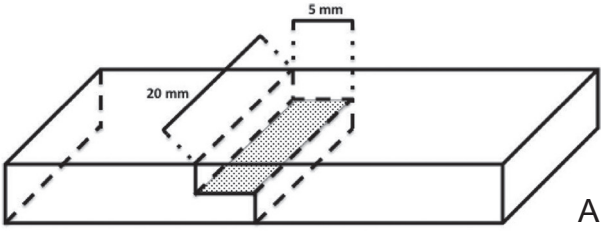


Fig. 1 Solutions A and B were separately connected to a spray set (CSL Behring), and they were sprayed via air pressure by compression with a microsyringe pump (Fusion 100, Chemyx Inc.). The horizontal distance from the spray tip to the board was set at 5 cm. X: Distance between the center of the coagulated glue and the tip of the dripped glue. Y: Transverse diameter of the coagulated glue. *Curved arrow*: Direction of the air for spray pressure.



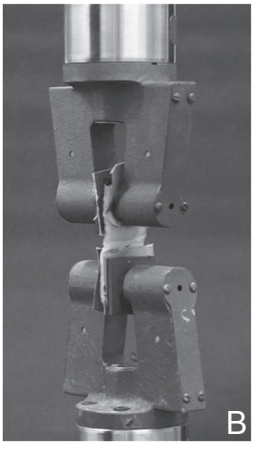


Fig. 2 A: Schematic diagram of the glued pig skin. Two sheets of pig skin were glued (*dot area*) by applying fibrin glue. B: The glued pig skin slices were pulled vertically at 10 mm/min, using a tensile tester.

V. Histological survey

The pig skin tissue fragments that were bonded together using the two FGs were then cut into 10-μm slices and stained with hematoxylin and eosin (H&E). The condition of the adhered skin slices was observed histologically using an optical microscope (Primo Star iLED, Zeiss, Tokyo).

VI. Statistical analysis

Statistical analyses were performed with SPSS software (SPSS Inc., Chicago, Illinois, USA), and numerical variables were analyzed using Student's *t*-test. The significance of the differences between the experimental conditions was determined using one-way analysis of variance followed by the Bonfferoni (Dunn) method. Values were presented as mean ± standard deviation, and $p < 0.01$ was considered statistically significant.

Results

I. Viscosity of FG

The viscosity of the fibrin monomer used in solution A of both FGs was significantly higher in the BP group (42.9 ± 2.16 vs. 18.7 ± 1.54 mPa•s, $p < 0.0001$; Fig. 3).

II. Clotting ability of FG

The dripping distance was significantly smaller for BP than BH, regardless of the spray pressure (7.5 mL/min: 33.8 ± 3.76 vs. 83.8 ± 16.7 mm, $p < 0.0001$; 15 mL/min: 44.7 ± 18.2 vs. 109.3 ± 19.3 mm, $p = 0.0001$). No statistically significant differences in spray pressure or dripping distance were observed between the FGs (7.5 mL/min, $p = 0.18$; 15 mL/min, $p = 0.03$; Fig. 4A). A study of the

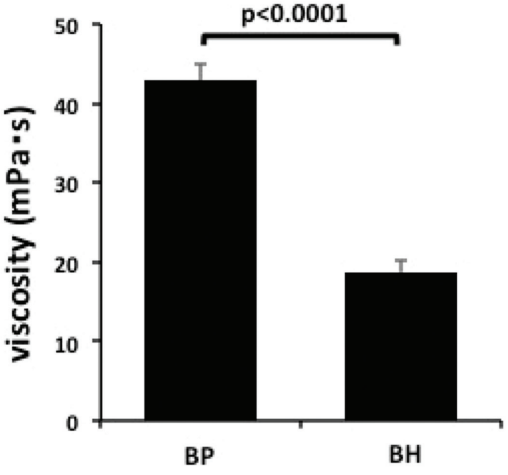


Fig. 3 Using solution A of both fibrin glue (FG) types, we measured the viscosity of the fibrin monomer using a rotational viscometer. The FG derived from blood donated in Europe and the United States (BP) group had statistically significantly higher viscosity than the FG derived from blood donated in Japan (BH) group. Values are presented as mean ± standard deviation (SD) (n = 6). BH: Bolheal®, BP: Beriplast®.

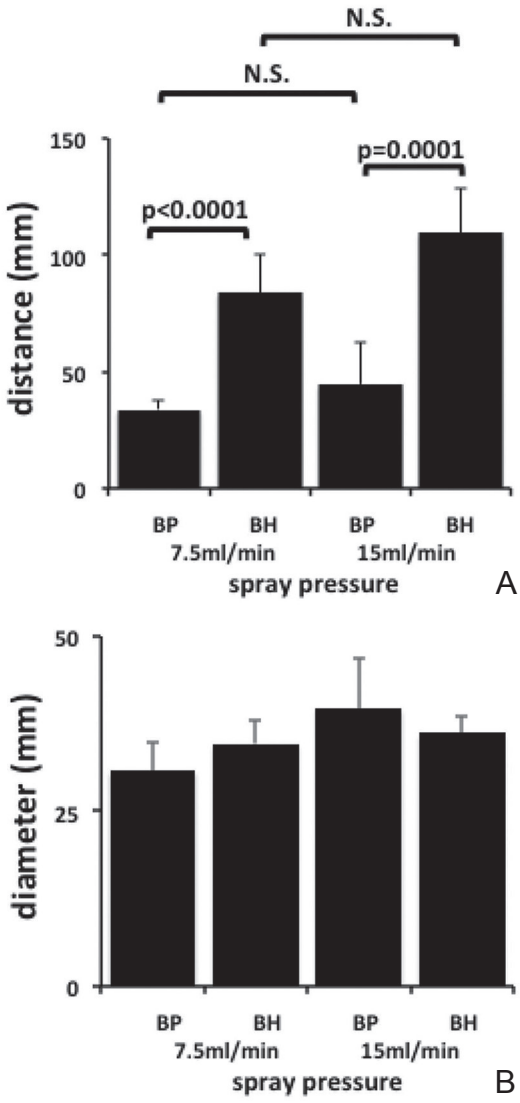


Fig. 4 A: Fibrin glue (FG) derived from blood donated in Europe and the United States (BP) had a statistically significantly smaller dripping distance than FG derived from blood donated in Japan (BH), regardless of spray pressure. No statistically significant differences in spray pressure and dripping distance were observed between the two FG types. Values are presented as mean ± SD (n = 6). B: The transverse diameter of FG that had coagulated on the vertical surface was not statistically significant different from the transverse diameter and FG type, regardless of spray pressure. Values are presented as mean ± SD (n = 6). BH: Bolheal®, BP: Beriplast®, N.S.: not significant, SD: standard deviation.

transverse diameters of FGs that had coagulated on a vertical surface showed no statistically significant differences between transverse diameter and FG type at both spray pressure values (7.5 mL/min: 30.8 ± 3.92 vs. 34.7 ± 3.20 mm, p = 0.95; 15 mL/min: 39.7 ± 7.31 vs. 36.1 ± 2.40 mm, p = 0.14; Fig. 4B). These findings suggest that although no differences were observed in the spread of each FG at different spraying pressures, there was a possibility that on a vertical surface, FG might drip as a result of differences in viscosity.

III. Adhesibility of FG

The adhesive strength when tissue slices were separated was statistically significantly greater in the BP group, suggesting that this FG type most likely had greater adhesive strength (1.99 ± 0.40 vs. 1.39 ± 0.31 gf/mm^2, p = 0.008; Fig. 5A).

IV. Histopathological findings

A histological study in which pig skin tissue fragments were stained with H&E revealed that the fragments were glued together closely in both groups (Fig. 5B, C); therefore, both types of FG achieved favorable histological adhesions.

Discussion

Fibrin is formed by mixing fibrinogen and thrombin, to achieve the necessary hemostatic effect and tissue-adhesive performance. The higher the viscosity of the FG, the lower its risk of dripping, which makes it easier to prevent unwanted adhesion to surrounding healthy tissue. Considering that the BP group had higher viscosity in our test, this type of FG is likely able to prevent accidental adhesions to healthy tissue. In the dripping test in a way that more closely approximated surgical conditions, the results showed less dripping of the FG in the BP group. This confirmed the high viscosity of BP, as did the viscosity test. Its superior usability has been reported when applied with healthy tissues and their periphery in some neurosurgical procedures.[1–3] However, aggravation of symptoms attributable to FG[7] and triggering of inflammation in healthy tissues[8] have also been reported. In the wrapping of cerebral aneurysms, parent artery narrowing was reported to be caused by FG.[9] Thus, high-viscosity FG that shows a minimum of dripping onto healthy tissues would appear preferable for hemostatic and adhesive operations in surgical fields that are sloping, cramped, and surrounded by healthy tissue, as in the wrapping of a cerebral aneurysm and in the transposition of offending vessels in microvascular decompression.

The two FG types have different preservation requirements when freeze-dried, and also for preservation period and temperature. Although only few reports describe the relationship between these requirements and fibrinogen activity,[10–12] as to what extent they derive from the methods by which FG is manufactured remains unclear. Since BP contains greater amount of thrombin, the viscosity of BP might be superior to that of BH, however, the viscosity test of solution A in this report was not related to thrombin. It can be assumed that in terms of the countries of origin, BP has numerous Anglo-Saxon donors; and BH, Asian donors. According to previous reports, fibrinogen concentrations may differ between races.[13,14] The relationship between fibrinogen polymorphism and

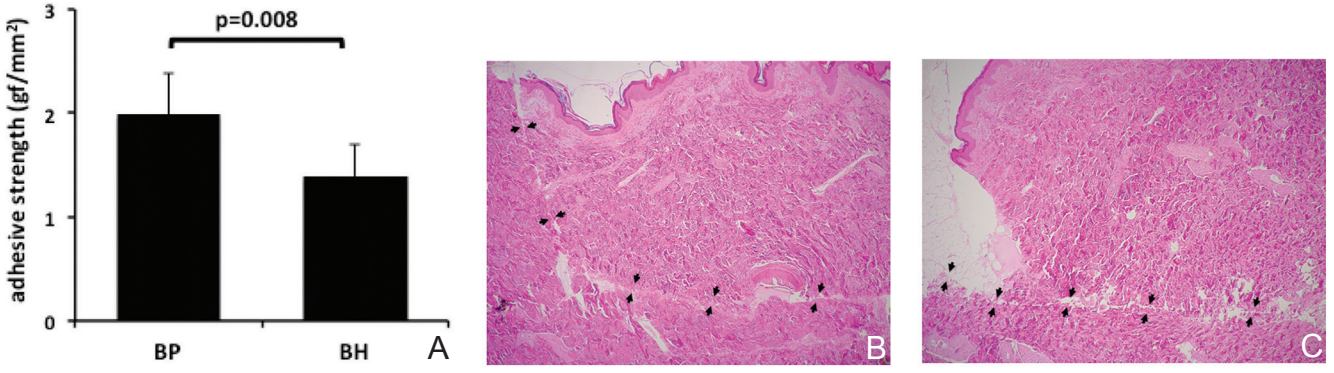


Fig. 5 A: The glued pig skin slices were pulled using a tensile tester. The adhesive strength at which the bond came off appeared to be significantly greater in the fibrin glue (FG) derived from blood donated in Europe and the United States (BP) than in the FG derived from blood donated in Japan (BH), indicating that BP group had greater adhesive strength. Values are presented as mean ± SD (n = 6). B, C: H&E staining of the pig tissue fragments bonded with fibrin glue. Both BP (B) and BH (C) could effectively glue the tissue fragments closely together. Original magnification ×40, *arrow*: bonding surface. H&E: hematoxylin-eosin, SD: standard deviation.

venous thromboembolism in Caucasians, and the relationship with sporadic cerebral hemorrhage caused by fibrinogen polymorphism in Chinese were reported.[15,16] Moreover, the influence of fibrinogen genes on the haplotype and structure of fibrin networks have also been reported,[17] suggesting the possibility that the time needed for fibrinogen to transform into fibrin and viscosity may vary owing to differences in the function of fibrinogen that are attributable to race and genetic makeup.

The tensile strength test revealed differences in adhesion strength between the two FGs. With the tissue fragments that were glued together, however, no major differences were found in the histological evaluations between the two groups. No relationships were found between the use of FG and cerebrospinal fluid (CSF) leakage.[18] Unless a strong external force is applied, the strength of the two FGs is highly similar. Meanwhile, a relationship was also reported between FG and the burst pressure of the dura mater in duraplasty,[19,20] indicating that a watertight closure may break if excessive CSF pressure is applied during the clotting of FG. Thus, FG with outstanding pressure resistance may also be needed for surgery where CSF pressure is applied. Methods of FG application other than spraying have also been reported. Nakajima et al. reported that in duraplasty using BH, the rubbing method showed greater pressure resistance than spraying.[21] Therefore, a FG-type with low viscosity that coagulates more slowly seems to be better suited for this type of usage. In clinical situations, FG is sprayed intermittently and repeatedly. If spraying is interrupted, BP is more likely to clot at the spray tip than BH. Additionally, our results raised the possibility that BP might be feasible for the pinpoint hemostasis of severe arterial bleeding in the narrow field, whereas BH might be feasible for the hemostasis of oozing at the time of dura closure.

Our study focused exclusively on the viscosity and adhesive performance between two FG types with the use of a spray device for BP, and was not performed in the human environment assuming the body temperature. Process patents also made it impossible to study the manufacturing method of each FG type; thus, further evaluations are needed. Our findings suggest, however, that each FG has its own distinct characteristics, which need to be investigated to optimize their use in different surgical situations.

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Conflicts of Interest Disclosure

The authors have no personal, financial, or institutional interest in any of the drugs, materials, or devices in the article. All authors who are members of The Japan Neurosurgical Society (JNS) have registered online Self-reported COI Disclosure Statement Forms through the website for JNS members.

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Address reprint requests to: Takuro Hayashi, MD, PhD, Department of Neurosurgery, National Hospital Organization Tokyo Medical Center, 2–5-1 Higashigaoka, Meguro-ku, Tokyo 152-8902, Japan.
e-mail: villacasa0416@gmail.com