Adhesive Strength of Autologous Fibrin Glue

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To establish an easy and rapid method for measuring the adhesive strength of fibrin glue and to clarify the factor(s) most affecting the strength, a study was made on the effect of the concentration of plasma components on the strength of cryoprecipitate (Cryo) prepared from a subject's own autologous plasma to be used as fibrin glue. The adhesive strength of the Cryo was measured with various supporting materials instead of animal skin using a tester of tension and compression. The results were as follows: (1) the strength of Cryo applied to ground flat glass (4 cm²) was significantly greater than that applied to clear glass, clear plastic, or smooth and flat wood chips; (2) the adhesive strength of Cryo depended on the concentration of thrombin with the optimal concentration being 50 units/ml; (3) the concentration of CaCl₂ did not affect the adhesive strength of Cryo; (4) the adhesive reaction was dependent on the temperature and the adhesive strength more quickly reached a steady state at 37 °C than at lower temperature; (5) the adhesive strength was correlated well with the total concentration of fibrinogen and fibronectin. These results indicate that the adhesive strength of Cryo can be easily and quickly evaluated using a tester and ground glass with thrombin at 50 units/ml, and that the adhesive strength of Cryo can be predicted from the total concentration of fibrinogen and fibronectin.

Key words fibrin glue; adhesive strength; fibrinogen; fibronectin; cryoprecipitate; autologous plasma

Fibrin glue consists of fibrinogen, coagulation factor XIII (F-XIII), thrombin, CaCl₂, and aprotinin as an inhibitor of the fibrinolytic system.¹⁾ Fibrin glue coagulates and adheres to bleeding tissues.²⁾ Several types of fibrin glue products are commercially available and are widely used as a biological adhesive in surgical operations.³⁾ However, these products have some problems.^{4,5)} The patients in whom the fibrin glue is used are placed at risk for infection by thermostable pathogenicities,^{6,7)} and for allergic reactions to subtype components of the glue.^{8–11)} To prevent these adverse effects, cryoprecipitate (Cryo) produced from the patient's own autologous plasma is recommended for use in fibrin glue.^{12,13)} In our previous paper,¹⁴⁾ we described that Cryo containing high concentrations of coagulation factors could be prepared easily and rapidly from a patient's autologous plasma (within 4–5 h) with shaking at 70 cycles/min, freezing at –20 °C, and defrosting in a 4 °C water bath. Generally, as the adhesive strength of fibrin glue is weak compared with that of a chemical adhesive such as Aron Alpha, it is difficult to measure. Some researchers have measured it using animal skin and a spring scale.^{15,16)} These procedures are complicated making it difficult to obtain reproducible results. The purpose of this study is to establish an easy and rapid measurement method for adhesive strength of fibrin glue and to clarify the factor(s) affecting the strength.

MATERIALS AND METHODS

Materials The thrombin, aprotinin, and CaCl₂ solutions used were Thrombin-Yoshitomi (10000 U/vial) (Yoshitomi Pharm. Indust., Osaka), Trasyrol (10000 U/ml) (Bayer Yakuhin Ltd., Osaka), and 2% CaCl₂-Otsuka (Otsuka Pharm. Ind., Tokyo), respectively.

Human plasma of healthy volunteers, excluded by alanine aminotransferase (ALT) test, were obtained from the Yamaguchi Red Cross Blood Center (Yamaguchi, Japan).¹⁷⁾

The assay kits used for fibrinogen, plasminogen, fibronectin, F-XIII and fibrin degradation products were ILtest

PT-Fibrinogen (Coulter Co., Tokyo), ILtest Plasminogen (Coulter), Fibronectin (Opsonic Protein) (Boehringer Mannheim Co., Tokyo), Latex Kit F-XIII (Hoechst Japan, Tokyo), and FDP reagent (ND) (International Reagent Co., Kobe), respectively. All chemicals used were obtained from commercial sources and were of analytical reagent grade.

Preparation of Cryo Preparation procedures were described in our previous paper in detail.¹⁴⁾ Namely, human plasma was treated under the following conditions. The plasma was frozen in a –20 °C freezer (MDF-U536, Sanyo Medica Systems Co., Osaka). It was shaken during the freezing period by a horizontal shaker (NR-3, Taitec Co., Saitama). The shaking speed was 70 cycles/min. The plasma was then defrosted in a cold water bath (4 °C). Defrosted plasma was centrifuged at 3000 rpm for 15 min at 0–4 °C, and then the supernatant was removed. The precipitation was collected as Cryo.

Preparation of Fibronectin-poor Cryo Primary, single donor plasma was divided into several vessels, and treated with a fifth, a tenth, or a twentieth of gelatin-coupled Celulofine (Chisso Co., Tokyo). Fibronectin was partially or completely removed from the human plasma, as it has a high affinity for gelatin.¹⁸⁾ Next, this plasma was treated as above for Cryo. The final precipitation was collected as fibronectin-poor Cryo.

Measurement of Adhesive Strength The adhesive strength of each prepared Cryo was measured as follows: two pieces of ground or clear flat glass (prepared from Micro slide glass of S-2215, Matsunami Glass Ind., Ltd., Osaka), clear vinyl chloride plates, or smooth and flat wood chips of 4 cm², bound to cubic wood with chemical adhesive, were prepared. Ten μ l of Cryo mixed with aprotinin (400 units/ml) was applied to one piece and 10 μ l of a given concentration mixture of thrombin and CaCl₂ to the other. The two surfaces were adhered and pressed at 50 g/cm² for 30 s. After 10 min, the materials adhered through Cryo were fixed in a tester of tension and compression (SV-55, Imada Manufact. Co., Ltd., Toyohashi) and the tensile strength of each Cryo preparation

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Fig. 1. Effect of Supporting Material on the Adhesive Strength of Cryo

Thrombin: 50 units/ml, CaCl_2 : 0.5%, temperature: 26 °C, * $p < 0.05$, ※ $p < 0.001$ (vs. ground glass, paired t -test). Mean $\pm$ S.D. ($n = 10$).

was monitored in the vertical direction for adhesive surface. The adhesive strength was determined as the maximum strength.

Analytical Methods The concentration of fibrinogen in each plasma sample and Cryo was determined using commercial kits supplied with the automated coagulation analyzer-ACL3000 (Coulter Co., Tokyo). Fibronectin was determined by the immunoprecipitation method. F-XIII was determined by the latex agglutination method.

Statistical Analysis Each value was expressed as the mean $\pm$ S.D. of experiments. Student's t -test was carried out for the statistical evaluation of a difference and a p value of 0.05 or less was considered significant.

RESULTS AND DISCUSSION

Effect of Supporting Material on the Adhesive Strength of Cryo To identify the most compatible supporting material with which to measure the adhesive strength of Cryo, Cryo was applied to ground glass, clear glass, clear vinyl chloride plate, or smooth surface wood and the strength was monitored. As shown in Fig. 1, the Cryo applied to ground glass was significantly stronger than that on other plates. As the Cryo applied to smooth surface wood was absorbed by the wood, the strength could not be measured. The contact surface of ground glass is the broadest of all the materials tested except wood, which may explain the superior strength. Moreover, the surface of the vinyl chloride plate is probably uneven compared with that of clear glass and the thickness of glue between the plates of the former is not uniform, so the adhesive strength of the former may be weaker than that of the latter. On the basis of these results, the adhesive strength was monitored using ground flat glass in all subsequent experiments.

Casali B *et al.* reported that the adhesive strength of fibrin glue containing 78 mg/ml of fibrinogen was 117 g/cm².¹⁵⁾ Discrepancies in measured adhesive strength may be due to different methods, since they used the Gottlob mouse skin method and we used the ground glass method. Namely, we measured the adhesive strength vertical to the adhesive surface, while they probably measured it horizontal to the surface.

Effect of Thrombin Concentration on the Adhesive

Fig. 2. Effect of Thrombin Concentration on the Adhesive Strength of Cryo

CaCl_2 : 0.5%, temperature: 26 °C, * $p < 0.05$, # $p < 0.01$, ※ $p < 0.001$ (vs. 50 units/ml, paired t -test). Mean $\pm$ S.D. ($n = 4$).

Strength of Cryo An experiment was performed to clarify the effect of thrombin concentration on the adhesive strength of the Cryo. As shown in Fig. 2, the maximum strength of Cryo was achieved at a thrombin concentration of 50 units/ml. Namely, the adhesive strength gradually increased with increases of thrombin concentration until 50 units/ml and then gradually decreased at concentrations above 50 units/ml. There was no significant difference in adhesive strength at 50 and 100 units/ml of thrombin. The reason why the adhesive strength gradually decreased with increases of thrombin concentration above 50 units/ml is probably that the association of fibrin produced by thrombin is inhibited with increases of thrombin (Zone phenomenon), or that the adhesion of polymerized fibrin on glass-applied thrombin is inhibited by excess thrombin.

Effect of CaCl_2 Concentration on the Adhesive Strength of Cryo To clarify the effect of CaCl_2 concentration on the adhesive strength of Cryo, various concentrations of CaCl_2 were applied to ground flat glass with or without thrombin at 50 units/ml and the strength of the Cryo was monitored. As shown in Fig. 3A, the adhesive strength of Cryo was very weak and could not be measured at each concentration of CaCl_2 without thrombin. On the other hand, the adhesive strength could be measured comfortably at each concentration of CaCl_2 with thrombin, being similar for each CaCl_2 concentration (Fig. 3B). These results indicate that the adhesive strength of Cryo was not affected by the concentration of CaCl_2 under our experimental conditions.

Effect of Temperature on the Adhesive Strength of Cryo To clarify the effect of temperature on the adhesive strength of the Cryo, the time course of adhesive strength of Cryo was monitored at 4, 26 (room temperature), and 37 °C. As shown in Fig. 4, the time courses differed remarkably depending on the reaction temperature. Namely, the adhesive strength increased gradually and was maximal approximately 10 min after Cryo was reacted with thrombin at 26 °C. The strength of Cryo was markedly and immediately increased at 37 °C. On the other hand, the strength of Cryo was more slowly increased at 4 °C. These results indicate that the adhesive reaction is dependent on the temperature and accelerated with increases in temperature. So, the adhesive strength

Fig. 3. Effect of CaCl₂ Concentration on the Adhesive Strength of Cryo

A: without thrombin, B: with thrombin at 50 units/ml, temperature: 26 °C. Mean ± S.D. (n=4).

Fig. 4. Time Courses of Adhesive Strength of Cryo at Various Reaction Temperatures

▲, 4 °C; ■, 26 °C; ●, 37 °C. Thrombin: 50 units/ml, CaCl₂: 0.5%, * $p < 0.05$, * $p < 0.001$ (vs. 26 °C, paired t -test). Mean ± S.D. (n=4).

Fig. 5. Relationship between Adhesive Strength and Concentration of Plasma Components in Cryo

Thrombin: 50 units/ml, CaCl₂: 0.5%, temperature: 26 °C. The correlation coefficient of fibrinogen, fibronectin, and F-XIII vs. adhesive strength is 0.909 ($p < 0.001$), 0.797 ($p < 0.01$), and 0.830 ($p < 0.01$), respectively.

should be monitored 10 min after Cryo is reacted with thrombin at 26 °C.

Effect of the Concentration of Plasma Components on the Adhesive Strength To clarify the effect of the concentration of fibrinogen, fibronectin, and F-XIII in Cryo on the adhesive strength, the relation between the adhesive strength and the concentration of each plasma component of various samples was studied. As shown in Fig. 5, for each plasma component, the concentration correlated well with the strength. Mino *et al.* reported that the adhesive strength of fibrin sealant correlated closely with the concentration of fibrinogen, and that the effect of F-XIII on the adhesive strength was dependent on the concentration of fibrinogen.¹⁶⁾ In this experiment, we recognized a relationship between the adhesive strength and fibronectin, or F-XIII, while these factors were correlated with fibrinogen (Fig. 6). So, it is unlikely that fibronectin and F-XIII are the main factors affecting the adhesive strength of Cryo.

Thus, to clarify the effect of fibronectin on the adhesive strength, fibronectin-poor Cryo was prepared and the relationship between the adhesive strength and the concentration of fibrinogen, fibronectin or F-XIII in each preparation was

Fig. 6. Relationship between the Concentration of Fibrinogen vs. F-XIII and Fibronectin

Thrombin: 50 units/ml, CaCl₂: 0.5%, temperature: 26 °C. The relation constant of fibrinogen vs. fibronectin, and F-XIII is 0.841 ($p < 0.01$), and 0.768 ($p < 0.01$), respectively.

examined. As shown in Fig. 7, the adhesive strength was correlated well with the concentration of fibrinogen ($r=0.951$) and fibronectin ($r=0.800$) in each Cryo, but the relationship

Fig. 7. Relationship between Adhesive Strength and Concentration of Plasma Components in Fibrinogen-poor Cryo

Thrombin: 50 units/ml, CaCl_2 : 0.5%, temperature: 26°C. The correlation coefficient of fibrinogen, fibronectin, and F-XIII vs. adhesive strength is 0.951 ($p < 0.001$), 0.800 ($p < 0.02$), and 0.297 ($p > 0.4$), respectively.

Fig. 8. Relationship between Adhesive Strength and Sum of Concentration of Fibrinogen and Fibronectin in Cryo

The correlation coefficient of fibrinogen and fibronectin vs. adhesive strength for the data of Fig. 5 (●), Fig. 7 (◆), and reference 21 (△) is 0.920 ($p < 0.001$), 0.893 ($p < 0.01$), and 0.927 ($p < 0.001$), respectively.

between the strength and the concentration of F-XIII was not so good ($r = 0.297$). It is well known that F-XIII is a stabilizing factor for fibrin polymerization. Inoue *et al.* described that F-XIII only slightly affected the adhesive strength of Cryo at a constant concentration of fibrinogen.¹⁹ So, F-XIII is not the main factor affecting the adhesive strength of Cryo at least according to our measurements. The reason for the good correlation between F-XIII and adhesive strength in Fig. 5 may be that the concentration of F-XIII corresponded to the concentration of fibronectin and fibrinogen (Fig. 6). The regression line of the strength vs. fibrinogen content and fibronectin content shifted markedly from the origin in the lower and upper direction, respectively. Thus, the relation between the adhesive strength and sum of content of fibrinogen and fibronectin was plotted. As shown in Fig. 8, a good correlation was observed between the adhesive strength and sum of both components. The regression line of the strength and sum of both components nearly crossed the origin. These results indicate that fibrinogen and fibronectin greatly contribute to the adhesive strength of Cryo and collaborate as biological adhesive. Generally, the concentration of fibrinogen in autologous fibrin glue is lower than that in commercial fib-

rin sealant,^{3,20} but the hemostasis effect on the operating field with the former is the same as or superior than that with the latter.¹² Much fibronectin is contained in the former but not in the latter. It is well known that fibronectin acts as an adhesion factor between fibrin and biological tissue or ground glass. The reason why the former is superior as adhesive sealant may be that much fibronectin is contained in it.

In summary, in an attempt to establish an easy method of measuring the adhesive strength of fibrin glue and to clarify the factor(s) most affecting this strength, a study was made on the effect of the concentration of plasma components on the strength of Cryo prepared from the subject's own autologous plasma. We obtained the following results. (1) The strength of Cryo applied to ground flat glass was significantly higher than that applied to other supporting materials. (2) The adhesive strength of Cryo was dependent on the concentration of thrombin with the optimal concentration being 50 units/ml. (3) The concentration of CaCl_2 did not affect the adhesive strength of Cryo. (4) The adhesive reaction was dependent on the temperature and the adhesive strength more quickly reached a steady state at 37°C than at lower temperature. (5) The adhesive strength correlated well with the total concentration of fibrinogen and fibronectin. These results indicate that the adhesive strength of Cryo can be easily and quickly evaluated using a tester and ground glass with thrombin at 50 units/ml, and that the strength can be predicted from the total concentrations of fibrinogen and fibronectin.

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