Follow-up papers - Experimental

Experimental use of an elastomeric surgical sealant for arterial hemostasis and its long-term tissue response

Shinichiro Oda\textsuperscript{a}, Shigeki Morita\textsuperscript{b,1,*}, Yoshihisa Tanoue\textsuperscript{a}, Masataka Eto\textsuperscript{a}, Takehisa Matsuda\textsuperscript{c}, Ryuji Tominaga\textsuperscript{a}

\textsuperscript{a}Department of Cardiovascular Surgery, Kyushu University, Fukuoka, Japan
\textsuperscript{b}Department of Thoracic and Cardiovascular Surgery, Faculty of Medicine, Saga University, 5-1-1 Nabeshima, Saga 849-8501, Japan
\textsuperscript{c}Kanazawa Institute of Technology, Kanazawa, Japan

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Abstract

Objective: Reliable suture line hemostasis should improve the outcome of aortic surgery. We examined the hemostatic effect and the tissue response of a novel elastomeric surgical sealant. Methods: Using porcine internal carotid arteries, we performed 16 end-to-end anastomoses with four stitches of simple interrupted sutures under full heparinization. The anastomoses were divided into two groups (eight anastomoses per group). Either novel sealant or fibrin glue was applied. The amount of bleeding was measured during the 30 s period after removing the vascular clamp. In a separate experiment, we applied the novel sealant around the abdominal aorta of rabbits (n=6) to assess the effect of the elastomeric property of the sealant on arterial wall histology. For comparison, we applied cyanoacrylate, which has no elastomeric property (n=6). A histological study was performed three months after the operation. Results: The novel sealant prevented arterial bleeding. The amount of bleeding from the anastomoses applied with novel sealant and fibrin glue was 0.12 ± 0.03 g vs. 91.8 ± 16.5 g, respectively (P<0.001). Thinning of the rabbit aortic wall was observed in the cyanoacrylate-treated abdominal aorta, whereas no thinning was observed in the novel sealant group. Histological examination revealed neither cell death nor necrosis in the novel sealant group. Conclusions: The novel sealant effectively prevented arterial bleeding from the anastomosis under full heparinization. In addition, the elastomeric property of the sealant prevented thinning of the aortic wall. The novel sealant may be a promising hemostatic agent for arterial anastomosis.

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1. Introduction

Bleeding is one of the most serious problems in cardiovascular surgery. Various surgical adhesives, glue or sealants have been used to prevent bleeding, and their application often reduces blood loss, minimizes frequency of transfusion, shortens operative time, reduces occurrence of post-operative bleeding, avoids the need for resternotomy, and decreases mortality [1–4]. However, especially in aortic surgery, because of post-bypass coagulopathy and tissue fragility, the hemostatic effectiveness of such adhesives is often unreliable, resulting in failure of bleeding control. Hence, high-performance or ideal surgical adhesive or sealant should not be linked with the blood coagulation cascade, should work well on highly moisturized tissues and should provide compliance matching with native arteries, all of which contribute to enhance hemostatic efficacy.

The authors previously designed a prototype sealant, which includes new concepts, new adhesion mechanism and chemistry, and conducted fundamental studies on performances vs. molecular parameters in a canine arterial model [5, 6]. We recently optimized performance and determined the final version of a highly reliable sealant (government-supervised clinical trial in Japan is almost completed). This novel elastomeric surgical sealant is a viscous liquid, which is a highly reactive copolymer of polyethylene glycol (PEG) and polypropylene glycol (PPG), in which both ends were capped with non-carcinogenic fluorinated hexamethylene disocyanate [7]. It is not necessary to mix any component to the sealant. The sealant starts to polymerize when it contacts to water. Following application to wet or moisturized tissues, the elastomeric sealant is spontaneously transformed to porous urethane rubber foam which functions as a hemostatic sealant for arterial anastomosis.

The characteristic features of this sealant, as compared with clinically used surgical adhesives or glue, such as fibrin glue, Bioglue and GRF glue, has high water absorption and high affinity towards water. These properties are advantageous for use on a moisturized or wet tissue, enabling a high degree of contact with topologically roughened tissue surface and formation of microporous elastomeric foam. The elastomeric nature of completely cured or hardened sealant provides much closer compliance with native arter-
ies than cyanoacrylate glue that becomes plastic as cured. This elasticity must minimize stress concentration generated at the site of anastomosis under high-pressure pulsatile arterial flow. Since the sealant developed is totally synthetic, occurrence of viral transmission associated with biological substances is ruled out.

In this study, we report how bleeding on highly moisturized arterial tissues is prevented with minimal stitching and full heparinization as compared with fibrin glue. Furthermore, we report how the architecture of sealant-coated arterial tissue is preserved at long-term implantation as compared with cyanoacrylate glue, which has no elastomeric property.

2. Materials and methods

2.1. Materials

The elastomeric sealant was prepared and provided by Sanyo Chemical Industries (Kyoto, Japan). Briefly, liquid copolymer of PEG and PPG (composition; 80:20 in weight; molecular weight; 4300 g/mol) was end-capped with fluorinated hexamethylene isocyanate. Fibrin sealant was purchased from Sanofi-Aventis (Tokyo, Japan). Cyanoacrylate, commercially available for surgery, was purchased from Daichi-Sankyo (Tokyo, Japan).

2.2. Animal care

All animals were treated in compliance with the ‘Guide for the Care and Use of Laboratory Animals’ published by the US National Institutes of Health (NIH publication 85-23, revised 1996). The protocols were reviewed by the Ethics Committee on Animal Experiments in the Faculty of Medicine, Kyushu University, and carried out under the Guidelines for Animal Experimentation in the Faculty of Medicine, Kyushu University and in accordance with the law (No. 105) and notification (No. 6) of the Japanese government.

2.3. Hemostatic efficacy

A total of 16 porcine internal carotid artery anastomoses were used. All pigs were anesthetized with ketamine (20 mg/kg intramuscularly). After endotracheal intubation, pigs were artificially ventilated with 100% oxygen. Anesthesia was maintained with inhaled isofluorane (1–3%), and muscle relaxation was induced with pancuronium (0.1 mg/kg intravenously). An arterial catheter was inserted into the femoral artery to monitor the arterial pressure and to take blood samples for analysis of activated coagulation time (ACT). ACT was maintained over 500 s by heparin injection during the procedure.

Both internal carotid arteries were exposed. After placement of a single purse-string, 7-0 monofilament polypropylene suture was placed distal to the transection site for a balloon catheter insertion, the vessel was clamped, transected, and an end-to-end anastomosis with four simple interrupted stitches was performed. A balloon catheter (Fr 3.3, balloon; Φ3.5 mm) was inserted from a purse-string suture site and inflated at the anastomosis to prevent collapse of the vessel and to adapt the suture line (Fig. 1).

To show the novel sealant’s efficacy, we determined to use extreme circumstances, which were four simple interrupted sutures. For this technique, it was difficult to keep three-dimensional configurations to maintain internal lumen. So we used endovascular balloon to prevent collapse of the vessel [8]. For the clinical use, the sealant should be applied to the anastomosis, just like fibrin glue. Endovascular balloon is unnecessary.

In the elastomeric sealant group (n=8 carotid artery anastomoses), the sealant was first placed on a silicon sheet, and then the anastomosis site was wrapped with the sealant-coated sheet around the anastomosis for 3 min (Fig. 1). After the silicon sheet was removed to place a thin-layer of sealant on the tissue, the vessel was declamped and the balloon was deflated. The amount of bleeding was measured 30 s after removing the vascular clamp. In the fibrin glue group (n=8 carotid arteries), the glue was directly coated on the anastomosis (Fig. 1) and, after 3 min, the vessel clamp was removed and then the balloon was deflated. The amount of bleeding was measured 30 s after removing the clamp.

2.4. Tissue response

Twelve Japanese white rabbits were used in this experiment. All rabbits were anesthetized with an intravenous injection of pentobarbital sodium (10 mg/kg) after premedication with intramuscular injection of xylazine (2.5 mg/kg), and the abdominal aorta was exposed. The rabbits were randomized into two groups. The elastomeric sealant (n=6) or the cyanoacrylate glue (n=6) was coated around the abdominal aorta. After three months, the coated sites and the non-coated normal aortic sites were excised, fixed in 10% formalin solution, and embedded in paraffin. Transmural sections were stained with hematoxylin-eosin and elastica van Gieson for microscopic examination. The cross-sectional area of the vessel (Av) and the lumen (Al) were measured. The mean thickness of the vessel wall at the wrapped site (Tw) was calculated based on the following relationship: Tw=(Av/π)1/2−(Al/π)1/2. The same measurements were made for each control section and the mean thickness of the vessel wall of the control (Tc) was obtained. The ratio of Tw/Tc was used to assess the thickness of the vessel wall [9].
2.5. Statistical analysis

Data are presented as the mean±standard error (S.E.) of the mean. Comparisons between the groups were assessed by the unpaired t-test. A P<0.05 was considered statistically significant.

3. Results

3.1. Arterial bleeding

The amount of blood that bled for 30 s after removing the clamp was 91.8±16.5 g for the fibrin glue group and 0.12±0.03 g for the elastomeric sealant group (n=8 for both groups, P<0.001). Macroscopically, the novel sealant effectively prevented arterial bleeding completely, where-as severe bleeding was noticed for the fibrin glue group. There was no statistical difference in arterial pressure between the elastomeric sealant group and the fibrin glue group (Table 1). For both groups, ACT was maintained over 500 s by heparinization during the procedure. As shown in Fig. 1, the elastomeric sealant-coated silicone elastomer film was wrapped on the anastomotic site.

3.2. Tissue response

To examine the long-term effect of sealant on arterial wall histology, a histochemical study was conducted in comparison with cyanoacrylate glue. Transmural sections (stained with elastica van Gieson) of sealant- or glue-coated rabbit aorta at three months post-implantation (Fig. 2a), showed that the thicknesses of the vessel wall for the elastomeric sealant group and the cyanoacrylate glue group was 103±11% and 58±4% of control, respectively (P<0.01). For the elastomeric sealant, the histological sections did not show any damage to the aortic wall (Fig. 2b). In the cyanoacrylate glue group, disappearance of endothelial and medial smooth muscle cells, and thinning of the internal elastic lamina and the medial elastic fibers were observed (Fig. 2b).

4. Discussion

In our previous study [7], the preliminary hemostatic efficacy was qualitatively reported on canine carotid artery end-to-end anastomosis with minimal stitching under full heparinization condition: neither bleeding nor leakage of the sealant into the lumen side of arteries was observed. In addition, neither intimal hyperplasia, cell death nor necrosis was observed up to three months post-implanta-

![Image](https://academic.oup.com/icvts/article-abstract/10/2/258/645246/10228964zg6461-guest-on-13-April-2019)

**Fig. 2.** Effects of the novel sealant on the vessel wall. (a) Representative photomicrographs of elastica van Gieson staining of the aorta in the novel sealant group and the fibrin glue group. (b) Representative photomicrographs of hematoxylin-eosin staining of the aorta in the two groups. L indicates the lumen of the aorta. Bar=20 μm.

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<thead>
<tr>
<th>Table 1</th>
<th>Arterial pressure and activated coagulation time before and after application of sealants</th>
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<td>Before application</td>
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<td>Novel sealant (n=8)</td>
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<tr>
<td>Systolic AP</td>
<td>105.6±8.7</td>
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<tr>
<td>Diastolic AP</td>
<td>67.1±8.2</td>
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<td>ACT (s)</td>
<td>&gt;500</td>
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Data are mean±S.E. of the mean. ACT, activated coagulation time; AP, arterial pressure; S.E., standard error.
chemistry) influence on thinning of the vessel wall using the cyanoacrylate.

High hemostatic efficacy of this elastomeric sealant at anastomotic sites under arterial pressure may be a function of the following. First, the liquid viscous sealant rapidly absorbs water on tissues, and subsequently flows into the topological void space on the tissue. The liquid viscous sealant does not solidify for a few minutes, allowing enough time to flow into the void space on the tissue, thus ensuring firm contact (called mechanical interlocking). Additionally, it is postulated that chemical bonding between amino group of the tissue surface and isocyanate group of the sealant strengthens the cohesiveness.

Since cyclic distention of arteries generated by pulsatile blood flow causes stress at anastomotic sites, compliant or elastomeric sealants favor the minimization of stress. In fact, anastomotic sites coated with elastomeric sealant synchronously pulsate with contiguous arteries, whereas no pulsation was noted for cyanoacrylate glue-coated arteries. This highly elastomeric property is derived from the inherent elasticity of polymerized sealant and its microporous structure which is produced by the formation of bubbles of carbon dioxide gas generated during the reaction of isocyanate group and water during polymerization of liquid sealant to elastomeric foam [7]. Our previous study showed that the cured elastomeric foam exhibits maximally eight-fold stretching under water.

In conclusion, we believe that the elastomeric sealant object of this study has definite promise for use in aortic surgery, where fragile tissues and coagulopathy lead to bleeding. High cohesiveness of the sealant under moisturized condition and full heparinization and its minimal tissue damage and lack of potential for viral transmission represent a significant advance over current adhesives. Since in our experience the application of the sealant produces technically elegant, simple and reliable arterial anastomoses, we believe that clinical application of this particular sealant could reduce morbidity and mortality in aortic surgery. The clinical trial study (targeted clinical case: dissected aorta) under the supervision of National Pharmaceuticals and Medical Devices Agency, Japan will be completed in the very near future.

References