Alaska Pollock-derived Gelatin Sealant has Higher Sealing Strength than, and Comparable Biocompatibility with, Fibrin Sealant in Porcine and Rat Dural Injury Models

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Study Design. Burst strength study in porcine dural models and functional and histological study in rat dural models.

Objective. This study aimed to investigate the sealing strength and biocompatibility of Alaska pollock-derived gelatin (ApGltn) and fibrin sealants in disrupted dural injuries.

Summary of Background Data. Disruption of the dura mater occurs during spine surgery, leading to cerebrospinal fluid leakage. Fibrin sealant is usually applied to ruptured sites; however, it lacks sealing strength. A novel biocompatible sealant composed of ApGltn was recently demonstrated to have good burst strength and biocompatibility in the porcine aorta.

Methods. Ten porcine dura maters with central holes were covered with ApGltn and fibrin sealants (five samples per group). The maximum burst strength of each sealant was measured, and histological examination was performed after burst testing. Twenty-seven dura maters of male Wistar rats were used for functional and histopathological evaluations. The rats were treated with three surgical interventions: defect + ApGltn sealant; defect + fibrin sealant; defect alone (nine rats per group). Macroscopic confirmation of the sealant, hindlimb motor function analysis, and histopathological examination were performed at two, four, and eight weeks after the procedure.

Results. The maximum burst strength of the ApGltn sealant was ~4.4 times higher than that of the fibrin sealant (68.1 ± 12.1 *vs.* 15.6 ± 8.7 mmHg; $P < 0.001$). Histological examination confirmed that the ApGltn sealant showed tight adhesion to the dural surface, whereas a gap was observed between the fibrin sealant and the dura mater. In the rat model, the ApGltn sealant resulted in spinal function and dural histological findings similar to those of the fibrin sealant.

Conclusion. The ApGltn sealant had a higher sealing strength than, and comparable effect on dura regeneration with, the fibrin sealant.

Key Words: Alaska pollock-derived gelatin, Alaska pollock-derived gelatin sealant, burst strength, cerebrospinal fluid, cerebrospinal fluid leakage, dura, dural injury, fibrin, fibrin sealant, sealing strength

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Disruption of the dura mater and arachnoid tissue occurs during neurosurgery, which leads to cerebrospinal fluid (CSF) leakage. The reported incidence of CSF leakage ranges from 0.8% to 32%.[1–3] Primary suturing of the dura is the standard technique for repair, and sealing materials are sometimes used to prevent CSF leakage, with or without suturing. The use of commercially available sealants for dural closure has been reported in the literature.[1,2,4] Among these, the fibrin sealant is commonly used because of its biocompatibility.[1] Many previous studies have used fibrin sealant for CSF leakage and showed the effectiveness of dura mater sealing; however, a disadvantage of fibrin sealant is its lack of sealing strength.[1,2,5] Other adhesives such as cyanoacrylate and biopolymers with aldehyde-based crosslinkers are used because of their high bonding strength.[6–9] However, the use of these materials is limited owing to their cytotoxicity and low biocompatibility.[4,9]

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Recently, a novel biocompatible sealant composed of Alaska pollock-derived gelatin (ApGltn), partially modified with various alkyl groups and a poly(ethylene glycol)-based crosslinker, was introduced.[10,11] The burst strength and biocompatibility of wet tissue have been demonstrated using a porcine aorta or lung model.[10–12] The burst strength of the ApGltn sealant was ~12 times higher than that of commercial fibrin sealant.[10]

The ApGltn sealant is a promising material, and its use in coating ruptured dura mater during spinal surgery is expected in the future. However, whether this sealant can be applied to ruptured dura mater, where prevention of spinal fluid leakage is needed, is unclear. The purpose of this study was to investigate the sealing strength and biocompatibility of this sealant in ruptured dura mater in porcine and rat models.

MATERIALS AND METHODS

Animal experiments were approved by our institutional animal committee (approval no. A2022-016).

Characteristics and Preparation of Sealants

ApGltn Sealant

The preparation and characterization of dodecyl group–modified ApGltn, a component of the ApGltn sealant, was performed according to previously reported procedures.[10,11] The resulting dodecyl group–modified ApGltn was combined with a biocompatible poly(ethylene glycol)-based four-arm crosslinker, pentaerythritol poly (ethylene glycol) ether tetrasuccinimidyl glutarate (4S-PEG). The pH of the modified ApGltn–0.1 M borate buffer solution was adjusted to 8, and a solvent of 4S-PEG was prepared with 0.01 M phosphate solution (pH 4). The solutions were mixed in equal volumes using a dual-injection device. ApGltn hardened within 60 s of injection.

Fibrin Glue

The fibrin glue (Bolheal®; KM Biologics, Kumamoto, Japan) used in this study was refrigerated (4°C) before use. Fibrinogen powder (40 mg) and coagulation factor XIII (37.5 IU) were reconstituted in an aprotinin solution (500 KIE/0.5 mL). Thrombin concentrate powder (125 IU) was dissolved in a calcium chloride solution (2.95 g/0.5 mL). Fibrinogen and thrombin solutions were cured by mixing each component in equal volumes.

Burst Strength and Histological Evaluation Using a Porcine Dural Defect Model

The primary outcome was the maximum burst strength of the ApGltn and fibrin sealants using a fresh porcine dural defect, according to the American Society of Testing and Materials (ASTM) F2392-04. Ten samples were tested to determine the burst strength of each sealant (n = 5 each) (Fig. 1). Tissue samples (diameter, 30 mm) were prepared. After opening a 3-mm pinhole in the center of each sample, the ApGltn and fibrin sealants (15-mm diameter and 1-mm thickness) were applied using a silicone ring (Fig. 2). The maximum burst strength was determined by running a saline solution through the system at a flow rate of 2 mL/min at 37°C and measuring the maximum value (Fig. 3). Then, the samples were fixed with 10% formalin neutral buffer solution (Wako Pure Chemical Industries, Ltd, Japan) and stained with hematoxylin and eosin (HE). Cross sections of the stained samples (slice thickness, 4 mm) were visualized using a BX51 light microscope (Olympus, Tokyo, Japan). The burst style with a gap between the dura and the sealant was examined.

Functional and Histological Evaluation Using a Rat Dural Defect Model

The secondary outcome was the functional and histological recovery of the repaired dura mater in a rat model. Male Wistar rats (Sankyo Labo, Tokyo, Japan; age, 8 wk, mean body weight at the time of surgery, 198 g; range, 190–212 g) were used for the dural rupture model.

Surgical Treatments of Dura Defect with the Sealant

All surgical procedures and surgical treatment assignments were performed by a single orthopedic surgeon. All rats were deeply anesthetized with intraperitoneal injections of midazolam (0.39 mL/kg; Sandoz, Tokyo, Japan), medetomidine (0.37 mL/kg; Zenoaq, Fukushima, Japan), butorphanol (0.49 mL/kg; Meiji Seika, Tokyo, Japan), and saline (3.74 mL/kg; Otsuka, Tokyo, Japan). A dorsal longitudinal skin incision was made, and the lamina was explored by splitting the paravertebral muscle. Laminectomy was performed at the T10 level using a bone rongeur, and the dura was exposed. A 3-mm defect in the dura was made using a 30-G needle to avoid damage to the spinal cord, and CSF leakage was observed. The defects were treated randomly using three surgical interventions: defect + ApGltn sealant, defect + fibrin sealant, and defect without sealant (n = 9 rats per group). In the surgical interventions involving defect + ApGltn sealant and defect + fibrin sealant, ~0.5 mL of the ApGltn or fibrin sealant was placed around the dura rupture site. One suture was placed in the surrounding muscle at the same level as the site of the dural injury. Then, the fascia and skin were closed with interrupted sutures, and the rats were allowed unrestricted motion. No rats were excluded due to death.

To evaluate the regeneration of the dura and spinal function, hind-limb motor function analysis was conducted at two, four, and eight weeks after the initial procedure. The rats were killed at two (n = 3 per group), four (n = 3 per group), and eight weeks (n = 3 per group) after surgery for macroscopic and histological examinations.

Macroscopic Examination

The macroscopic appearance of the tissue surrounding the dural injury site was evaluated weekly. The resorption of the ApGltn and fibrin sealants was also examined.

Hind-limb Motor Function Analysis

Hind-limb motor function was tested using the open-field Basso, Beattie, and Bresnahan (BBB) locomotor scale.[13] BBB scores were measured two, four, and eight

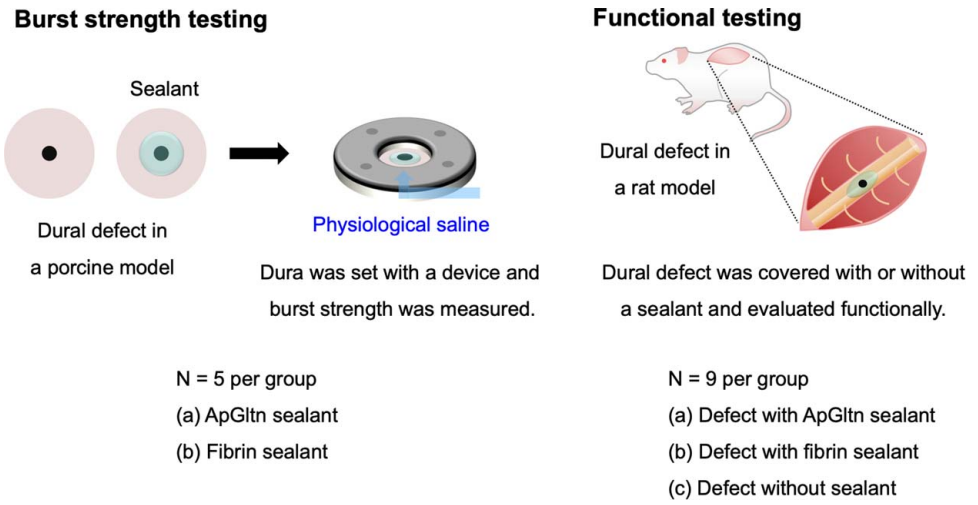

Figure 1. Burst strength testing and functional testing using the disruption of the dura mater in porcine and rat models. ApGltn indicate Alaska pollock-derived gelatin.

weeks after the initial procedure, with 0 denoting total loss of hind-limb movement and 21 denoting normal function.

Histological Examination

Three of the nine rats from each group were killed at two, four, and eight weeks (27 rats in total) for histological examination. The spinal cord was resected along with the dura mater and fixed in 10% formalin buffer solution for at least 3 days. Tissue specimens were cut stepwise at 2-mm intervals and embedded in paraffin. Paraffin-embedded tissues were cut into 4-mm-thick slices and stained with HE. A pathologist, blinded to clinical information, selected one representative section that contained the border region between the spinal cord and the dura mater for each rat and performed a semiquantitative evaluation of pathological changes, including inflammation, myelitis, neuronal damage, edema, desmoplasia, vascularization, necrosis, foreign body reaction, and adhesion between the dura and the surrounding tissues. These findings were classified into four categories according to a modified previous method (0, none; 1, mild; 2, moderate; 3, severe) (Table 1).[9,14,15] The average score for each of the three rats was calculated.

Statistical Analysis

Student's *t*-test was used to evaluate the burst strength of the ApGltn and fibrin groups. The sample size for burst testing was determined according to previous reports.[10,11,16] An analysis of histological scores involved the use of two-way repeated analysis of variance and Tukey's post hoc comparisons to assess intergroup differences. Kruskal–Wallis tests were utilized to evaluate intragroup differences across three evaluation periods. A post hoc power analysis was conducted to confirm whether the sample size was adequate to detect a significant

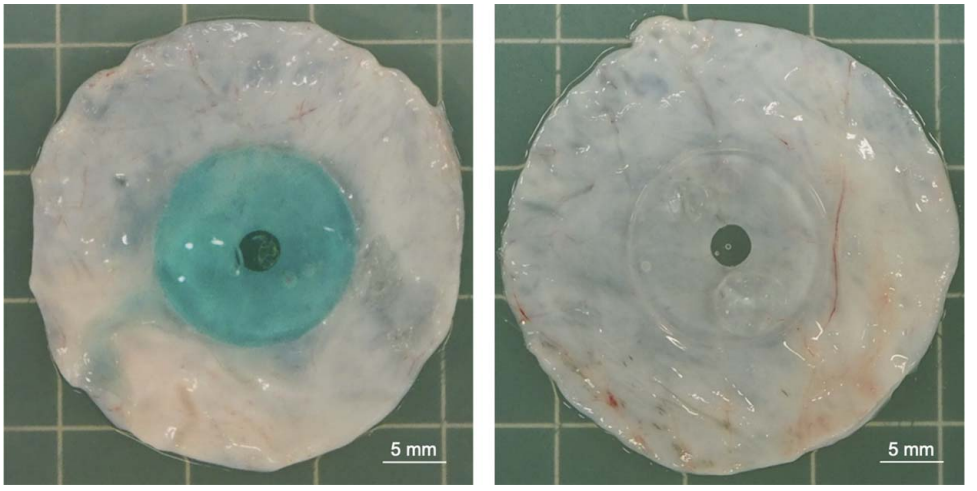

Figure 2. Fresh porcine dura was used in this study. The sealants were applied with a diameter of 15 mm and thickness of 1 mm. (left) ApGltn sealant. (right) Fibrin sealant.

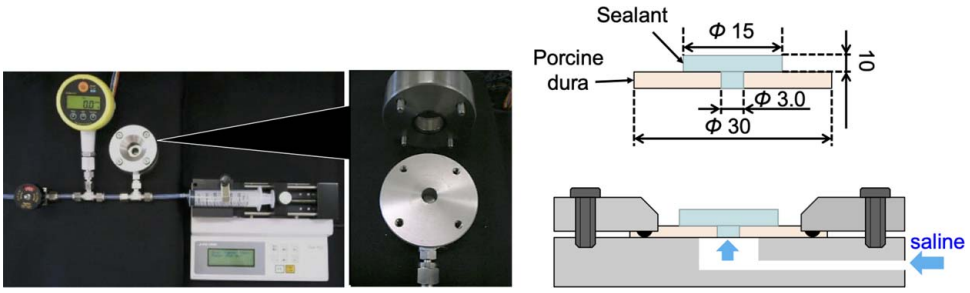

Figure 3. (left) Measurement equipment of the burst strength of sealants according to American Society of Testing and Materials F2392-04. (right) System for evaluating burst strength.

difference ($\alpha = 0.05$). The effect sizes are expressed as mean ± SDs. The power analysis demonstrated a statistical power of 100% for burst testing. Statistical significance was set at $P < 0.05$.

RESULTS

Burst Strength Using a Fresh Porcine Dural Defect Model

Burst Strength

The maximum burst strengths of the ApGltn and fibrin sealants (68.1 ± 12.1 and 15.6 ± 8.7 mmHg, respectively) significantly differed ($P < 0.001$). Macroscopic examination of the burst behavior showed that the ApGltn sealant ruptured and saline leaked from the ruptured site in all five samples. In the fibrin group, the sealant detached from the dura surface, and saline leaked from the interface between the fibrin sealant and the dura mater in all five samples.

Histological Findings After the Burst Testing

Histological images of the tissues stained with HE after burst testing are shown in Figure 4. The ApGltn sealant ruptured and adhered tightly to both the defect and the surrounding dura in all five samples (Fig. 4, left). A gap was observed between the fibrin sealant and the surface of the dura, both in the defect and in the surrounding dura, in all five samples (Fig. 4, right). These findings are consistent with the macroscopic examination of the burst behavior.

Functional Evaluation Using a Rat Dural Defect Model

Macroscopic Examination

Scar tissue was observed around the dural injury site in all three groups at each examination. ApGltn remained on the dura mater in three rats at two weeks and in two rats at four weeks and was resorbed in three rats at eight weeks. The fibrin sealant remained on the dura mater in one rat at two weeks and was resorbed in three rats at four and eight weeks (Fig. 5). Both the ApGltn sealant and fibrin sealant groups demonstrated the absence of CSF leakage in all rats at two, four, and eight weeks, postoperatively.

Hind-limb Motor Function Analysis

All rats in the three procedures showed a BBB score of 21 points at two, four, and eight weeks after the initial repair, indicating no loss of hind-limb motor function.

Histological Examinations

The results of the semiquantitative analyses of the histological findings are summarized in Table 2. Mild, moderate, and severe inflammatory cell infiltration and myelitis were observed in all three groups at two weeks. At four and eight weeks, the inflammation gradually became

TABLE 1. Grading System Used for Quantifying Histological Findings

	0	1	2	3
Inflammation	No cell/few inflammatory cells	Mild inflammatory cells	Moderate inflammatory cells	Severe inflammatory cells
Myelitis	None	Few fibroblasts	Moderate fibroblasts	Severe fibroblasts
Neuronal damage	None	Mild neuronal disintegration	Moderate neuronal disintegration	Severe neuronal disintegration
Edema	None	Mild rarification of the intercellular tissue	Moderate rarification of the intercellular tissue	Severe rarification of the intercellular tissue
Desmoplasia	None	Thin layer	Moderate thickness	Thick layer
Vascularization	None	Few new capillaries	Moderate capillaries	Dense capillaries
Necrosis	None	Mild	Moderate	Severe
Foreign body reaction	None	Mild giant cells	Moderate giant cells	Severe giant cells
Dural adhesion	None	Mild	Moderate	Severe

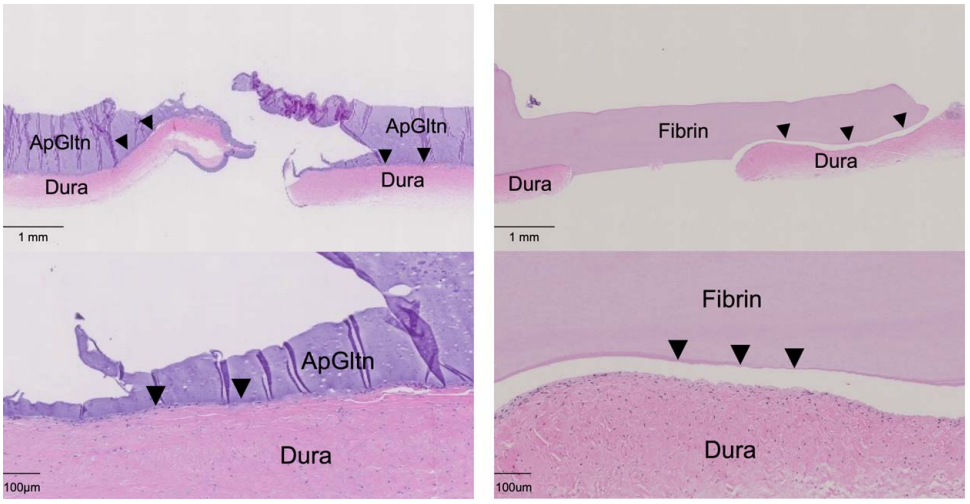

Figure 4. Histological images of the tissue after burst testing. (left) ApGltn; The ApGltn adhered tightly to the dura (arrowhead). (right) Fibrin; A gap was observed between the fibrin and the dura (arrowhead).

mild or disappeared in the ApGltn group, whereas it only lightly decreased or was unchanged in the control and fibrin groups. Mild or no neuronal damage or edema of the spinal cord was observed in all three groups every week. All three groups had mild to severe desmoplasia and vascularization at two weeks, but these findings were moderate or absent at eight weeks. ApGltn showed significantly more vascularization than the control group ($P = 0.03$). No soft tissue necrosis was observed in the three groups. Dural adhesion between the dura and connective tissues was mild or absent in the ApGltn group and mild or moderate in the fibrin group at two, four, and eight weeks with significant differences ($P = 0.02$). No significant differences were found at the three evaluation time points in each group, except desmoplasia in fibrin group between two and eight weeks ($P = 0.01$). Representative histological images of HE staining at two, four, and eight weeks are shown in Figure 6.

DISCUSSION

In this study, the sealing strength and histological recovery of dural defects were compared between ApGltn and fibrin sealants. The burst strength of the ApGltn sealant was ~4.4 times higher than that of the fibrin sealant. Histological examination confirmed that the ApGltn sealant adhered tightly to the dural surface compared with the fibrin sealant. These results indicated that ApGltn sealant showed cohesion failure when applied to the dura surface, which means that the interfacial strength between the cured ApGltn sealant and the dura tissue was higher than that of the fibrin sealant. Compared with the fibrin sealant, the ApGltn sealant did not prevent spinal function or dura mater regeneration, suggesting the biocompatibility of the ApGltn sealant.

Taguchi *et al* developed the first hydrophobically modified ApGltn-based biocompatible sealant and reported sufficient burst strength for its clinical application in burst porcine aortas and in an air-leak model of a rat lung.[10] The burst strength of the ApGltn sealant was 11.6 times higher than that of a commercial fibrin sealant (341 vs. 29 mmHg). Yamaoka *et al* validated the burst strength of ApGltn and fibrin sealants using an air-leak porcine lung.[12] The burst pressure of ApGltn sealant was significantly higher than that of fibrin sealant (52 vs. 38 cm H_2O). Our results are consistent with those of previous

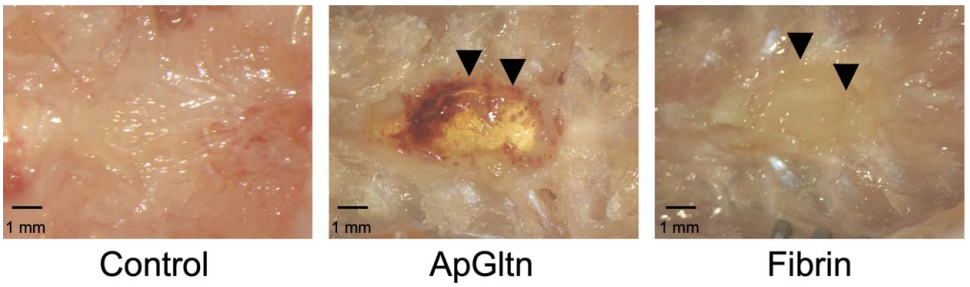

Figure 5. Macroscopic examination of the dura after each procedure at two weeks. The ApGltn (arrowhead) remained on the dura mater in all three rats, and the fibrin sealant (arrowhead) remained in one of three rats.

TABLE 2. Semiquantitative Analyses of the Histological Findings

	2 wk (n = 3 per group)			4 wk (n = 3 per group)			8 wk (n = 3 per group)		
	Control	**ApGltn**	**Fibrin**	**Control**	**ApGltn**	**Fibrin**	**Control**	**ApGltn**	**Fibrin**
Inflammation	1.7 ± 0	2.3 ± 0.5	2.3 ± 0.5	1.0 ± 0.8	1.3 ± 0.5	1.7 ± 0.5	1.7 ± 0.5	1.0 ± 0	2.0 ± 0
Myelitis	1.0 ± 1.4	1.3 ± 1.3	1.0 ± 0	1.0 ± 0.8	0.7 ± 0.5	1.0 ± 0	1.7 ± 0.5	0.3 ± 0.5	1.3 ± 0.5
Neuronal damage	0.7 ± 0.9	1.0 ± 0.8	1.0 ± 0	0.7 ± 0.9	0.3 ± 0.5	1.3 ± 0.5	1.7 ± 0.5	0 ± 0	1.0 ± 0
Edema	0 ± 0	0.7 ± 0.5	1.0 ± 0	0.3 ± 0.5	1.0 ± 0	0.3 ± 0.5	0.3 ± 0.5	0.3 ± 0.5	0.7 ± 0.5
Desmoplasia	2.0 ± 0.8	1.7 ± 0.5	3.0 ± 0	1.0 ± 0.8	2.0 ± 0	2.0 ± 0	1.3 ± 0.5	1.0 ± 0	1.0 ± 0
Vascularization	1.7 ± 0.5	1.7 ± 0.5	2.0 ± 0	0.3 ± 0.5	2.0 ± 0.8	1.3 ± 0.5	0.3 ± 0.5	1.7 ± 0.5	1.0 ± 0.8
Necrosis	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Foreign body reaction	0.3 ± 0.5	1.3 ± 0.9	0.7 ± 0.5	1.0 ± 1.4	0.3 ± 0.5	0.3 ± 0.5	1.0 ± 0.8	2.0 ± 0.8	1.7 ± 0.5
Dural adhesion	0.7 ± 0.9	0.7 ± 0.5	1.3 ± 0.5	0.7 ± 0.5	0.3 ± 0.5	1.7 ± 0.5	2.3 ± 1.2	1.0 ± 0	2.0 ± 0

Values are presented as mean ± SD.

studies in that the breaking strength of the ApGltn sealant was higher than that of the fibrin sealant. Doormaal *et al* evaluated burst strength using a fresh porcine dura model to compare the sealing effect of nine different sealants for dural closure using the same methods as our study (ASTM F2392-04; 30 mm in diameter with a 3-mm hole of the dura and sealant with a diameter of 15 mm and thickness of 1 mm).[2] Adherus® (polyethylene glycol-based hydrogel) had the highest burst pressure (87 ± 47 mmHg), followed by Tachosil® (hemostatic collagen; 71 ± 16 mmHg); Tisseel® (fibrin sealant) showed a significantly lower burst pressure (12 ± 9 mmHg) than these two sealants. Although the type of fibrin sealant used in our study was different, its breaking strength of the fibrin sealant was low when applied to porcine dural defects. Normal adult CSF pressure is ~5–15 mmHg.[17,18] The use of a sealant with greater breaking strength than intracranial pressure is desirable to prevent CSF leakage.

Histologically, the ApGltn sealant tightly adhered to the dural surface, and the bond was ruptured only by the destruction of the bulk ApGltn sealant. In contrast, a gap was observed between the fibrin sealant and the surface of the dura, suggesting weak interfacial strength between the sealant and the dura. This burst style is consistent with previous histological studies that showed tight adhesion of the ApGltn sealant in the porcine aorta or lung burst model.[11,12] These results were due to increased burst strength and hydrophobic interactions between the dodecyl group of the ApGltn and extracellular matrix proteins.[11] Under wet conditions, tight adhesion of the ApGltn sealant contributes to the prevention of CSF leakage.

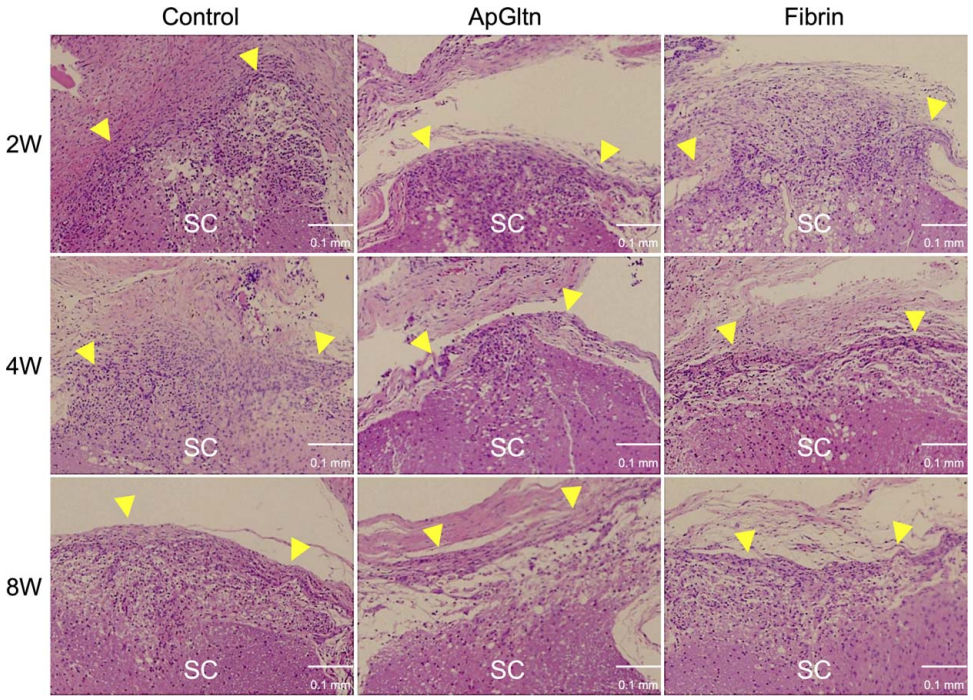

Figure 6. Histological images of the rat dura mater (arrowhead) and spinal cord after burst testing at two, four, and eight weeks. ApGltn indicates Alaska pollock-derived gelatin; SC, spinal cord.

Previous studies that used fibrin sealants in animal models have demonstrated that fibrin sealants do not prevent the regeneration of the dura and lead to axonal damage.[1,14] In the present study, the ApGltn sealant demonstrated the same functional and histological findings of the dura mater as those of the fibrin sealant. Previous studies have demonstrated that severe inflammatory reactions are not observed at the bonding site of ApGltn,[11,16,19] which is consistent with the results of our study. This may be because the hexanoyl group induces weak inflammation in the tissue, resulting in the secretion of inflammatory cytokines or growth factors.[20] Masuda *et al* demonstrated that rat sciatic nerves repaired with ApGltn sealant showed similar recovery of axons as those repaired with sutures and fibrin sealant.[21] Our data suggest that the ApGltn sealant can be applied not only to the peripheral nerve but also to the central nerve field. Mizuno *et al* showed that ApGltn sealant promotes cell migration and acts as a scaffold for tissue migration, which might lead to vascularization around the dura.[22] Because it is composed of gelatin, this sealant does not prevent tissue regeneration. Furthermore, Mizuta *et al* showed that ApGltn sealant acts as an anti-adhesion barrier on the target surface to prevent adhesion.[23] This may have contributed to the prevention of dural adhesions in the current study.

In addition to its burst strength, the ApGltn sealant has other advantages over fibrin glue. The fibrin sealant was resorbed in ~two weeks, whereas the ApGltn sealant was resorbed in ~four–eight weeks, suggesting a longer-lasting adhesive capacity.[10,12,21] The duration of dural repair in rats is considered to be three to four weeks or longer.[8,24] Sealants with long-lasting adhesive capacity are desirable for repairing the dura mater to prevent CSF leakage. The fibrin sealant is composed of fibrinogen (lyophilized pooled human concentrate) and thrombin; therefore, viral infections may occur. The ApGltn sealant is composed of biocompatible ApGltn purified from Alaska pollock skin by alkali treatment; thus, the risk of viral infection may be reduced. Furthermore, ApGltn is derived from the waste products of Alaska pollock skin; therefore, the cost of these materials is quite low. In clinical practice, the cost of ApGltn sealants is lower than that of fibrin sealants. Based on these observations and the results of this study, we believe that ApGltn could be used as a substitute for fibrin glue in the future.

ApGltn is prepared from Alaska pollock skin through demineralization and alkaline treatment; therefore, it is used in the same manner as previously approved bovine-derived and porcine-derived gelatin. The chemical structure and molecular weight of the cross-linker (4S-PEG) are the same as those of FDA/Japanese FDA-approved sealant (DuraSeal®) used in brain surgery. The difference between ApGltn sealant and DuraSeal lies in the adhesive components (ApGltn: Dodecyl-group modified ApGltn, DuraSeal: trilysine). We believe that ApGltn sealant could overcome the regulatory challenges of its use in humans. Clinical trials on humans will be needed in the future.

The ApGltn sealant has greater sealing strength than fibrin glue. The functional and histological findings in the spinal cord and dura mater are similar to those of the fibrin glue. To prevent CSF leakage, ApGltn is a promising material for disrupting the dura mater in clinical settings.

➢ Key Points

- ❑ The present study investigated the sealing strength and biocompatibility of this sealant in ruptured dura mater in porcine and rat models.
- ❑ The burst strength of the ApGltn sealant was 4.4 times higher than that of the fibrin sealant. Histological examination confirmed that the ApGltn sealant adhered tightly to the dural surface compared with the fibrin sealant.
- ❑ Compared with the fibrin sealant, the ApGltn sealant did not prevent spinal function or dura mater regeneration, suggesting the biocompatibility of the ApGltn sealant.
- ❑ ApGltn is effective in preventing cerebrospinal fluid leakage and seal disruption of the dura mater.

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