ABSTRACT

Objectives: Watertight dural closure is imperative after neurosurgical procedures because inadequately treated leakage of cerebrospinal fluid (CSF) can have serious consequences. In this study, the authors test the use of a new gelatin glue as a dural sealant in *in vitro* and *in vivo* canine models of transdural CSF leakage.

Methods: The *in vitro* model was sutured semicircles of canine dura mater and artificial dural substitute. The sutures were sealed with gelatin glue (n=20), fibrin glue (n=20) or a polyethylene glycol (PEG)-based hydrogel sealant (n=20). Each sample was set in a device to measure water pressure, and pressure was increased until leakage occurred. Bonding strength was subjectively evaluated. The *in vivo* model was dogs who underwent dural excision and received either no sealant (control group; n=5) or gelatin glue sealant (n=5) prior to dural closure. Twenty-eight days post-surgery, the maximum intracranial pressure was measured at the cisterna magna using Valsalva maneuver and tissue adhesion was evaluated.

Results: The water pressure at which leakage occurred in the *in-vitro* model was higher with gelatin glue (76.5±39.8 mmHg) than with fibrin glue (38.3±27.4 mmHg, P<0.001) or the PEG-based hydrogel sealant (46.3±20.9 mmHg, P=0.007). Bonding strength was higher for the gelatin glue than fibrin glue (P<0.001) or PEG-based hydrogel sealant (P=0.001). The maximum intracranial pressure in the *in-vivo* model was higher for the gelatin glue group (59.0±2.2 mmHg) than the control group (13.8±4.0 mmHg, P<0.001). Tissue adhesion was lower for the gelatin glue group than the control group (P=0.005).

Discussion: The new gelatin glue provides an effective watertight closure when used as an adjunct to sutured dural repair.

KEY WORDS: Cerebrospinal fluid leak, dural repair, dural sealant system, gelatin glue

RUNNING TITLE: Gelatin glue dural sealant

1. Introduction

Cerebrospinal fluid (CSF) leakage is one of the most challenging and potentially dangerous complications of cranial and spinal surgeries, and can have serious consequences, including meningitis, arachnoiditis, and epidural abscess[1-3]. Appropriate closure of the dura mater is very important in preventing CSF leakage in neurosurgical practice, as it constitutes a barrier on the brain surface. Dural suture remains the most frequently used method of closure, however, suture techniques are difficult to carry out, particularly when defects are in relatively inaccessible areas or surrounded by friable dura. This has led some surgeons[1-5] to advocate the use of other techniques such as sealing the sutures with fibrin glue[6,7] or polyethylene glycol (PEG)-based hydrogel dural sealant[8]. However, these commercially available sealants have some limitations, including low bonding strength, possible virus transmission and troublesome preparation[9]. The available methods of dural closure are therefore still sub-optimal. To address this problem, we created a sealant system that uses the common biomaterial, gelatin. In medical applications, gelatin has been extensively used for pharmaceutical capsules, drug delivery systems, hemostatic agents such as Gelform® (Pfizer, NY) and Floseal® (Baxter Healthcare Corporation, Freemont, CA), and surgical adhesives such as GRF® (Cardical, Technopole, Sainte-Etienne, France). The gelatin glue in our sealant system consists of 26 wt% gelatin and 1 wt% glutaraldehyde (GA) solutions, and exhibits higher bonding strength than fibrin glue and low cytotoxicity[10].

In a preliminary experiment based on our previous study that used an *in vivo* rat model, we found that the gelatin glue was better at preventing CSF leakage than fibrin glue (unpublished data). In the present study we use a large animal model to test the efficacy of the gelatin glue for preventing CSF leaks and tissue adhesion in comparison with current dural closure sealant systems. We hypothesized that gelatin's feasibility towards clinical application is high.

2. Methods

This study was approved by the Advanced Medical Research Center of Nara Medical University in Japan. All surgical procedures were conducted under routine sterile conditions.

2.1 Materials

Medical grade gelatin (Medigelatin®) was supplied by Nippi Inc. (Shizuoka, Japan) and was extracted from the porcine skin to have an isoelectric point of five. Phosphate-buffered saline and 25 wt% GA solution were purchased from Wako Pure Chemical Inc. (Osaka, Japan). All agents were used as obtained. Fibrin glue (Beriplast®, CSL Behring) and PEG-based hydrogel sealant (DURASEAL®, COVIDIEN) were purchased from Wakenyaku Co. Ltd. (Osaka, Japan). Doubly-distilled water was used for all preparation. Gelatin solution (26 wt%) was prepared using phosphate-buffered saline at pH 7. Gelatin and GA (1 %) solutions were preheated to 45°C using an application device and applied to the dura mater simultaneously with rubbing[10] so that they mixed well and penetrated into the suture holes.

2.2 *In-vitro* study

A Beagle dog (5 years old, mean body weight 10.5 kg) was euthanized, and the dura mater was removed. The canine dura mater and a bioabsorbable artificial dural substitute that was made of a film from copolymer of L-lactide, ε-caprolactone and polyglycolic acid felt (SEAMDURA®, GUNZE Co. Ltd, Japan) were cut into semicircles with a diameter of 24 mm. Dura mater samples were made by running simple suturing semicircles of the canine dura mater and the artificial dural substitute at intervals of 3 mm with 4-0 NUROLON® (Johnson & Johnson K.K.) under the microscope and were assembled into the apparatus for testing burst water pressure (Figure 1). Water pressure was measured using a pressure gauge (Valcom Co., Ltd, Osaka, Japan). A sample was randomly selected and set in the apparatus (Valcom Co., Ltd, Osaka, Japan). The pressure chamber was designed as a circular box of 10 mm inner diameter. (Figure 1). The uniform water leakage at a water pressure of 10 mmHg was checked. The sample surface was then wiped dry, and 200 μL of sealant was applied to seal the sutures. Three sealant groups were created randomly: new gelatin (n=20); fibrin (Beriplast®, CSL Behling; n=20); and PEG-based hydrogel (n=20). Five minutes after sealant application, water pressure was applied at 10 mmHg. Leakage was characterised by the observation of colored water on the dural menbrane surface. We also identified a stable maximum pressure value from pressure gauge, which indicated the occurrence of leakage

more precisely. If water did not leak, the pressure was raised 5 mmHg until leakage occurred. The maximal water pressure reached before water leakage occurred from the sutured position was used as a measure of sealing effect. Samples were reinflated with 10% formalin and immersed in the same solution. After fixation, each defect site was resected, embedded in paraffin, sectioned, and subjected to hematoxylin and eosin stain. A veterinarian pathologist diagnosed whether all sealants were tightly adhered to the dural samples.

To investigate the bonding strength of each sealant, 100 μL of sealant was applied to the dura mater sample. After 5 min, the sealant was slowly removed using blunt forceps. The force required to separate the glue from the dura mater sample was subjectively rated on a five-point scale blindly to the group (0, not gluing/sliding; 1, mild; 2, moderate; 3, severe; and 4, very severe) and used as a measured of bonding strength.

2.3 *In-vivo* study

Ten Beagle dogs (5 years old, mean body weight 11 kg) were housed at room temperature and given standard feed. No animals had focal neurological deficits before surgery. Dogs were intubated under general anesthesia and managed under a respirator. The scalp over the right frontoparietal area was shaved and treated with 70% ethyl alcohol. A curved incision was made into the skin in this region, the temporal muscle was reflected laterally, and an oval bone flap was raised with a high-speed drill. The dura mater was kept intact during this procedure. The dura mater was then opened transversely for 2 cm under the operating microscope. Several areas of the arachnoid were incised to allow CSF leakage. running sutures (4-0 NUROLON®) were placed at precise intervals of 2 mm to close the dura mater incision under the microscope. Dogs were then randomized to one of the following two treatment groups: control (N=5) and gelatin glue sealant (N=5). In the control group no sealant was applied after dural closure. In the gelatin glue sealant group the gelatin glue was applied extradurally after dural closure just on the incision (average thickness, 2.0 mm; range over five dogs, 1.0–2.9 mm). Margins of at least 5 mm on each side of the suture were covered by the sealant application. Application of the gelatin glue took only a few seconds. In the gelatin glue sealant group, the durotomy sites were tested for CSF leakage with a Valsalva maneuver at 20 cmH_2O 20 minutes after glue application. All 5 dogs were not observed CSF leakage. Absence of CSF leakage with this maneuver predicts postoperative success[11-14]. The bone flap was replaced and secured with 3-0 polyglactin 910 sutures

(VicrylTM, Ethicon, Inc., Johnson & Johnson, Somerville, NJ). The temporal muscle, fascia, and galea were repaired with a running 3-0 polyglactin 910 suture in layers, and the scalp was closed with 3-0 nylon suture (Ethilon, Ethicon, Inc.). Finally, the skin was closed with 2-0 nylon sutures. All surgical procedures were performed without complications.

After surgery, dogs were monitored until full recovery from anesthesia. They were then observed and behavior, general health (e.g., incision healing and appetite) and possible central nervous system abnormalities (e.g., somnolence, unstable gait and seizures) were assessed. Animals were managed by veterinary treatments, including administration of antibiotics and appropriate pain relief.

Twenty eight days post-surgery, anesthesia was again induced using a general inhalant, and each incision was carefully reopened. The bone flap was raised by a neurosurgeon who was blind to the treatment group. The neurosurgeon subjectively rated the extent of the adhesion formed between the dura mater and the bone flap blindly using a five-point scale (0, no adhesions; 1, mild adhesion/filmy adhesions, easily removed by blunt dissection; 2, moderate adhesion/fibrous adhesions, easily dissected; 3, severe adhesion/thick fibrous adhesions, dissectible; and 4, very severe adhesion/thick fibrous adhesions, not dissectible without damage the adherent tissue) according to a previous report.[1]

The cisterna magna was cannulated percutaneously with a 20-gauge, 3.5-inch spinal needle to directly measure intracranial pressure as an initial intracranial pressure. A Valsalva maneuver was performed while CSF leakage was visually assessed under the operating microscope. During the Valsalva maneuver, the maximum intracranial pressure reached on the manometer was recorded. The intracranial pressure at which CSF leaked onto the dural surface was recorded as the maximum intracranial pressure. To avoid brain stem herniation, maximum intracranial pressure was stopped at 60 cmH_2O, even if no CSF leakage had been noted.

After all examinations had been performed the dogs were heparinized and euthanized. The treatment sites were extracted and pressure-perfused with formalin after saline rinsing. Tissues were embedded in paraffin, cut, and stained with hematoxylin and eosin for evaluation by a veterinarian pathologist who was blind to the treatment group. The focus of the microscopic examination of the tissue samples was tissue healing with special attention to neurocompatibility, dural thickness, implant absorption, and dural–cerebral cortex scar

(adhesion) formation.

2.4 Statistical Analyses

The data shown represent mean values ± standard deviation. For the *in-vitro* study, the water pressure at which leakage occurred was compared across treatments (new gelatin glue *vs.* fibrin glue, and new gelatin glue *vs.* PEG-based hydrogel dural sealant) using Turkey tests, and the bonding strength score was compared using Kruskal Wallis test. For the *in-vivo* study, the initial / the maximum intracranial pressures were compared across groups (control; gelatin glue sealant) using independent sample t-tests for non-normally distributed means, and the adhesion score was compared using Mann Whitney U test. Statistical significance was concluded at an error probability of $p<0.05$. All statistical comparisons were performed using Sigma-Stat software (Jandel Scientific, Erkrath, Germany).

3. Results

3.1 *In vitro* study

The water pressure at which leakage occurred from the sutured position was significantly higher with the new gelatin glue (76.5 ± 39.8 mmHg) than with fibrin glue (38.3 ± 27.4 mmHg, $P<0.001$) or with PEG-based hydrogel dural sealant (46.3 ± 20.9 mmHg, $P=0.007$)(Figure 2). The bonding strength was significantly higher with the new gelatin glue than with fibrin glue ($P< 0.001$) or PEG-based hydrogel sealant ($P= 0.001$)(Figure 3). Histologically, it was confirmed that all sealants were tightly adhered to the dura samples in all sections (Figure 4).

3.2 *In vivo* study

All animals survived until the scheduled second surgery. No treatment-related clinical observations, neurologic effects, or body weight or clinical pathology changes were identified. In the control group, all durotomy sites were spontaneously leaking CSF at closure. In the gelatin glue sealant group, rapid sealant polymerization of the gelatin glue allowed complete duraplasty patching and the surrounding dura mater coverage without runoff. Twenty-eight days post-surgery, the initial CSF pressure at the cisterna magna prior to the Valsalva maneuver was similar in the control and gelatin glue groups (6.4 ± 2.9 and 7.8 ± 1.3 mmHg respectively, $P= 0.351$). The maximum intracranial pressure of the new gelatin glue group (59.0 ± 2.2 mmHg) was significantly higher than that of the control group (13.8 ± 4.0 mmHg,

P< 0.001) (Figure 5). The adhesion between the bone and the dura mater was severe in all animals in the control group and there was significantly less adhesion in the new gelatin glue group (P= 0.005) (Figure 6).

The majority of gelatin glue was degraded by 28 days post-surgery, and the residual small amounts of gelatin glue were detected with fibrous tissue formation. Histologically, one layer of mesothelial cells covered the residual gelatin glue. Infiltration of foreign body giant cells, lymphocytes, and fibroblasts was observed around the glue. In the gelatin glue group, no necrotic tissue or hemorrhage was evident (Figure 7a), and the gelatin glue was completely covered by thick fibrous tissue with decreased numbers of histiocytes and fibroblasts and an increased amount of collagen fibers (Figure 7b). Any brain modification was observed in the gelatin glue group. In the control group, inflammatory cells had penetrated into the brain (Figure 7c), there was encapsulation by a thick fibrocellular layer, and infiltration of histiocytes, lymphocytes, and fibroblasts. The treated area had been replaced by thick fibrous tissues with slight infiltration of lymphocytes (Figure 7c).

4. Discussion

In the present study, newly developed gelatin glue was used as a dural closure sealant to prevent CSF leakage. We demonstrated that this gelatin glue could withstand significantly higher water pressure without leaking than other currently established sealants. This supports Suzuki *et al.*, who reported that the burst water pressure of gelatin glue applied on pricked vascular grafts was significantly higher than that of fibrin glue applied by rubbing[10]. We also demonstrated that the newly developed gelatin glue adhered to the dura mater with greater strength than other sealants, and possessed other properties that are desirable in a dural sealant (degrades in living body, induced no harmful foreign body reaction, is easily stored and readily available when needed) [10].

The performance of sealants depends on the watertightness property of the sealant itself and the adhesion quality of sealant with the dura mater. Indeed in the present *in vivo* study, we did not observed CSF leakage 28 days after operation. Although the dura has its own healing properties, the gelatin glue prevents delayed CSF leakage only margins of 5 mm on each side of the suture are covered by the gelatin glue at the operation, which means the gelatin glue has appropriate biomechanical property to living dura mater. These results can be

explained by the unique molecular characteristics of gelatin. It is present in solution primarily as random chains, and this structure leads to chain entanglement, which results in strong adhesion. Furthermore, the gelatin molecule consists of a variety of comonomers that involve hydrophobic, polar, and negatively/positively charged amino acids, which facilitate particular interactions with the surface to be glued[10].

The appeal of the new gelatin glue as a dural sealant is compelling when the properties are considered in view of other techniques that are available for dural closure and duraplasty. Previous experimental studies that have documented the effectiveness of fibrin glue and other tissue adhesives for sealing CSF leakages have been performed on relatively small dural defects.[17,26] Fibrin glue is well-known as a material for neurosurgical practice; however, it has some shortcomings, including possible virus transmission, low adhesive strength, troublesome preparation and high cost[9]. Fluid collection occurred in 26% of patients when fibrin glue was used, and it requires dry surfaces to polymerize, lacks sufficient mechanical strength, and is not easily handled[9]. In the present study we ensured that the dura mater surfaces were dry for both *in vitro* and *in vivo* studies to optimize the environment for fibrin glue. Despite this, fibrin glue was less adhesive than gelatin glue.

Synthetic PEG-based hydrogel sealant is increasingly used to facilitate watertight repair of cranial and spinal dural defects and prevent CSF leakage[16-20]. It has been demonstrated to be both safe and effective in clinical studies[16,17]. However, this sealant also has some problems. As body fluid is imbibed, the volume of hydrogel may increase up to 50%, leading to a risk of compression of neural elements[20]. Hydrogel-entrapped hematoma has also been reported[20].

Commercially available sealants contain aldehyde (GRF® and Bioglue®, CryoLife, Inc., Kennesaw, GA) have high adhesive strength but their applications are limited, because of late complications and adverse events[21-23]. GA is a highly reactive molecule with low molecular weight, and is therefore potentially toxic. However, it functions as a crosslinker of gelatin molecules through Schiff base formation to enhance the resistance against enzymatic degradation of the glue. GRF® is composed of 37.5% gelatin, 12.5% resorcin, 16.7% formaldehyde and 2.5% GA solutions, and Bioglue® is composed of 45% albumin and 10 % GA solutions. Our new gelatin glue consists of a relatively low GA solution (1%), and the final concentration of aldehyde in the new gelatin glue is much lower than in GRF® and Bioglue®. In the present *in vivo* histological study, the degree of inflammation at the junction

between the dura mater and the brain tissue, which consisted of dense and diffuse lymphocytic infiltrates was similar in the two groups. Both groups showed similar bony remodeling. These results are consistent with a previous report that showed lower cytotoxicity of GA in a gel extract than of free GA, which indicates that GA in a gel extract is partially bound to gelatin molecules.[11]

Biological materials such as collagen and gelatin of cow origin have a risk of bovine spongiform encephalopathy, which arises from an abnormal prion[24]. However, the World Health Organization and the World Organization for Animal Health support the safety of gelatin, because susceptibility to gelatin is not detected in the skin and the bone, and strong alkali and acid processes in production of gelatin can inactivate pathogens[25]. The source of the gelatin used in this study and the manufacturing process of gelatin granules are certified according to the bovine spongiform encephalopathy safety regulations in the USA and the European Union, both of which have a high level of safety requirements with regard to the transmission risk of Creutzfeldt-Jacob disease. In addition, gelatin granules of bovine origin have recently been recertified as bovine spongiform encephalopathy-safe based on a new European Union regulation. We therefore believe that the gelatin glue would be safe for use in medical products.

The conclusions of this study are limited because the results were obtained from an *in vitro* and a living-dog model. In the *in vitro* study, the inner diameter of the pressure chamber is relatively small, which may influence the frequency of CSF leakage. In the *in vivo* study, we did not examine the efficacy of the other commercial available sealants compared to that of the gelatin glue, therefore, controlled study is required. Furthermore, the canine dura mater is relatively thin compared with the human one, therefore the results may not be accurately reflect the results that would be observed in a human clinical setting with neurosurgical patients. Further clinical investigation should be undertaken to clarify this issue.

5. Conclusion

The present study shows that the new gelatin glue could resist a higher water pressure and had stronger adhesion to the dura mater than existing products, and reduced tissue adhesion *in vivo*. Thus, this new gelatin glue may significantly improve the safety and efficacy of neurosurgical dural closure. Further human clinical investigation should be undertaken to

clarify this issue.

7. References

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Figure Legends

Figure 1. A schematic depicting the apparatus used to test burst water pressure. A sutured dural sample was set in the apparatus and the burst water pressure was measured by gradually increasing the water pressure applied to the sample.

Figure 2. The burst water pressure of *in vitro* dural samples sealed with fibrin, polyethylene glycol (PEG) or gelatin (n = 20 for each group). The data shown represent mean values ± standard deviation.

Figure 3. The subjective bonding strength score of fibrin, polyethylene glycol (PEG) and gelatin (n = 20 for each group) glues on *in-vitro* dural samples. The data shown represent mean values.

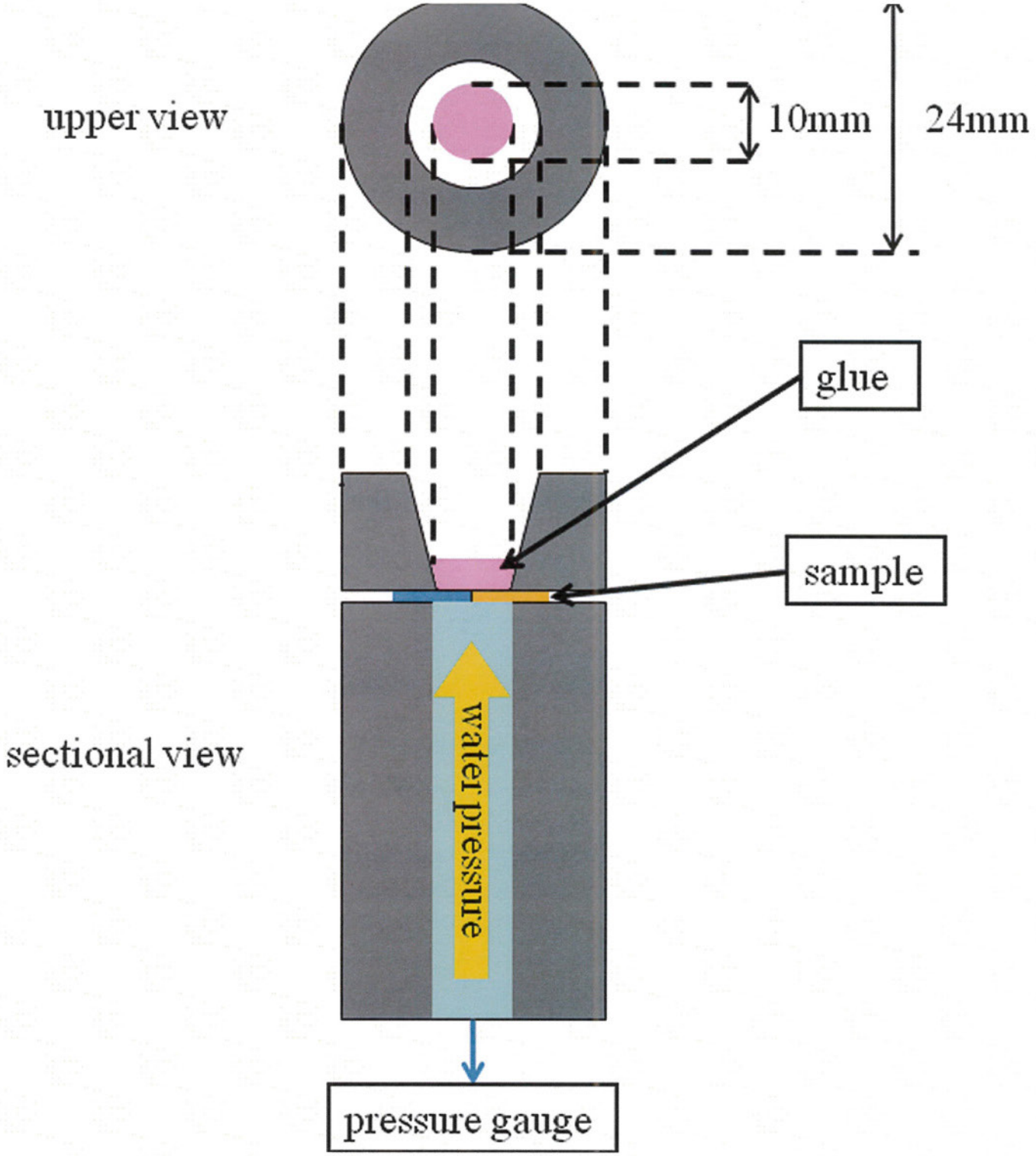
Figure 4. Photomicrographs of longitudinal-sections in *in-vitro* models of fibrin glue (a), polyethylene glycol-based hydrogel dural sealant (b) and new gelatin glue (c) on canine dura mater after staining with hematoxylin and eosin. The upper part of each image is the sealant and the lower part is the canine dura mater. White arrow indicates close apposition between the dura mater and the sealant. Original magnification ×400 (bar=100 μm).

Figure 5. The initial (blue) and the maximum (red) intracranial pressure (red) measured in *in vivo* canine models 28 days after dural excision surgery in which no sealant was applied prior to dural closure (control; n=5) or the new gelatin glue was applied prior to closure (gelatin; n=5). The data shown represent mean values ± standard deviation.

Figure 6. Subjective rating of the adhesion between the dura mater and the bone tissue in *in vivo* canine models 28 days after dural excision surgery in which no sealant was applied prior to dural closure (control; n=5) or the new gelatin glue was applied prior to closure (gelatin; n=5). The data shown represent mean values.

Figure 7. Microscopic views of the operated sites in *in-vivo* canine models 28 days after dural excision surgery in which new gelatin glue was applied prior to closure (a, b) or no sealant

was applied prior to dural closure (c). Samples were stained with hematoxylin and eosin. In the gelatin group (a, b), the majority of gelatin glue was dissolved and fibrous tissue was observed (red arrow). There were a few inflammation sites. In the control group (c), development of granulation tissue was observed, but there was only slight inflammatory cell permeation. Original magnification: a; ×200 (bar=50 μm), b; ×400 (bar=20 μm), c; ×400 (bar=20 μm).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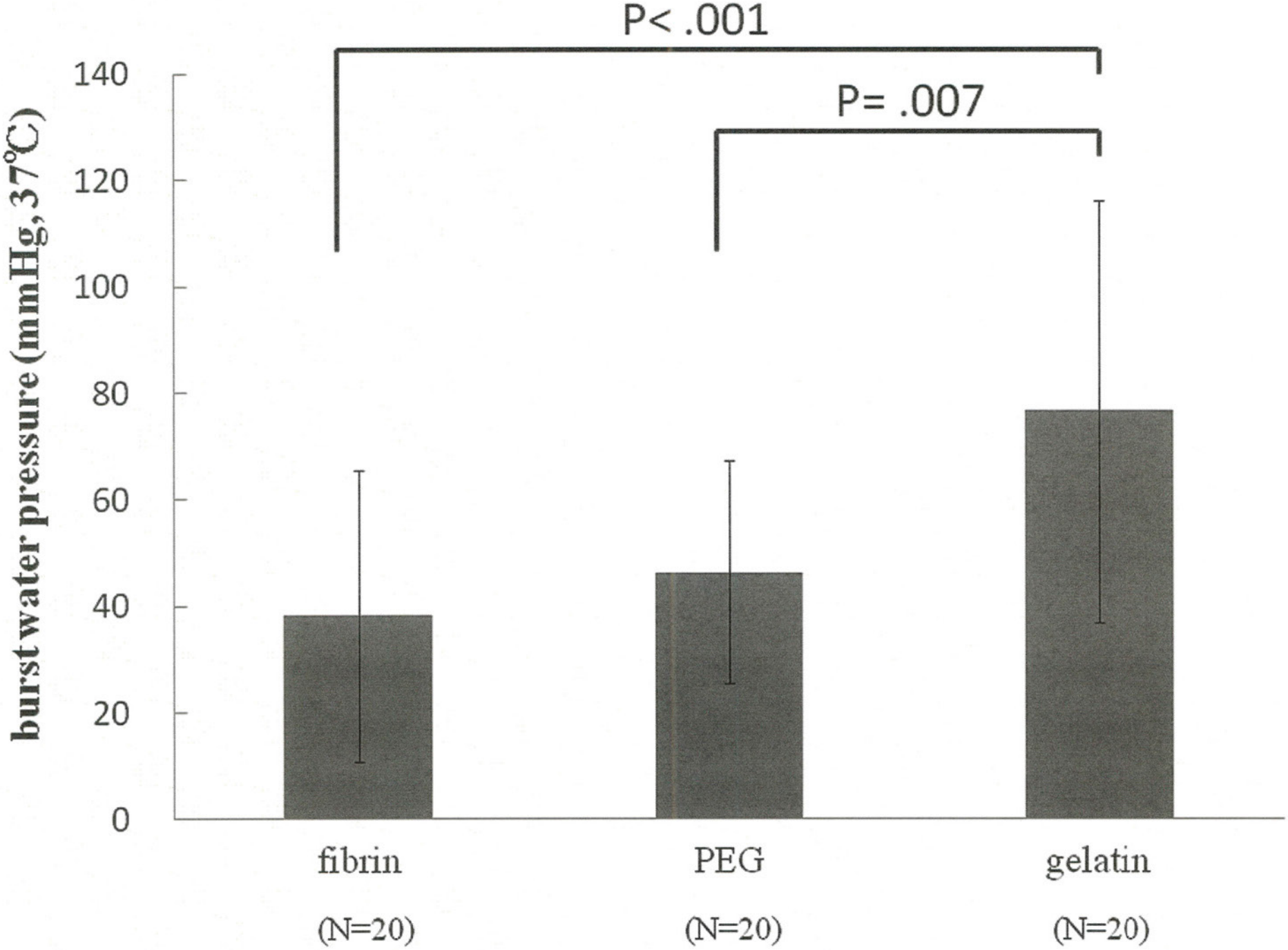
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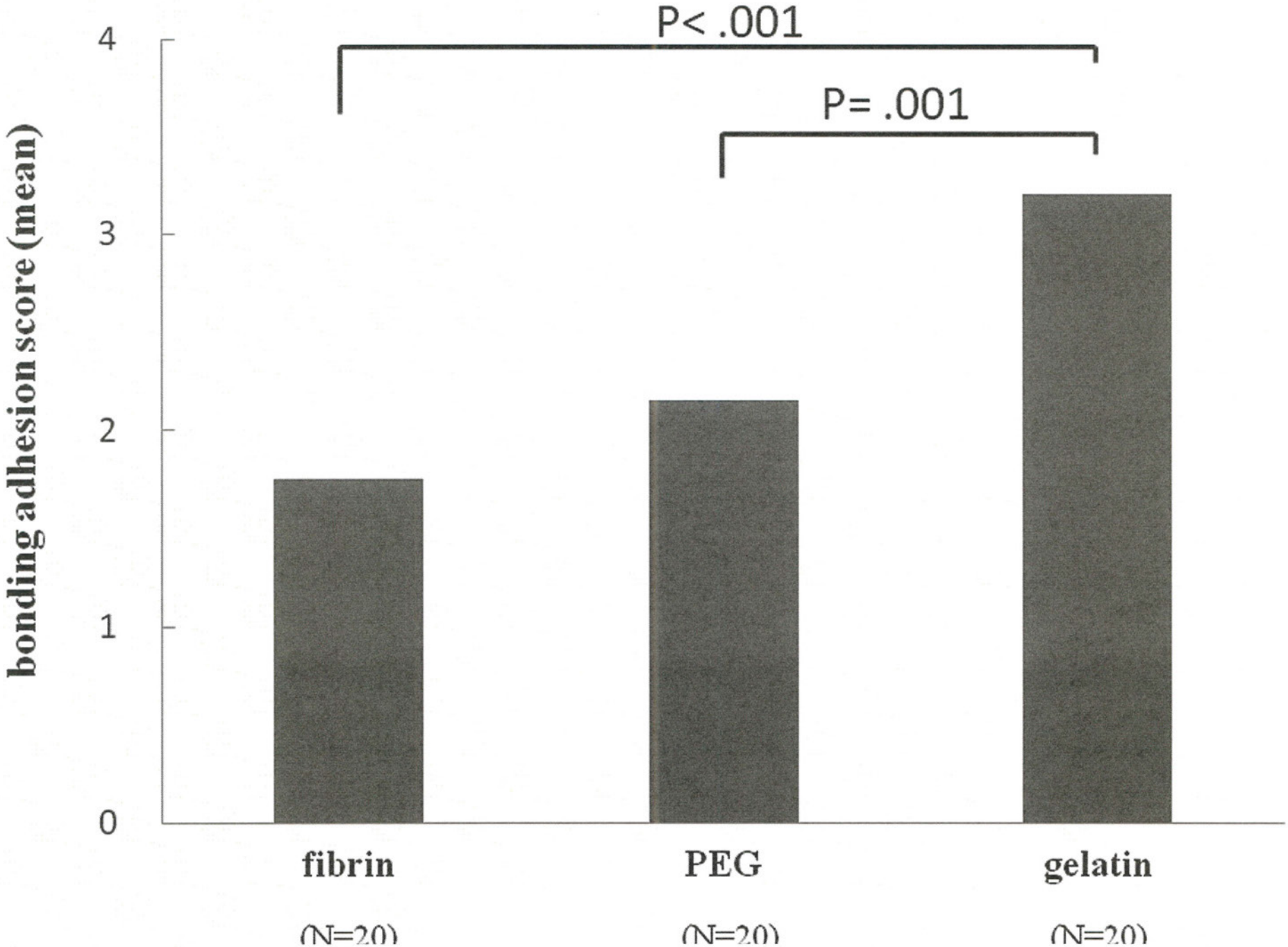
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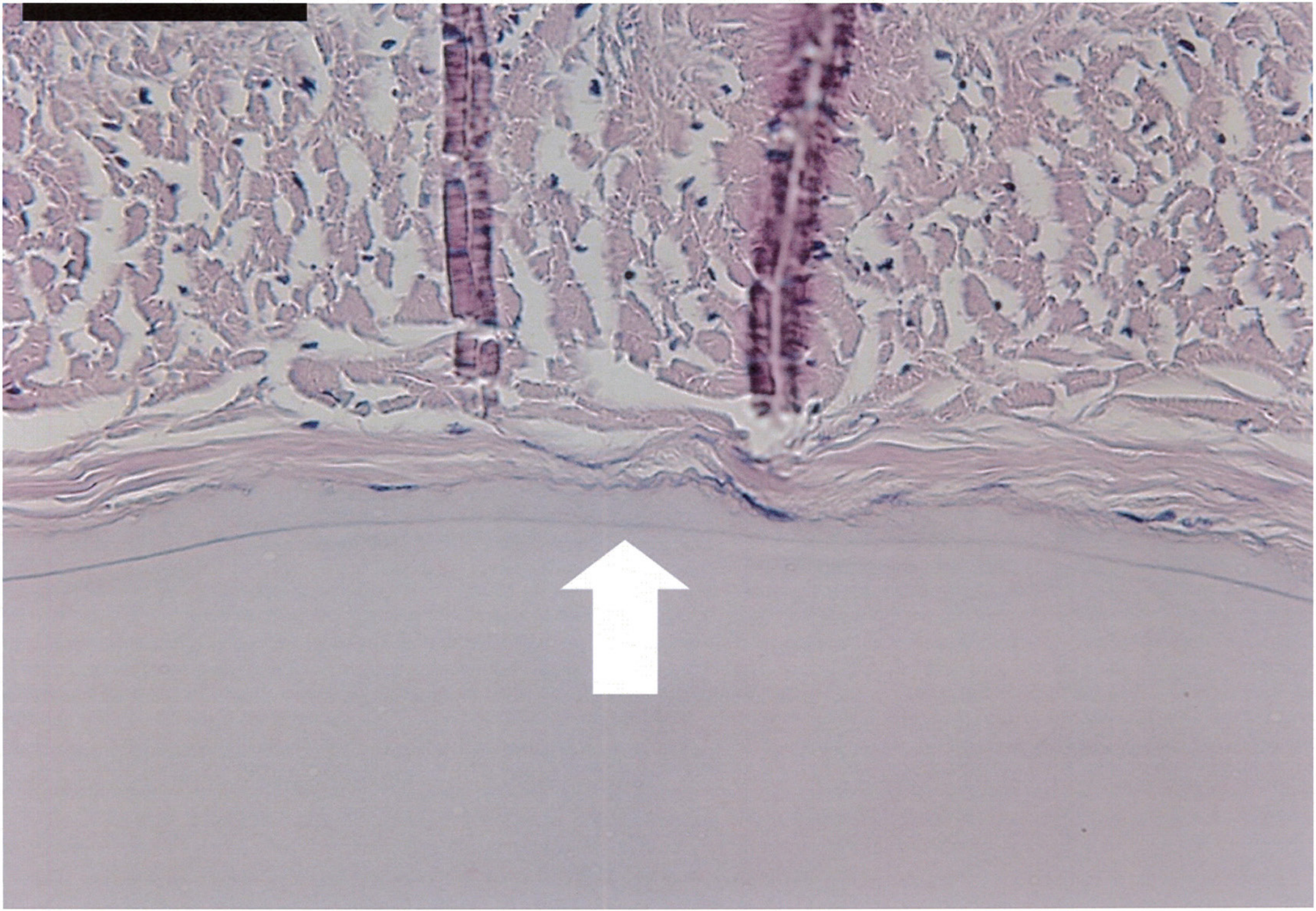

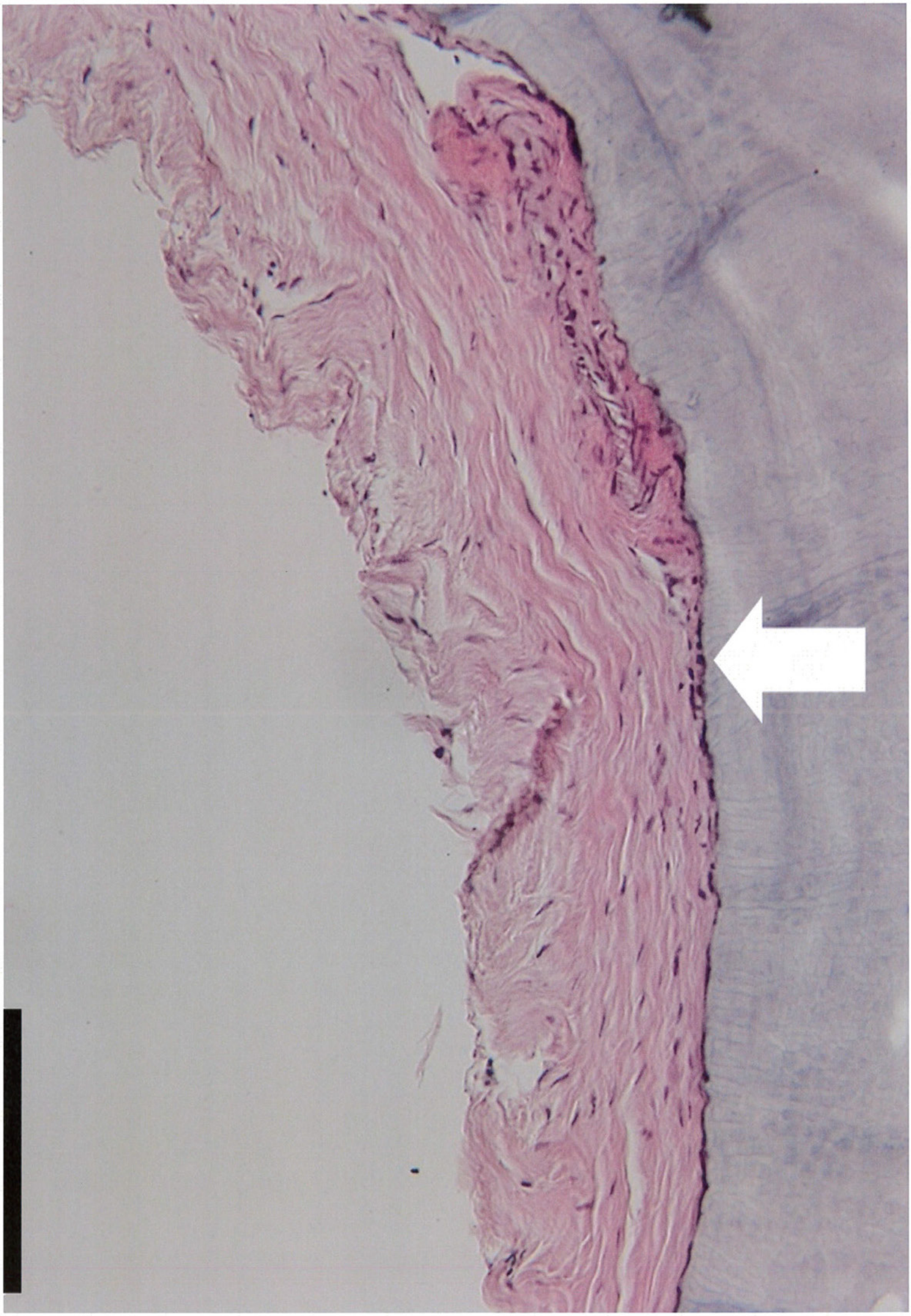

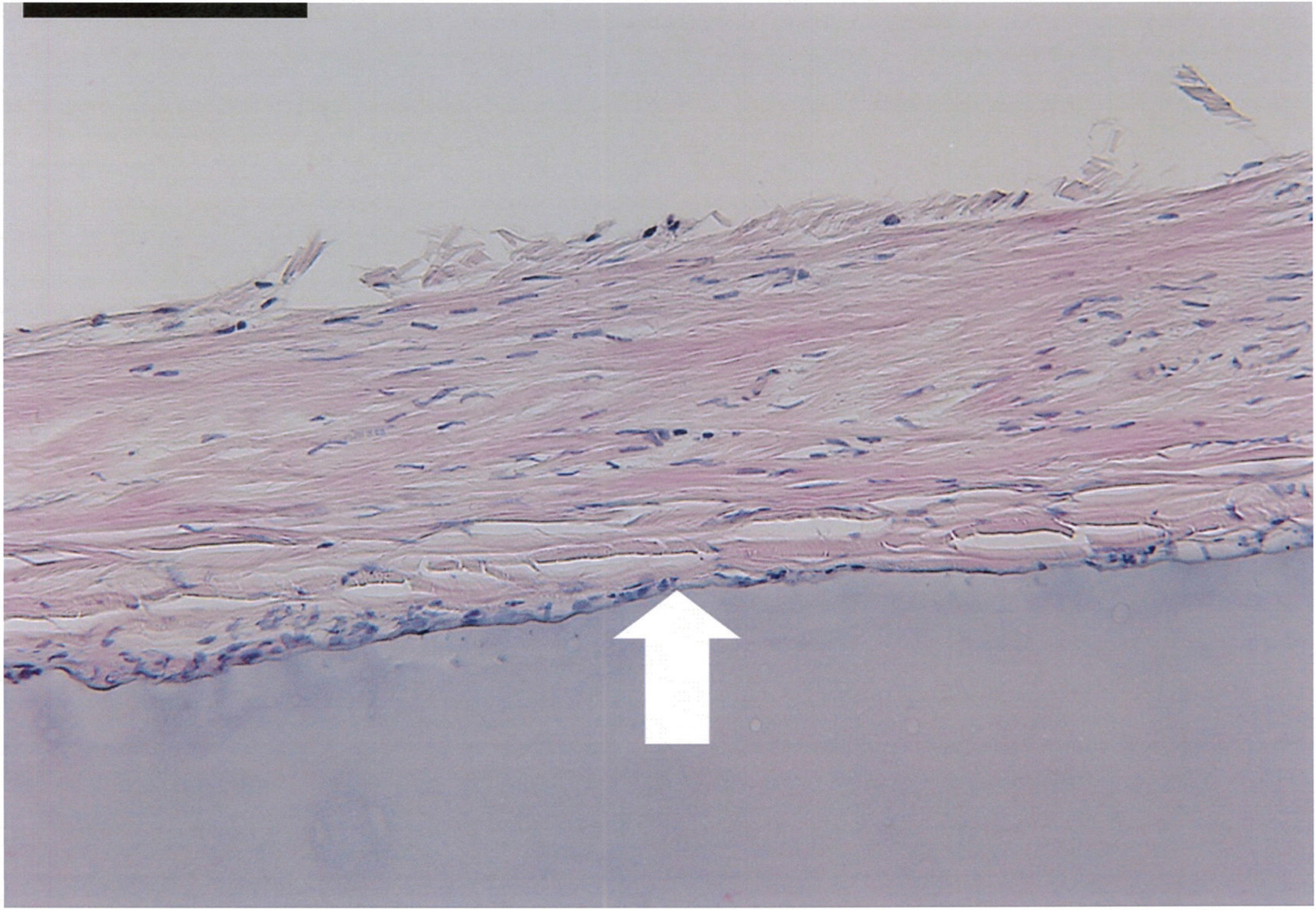

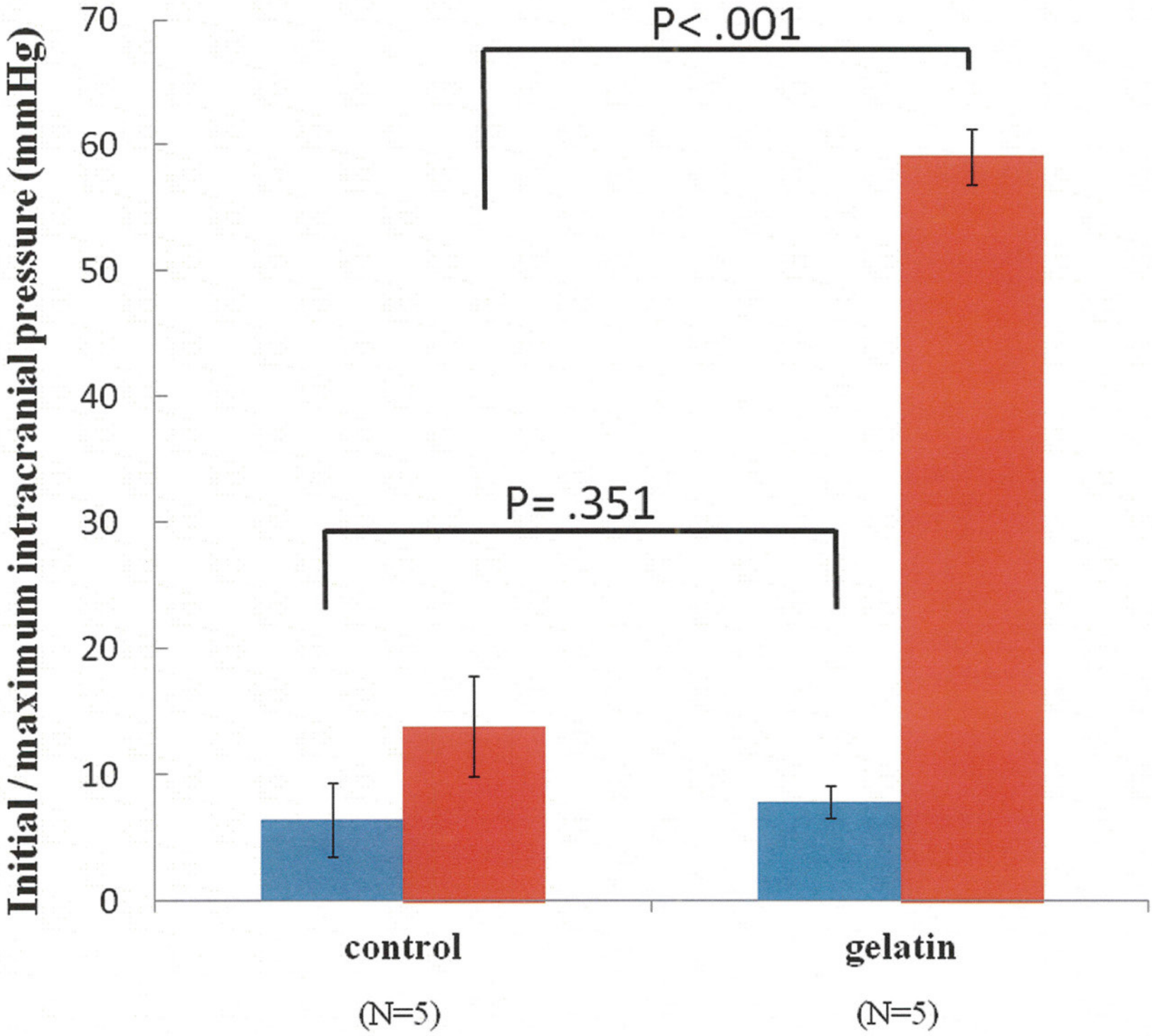
Initial / maximum intracranial pressure (mmHg)
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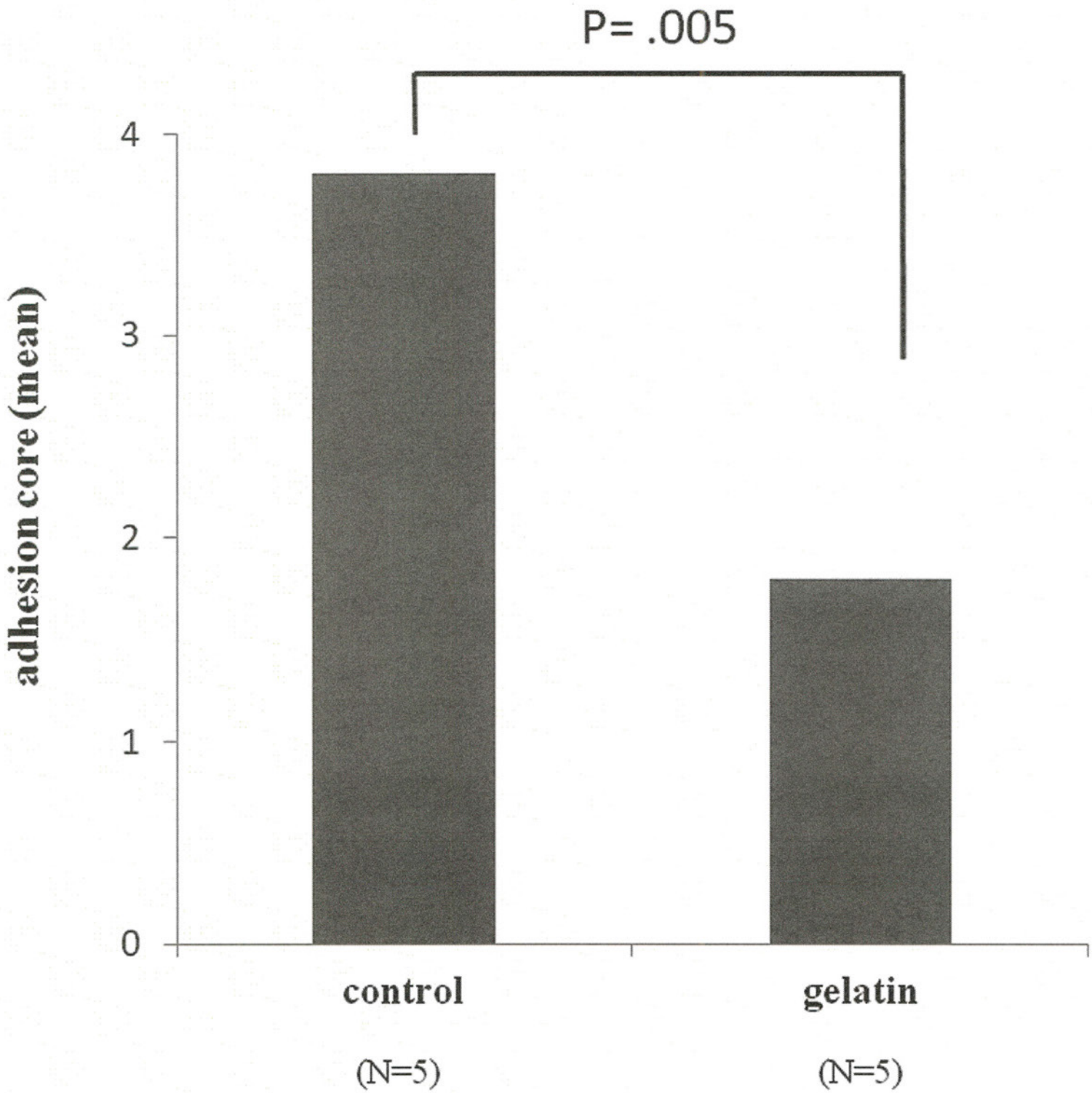
P< .001
P= .351
control
(N=5)
gelatin
(N=5)


P= .005
adhesion core (mean)
4
3
2
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control
gelatin
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