Journal of Clinical Neuroscience xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Journal of Clinical Neuroscience

journal homepage: www.elsevier.com/locate/jocn

Clinical Study

# Application of atelocollagen sheet for sellar reconstruction

Yuko Goto [a,b], Satoru Oshino [b], Takeshi Shimizu [b], Youichi Saitoh [a,b,*]

[a] Department of Neuromodulation and Neurosurgery, Graduate School of Medicine, Osaka University, 2-2, Yamadaoka, Suita, Osaka 565-0871, Japan
[b] Department of Neurosurgery, Graduate School of Medicine, Osaka University, Osaka, Japan

ARTICLE INFO

*Article history:*
Received 23 December 2014
Accepted 18 August 2015
Available online xxxx

*Keywords:*
Atelocollagen
Cerebrospinal leakage
Fibrin glue sealant
Sellar reconstruction

ABSTRACT

We aimed to evaluate combined use of atelocollagen sheet and fibrin glue for sellar reconstruction. Experiment 1: A plastic chamber was prepared with a hydroxyapatite lid with a hole of 10 mm in diameter at its center, covered with a Gore-Tex sheet (W.L. Gore & Associates, Tokyo, Japan) 15 mm in diameter and sealed with a combination of fibrin glue sealant and either atelocollagen sheet or polyglycolic acid (PGA) sheet. Air was injected into the chamber and the pressure at which air leakage occurred was measured under each situation. Mean (± standard deviation) leakage pressure was 816 ± 162 mm$H_2O$ for atelocollagen sheet (n = 5), significantly higher than the 557 ± 130 mm$H_2O$ for PGA sheet (n = 5, $p < 0.05$, Wilcoxon test). Experiment 2: Bilateral 5 mm bone windows were made in the temporal bone in eight rats. The surgical cavities were filled with one of four materials (fibrin glue only; fibrin glue and atelocollagen sheet; PGA sheet; or autologous fat tissue). Histological changes including the status of implanted materials and inflammatory responses were investigated 2 and 5 weeks after the procedures. Both atelocollagen and PGA sheets remained at 5 weeks after implantation, whereas fibrin glue and fat tissue were absorbed and undetectable at 2 weeks. Inflammatory cell accumulation was less around the atelocollagen sheet compared to the PGA sheet. The combination of atelocollagen sheet and fibrin glue sealant showed sufficient adhesion force and favorable tissue affinity, suggesting this combination as a feasible material in sellar reconstruction.



## 1. Introduction

The evolution of surgical techniques and instruments has enabled wider opening of the dura mater and bone windows for aggressive tumor resection in skull base surgery, including extended transsphenoidal surgery (TSS) [1]. Various approaches to sellar reconstruction have been applied to prevent cerebrospinal fluid (CSF) leakage after extended TSS [1–5]. The current methods for sellar reconstruction involve the use of synthetic materials, including artificial dura mater, silicone plates, titanium mesh and hydroxyapatite cement, oxidized cellulose, absorbable gelatin, collagen and bioabsorbable polyglycolic acid (PGA) mesh sheet, or autologous tissues (fat tissue, fascia, and pedicle mucosa) [4,6–9]. Reported rates of postoperative CSF leakage have ranged from 1.5–8.7% in recent reports, and prevention remains a challenge for neurosurgeons [4–6,9–11]. The surgical field during extended TSS is deep and narrow, necessitating safe, stable and convenient techniques for sellar reconstruction. CSF leakage occurs when intracranial pressure is high or when there has been spontaneous, traumatic, or iatrogenic violation of the dura. After sellar reconstruction, inflammation will cause erosion of the dura, and in that situation, normal physiologic increase in CSF pressure by coughing or straining can cause CSF leakage [12]. Previous studies have demonstrated that the CSF pressure during Valsalva maneuver is elevated more than 250 mm $H_2O$, and the maximum level was 470 mm $H_2O$ [13]. According to this study, dural substitute should be durable to greater than 500 mm $H_2O$. To prevent CSF leakage after TSS, the method for sellar reconstruction should achieve pressure durability with minimal inflammatory change.

Bioabsorbable PGA mesh sheets (Neoveil; Gunze, Kyoto, Japan) (Fig. 1) have been used in lung surgery to prevent air leakage at the interlobar surfaces of the lungs without suturing and offer good biocompatibility [14]. In the neurosurgical field, PGA sheet combined with fibrin glue has also been used to reinforce dural sutures [15]. However, in our experience, PGA sheet combined with fibrin glue offers insufficient adhesive ability for sellar reconstruction to prevent CSF leakage during TSS.

We have recently applied a cotton-type collagen hemostat, Integran (Nippon Zoki Pharmaceutical and KOKEN, Tokyo, Japan) (Fig. 2A) combined with fibrin glue in the final step of sellar reconstruction (Fig. 2B, C). This surgical material is an absorbable

* Corresponding author. Tel.: +81 6 6210 8435; fax: +81 6 6210 8434.
*E-mail address:* neurosaitoh@mbk.nifty.com (Y. Saitoh).

http://dx.doi.org/10.1016/j.jocn.2015.08.039
0967-5868/

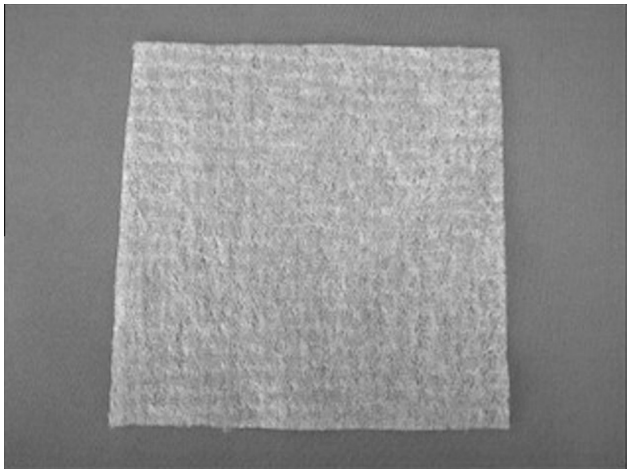

**Fig. 1.** The bioabsorbable polyglycolic acid mesh sheet used in this study (Neoveil; Gunze, Kyoto, Japan).

collagen hemostat composed of cotton fiber-like spun atelocollagen derived from calf dermis and chemically cross-linked with a polyepoxy compound. This atelocollagen sheet seems to offer stronger adhesion compared to other surgical materials. Our interest has thus moved to changes of this atelocollagen sheet after adhesion, particularly whether it is rapidly absorbed or remains intact, and its effects on inflammation. The present study examined the adhesive properties and histological changes induced by using the combination of atelocollagen sheet and fibrin glue.

## 2. Materials and methods

### 2.1. Experiment 1

Experiment 1 compared the adhesive properties between the combinations of fibrin glue and either atelocollagen sheet or PGA sheet.

A specially made 200 mL plastic chamber (diameter, 38 mm) was used in this experiment. The inside of the chamber could be pressurized by air infusion through an opening in the side wall. The lid of the chamber comprised hydroxyapatite with a 10 mm hole in the center (Fig. 3).

First, a 15 mm diameter, round Gore-Tex sheet (W.L. Gore & Associates, Tokyo, Japan) was placed over the hole without fixation. Two combinations of fibrin glue (Bolheal; Kaketsuken, Kumamoto, Japan) and either atelocollagen sheet or PGA sheet were then

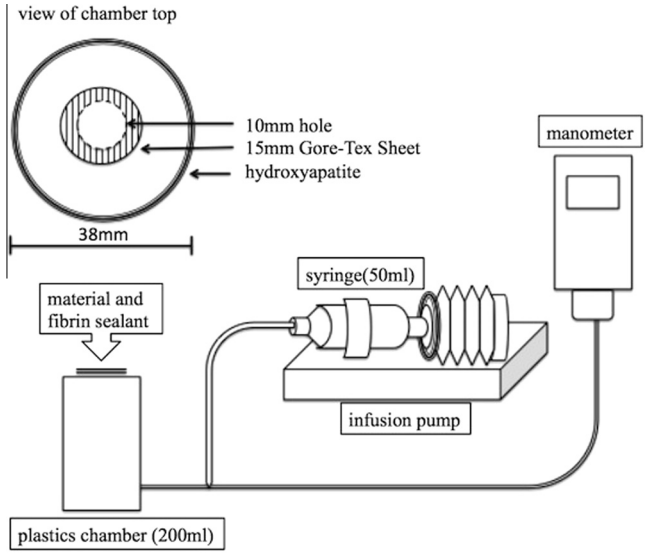


**Fig. 3.** The chamber system used for Experiment 1. The chamber is 38 mm in diameter and made of hydroxyapatite, with a 10 mm diameter hole in the center. The hole is covered with a 15 mm diameter Gore-Tex sheet (W.L. Gore & Associates, Tokyo, Japan) and sealed with a combination of fibrin glue sealant and either atelocollagen sheet or polyglycolic acid sheet.

applied as sealant. The fibrin glue consisted of Solution A and B. Solution A contains fibrinogen (80 mg/ml), blood coagulation factor XIII (75 units/ml), and aprotinin (1000 KEI/ml), whereas Solution B contains thrombin (250 units/ml) and calcium chloride (5.9 mg/ml). The Gore-Tex sheet was covered by a circle of atelocollagen sheet or PGA sheet 25 mm in diameter. Solution A (1 ml) and B (1 ml) were then applied over the entirety of each material in an identical method.

After a 5 minute interval for stabilization of the fibrin glue sealant, air was infused into the chamber at a rate of 50 ml/minute. The mean pressure at which air leakage occurred was compared between combinations (n = 5 each).

### 2.2. Experiment 2

Experiment 2 studied the histological change after implantation.

Female Wistar rats (10–11 weeks old) were purchased from Japan SLC (Shizuoka, Japan), housed under a 12 hour light-dark cycle and allowed free access to food and water.

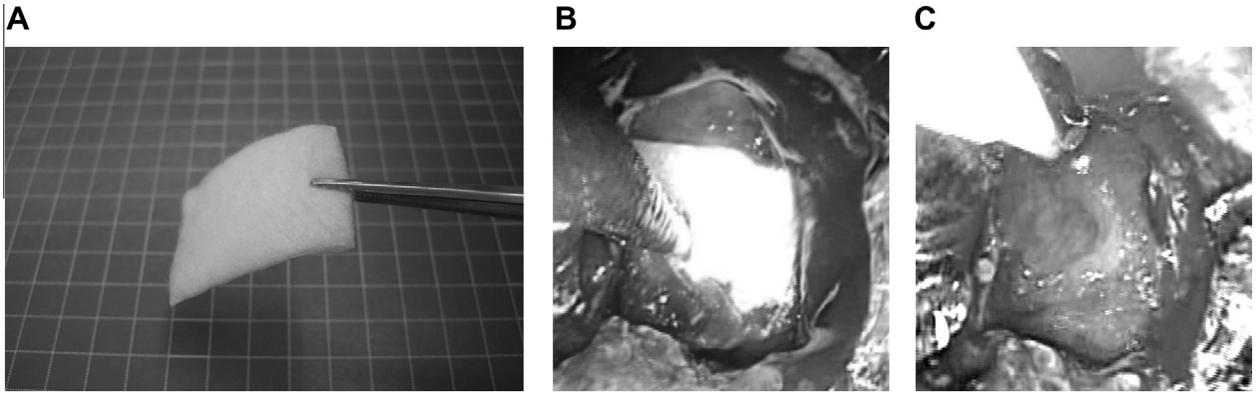


**Fig. 2.** (A) The absorbable hemostat cotton fiber-like spun atelocollagen sheet (Integran; Nippon Zoki Pharmaceutical and KOKEN, Tokyo, Japan). (B, C) Intraoperative photographs of a representative case using atelocollagen sheet for sellar reconstruction. After removal of a pituitary adenoma, sealing is achieved with a 15 mm square collagen sheet cut to fit to the area (B) and fibrin glue sealant is applied through a long nozzle to the atelocollagen sheet (C).

Please cite this article in press as: Goto Y et al. Application of atelocollagen sheet for sellar reconstruction. J Clin Neurosci (2016), http://dx.doi.org/10.1016/j.jocn.2015.08.039

Each rat was placed with the head fixed under complete sedation and analgesia with intraperitoneal injection of midazolam (2 mg/kg), butorphanol (2.5 mg/kg), and xylazine (1.0 mg/kg). After making a midline scalp incision, the temporal muscles on both sides were reflected to expose the temporal bone. A bone window 5 mm in diameter was then made in the temporal bone and the dura was exposed. We used both sides of the temporal bone to apply the two treatments separately, one on each side in each rat.

To fill the cavities, the following three treatments were applied: (1) atelocollagen sheet and fibrin glue (CLG group); (2) PGA sheet and fibrin glue (PGA group); and (3) fibrin glue and autologous subcutaneous fat tissue taken from the back of the same rat's neck (fat group). Each material was customized to form a $5 \times 5$ mm square and packed into the cavity followed by the fibrin glue (Solution A, 0.2 ml; Solution B, 0.2 ml) dripped simultaneously. As a control, the cavity was filled using the same volume of fibrin glue only (control group). The skin flap was closed using a surgical stapler. Four rats had atelocollagen sheet on the right side and only fibrin glue on the left side, and another four rats had PGA sheet on the right side and fat on the left side.

### 2.3. Histological examination

Rats were sacrificed with an overdose (150 mg/kg) of pentobarbital administered intraperitoneally at 2 or 5 weeks. After fixation in formalin and decalcification, histological changes around the implanted materials were investigated.

First, we inspected whether the materials remained intact at each measurement point. If remaining, we counted cell numbers around the materials under light microscopy (BX53; Olympus, Tokyo, Japan) with hematoxylin and eosin staining to gauge the inflammatory response (Fig. 4). Cells were counted using morphometric software (WinRoof; Mitani, Fukui, Japan) in two different sequential microscopic fields between the exposed dura mater and materials (Fig. 5A).

This protocol was approved by the Ethical Board of the Animal Care and Use Committee at Osaka University (permission #24-002-002).

### 2.4. Statistical analysis

Statistical analysis was performed using JMP Pro version 10.0.2 software (SAS Institute, Cary, NC, USA). All results are expressed as mean ± standard deviation.

## 3. Results

### 3.1. Experiment 1: leakage pressure of fibrin glue sealant combined with hemostat sheets

Mean leakage pressure was 816 ± 162 $mmH_2O$ in the atelocollagen group, significantly higher than that in the PGA group (557 ± 130 $mmH_2O$; $p < 0.05$, Wilcoxon test). At leakage, the fibrin layer itself was not disrupted, but part of the layer was stripped from the bottom. Once air leakage began, the fibrin layer surrounding the tract was immediately stripped from the bottom, forming a site for further leakage.

### 3.2. Experiment 2

None of the rats developed subcutaneous hematoma, CSF collection, or wound infection during the observation period. Implanted atelocollagen and PGA sheets remained in the surgical cavity at both 2 and 5 weeks, whereas both fibrin glue and fat were completely absorbed by 2 weeks.

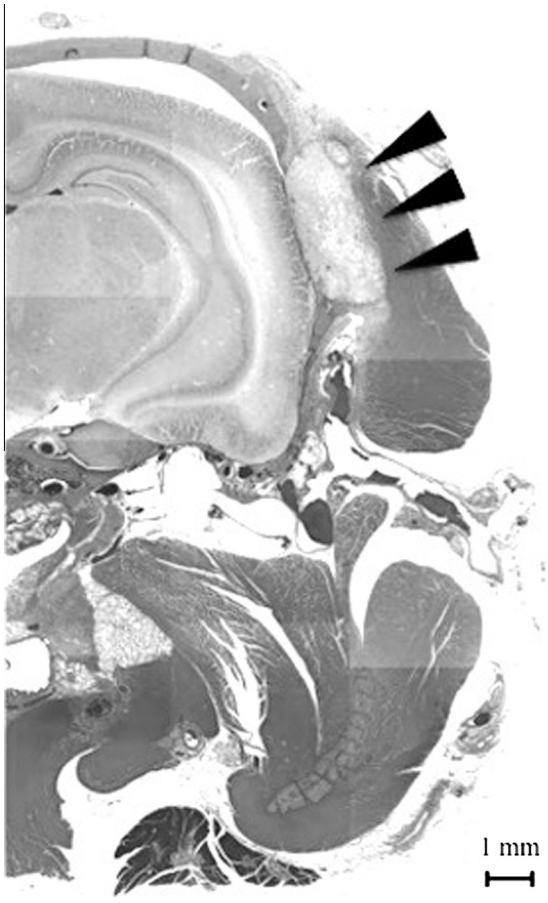


**Fig. 4.** Hematoxylin and eosin staining of a coronal section of a rat head. The fenestration site at the temporal bone was covered by a 5 mm square of atelocollagen sheet (arrowheads) with fibrin glue sealant and examined at 2 weeks.

Next, we compared inflammatory changes around the remaining atelocollagen and PGA sheets at 2 and 5 weeks. In the CLG group, mean cell count was 952 ± 415 at 2 weeks, decreasing significantly to 254 ± 38 at 5 weeks ($p < 0.05$, Mann-Whitney U-test) (Fig. 5B, C). In the PGA group, mean cell count was 881 ± 123 at 2 weeks, decreasing slightly to 619 ± 89 at 5 weeks. Accumulation of macrophages was observed only around the PGA fibers at both observation points, and multinucleated giant cells were observed around the PGA fibers at 5 weeks (Fig. 5D, E). In all groups, regenerative bone was observed at the edge of surgical cavity at 2 weeks, but had not progressed at 5 weeks.

## 4. Discussion

In the present study, the adhesive properties and histological changes of atelocollagen sheet combined with fibrin glue sealant were evaluated in models of TSS. Advantages of combining fibrin glue sealant and hemostatic materials for sellar reconstruction have been reported [2,15]. These surgical materials allow maintenance of high concentrations of fibrin glue sealant components at local sites [2], which may reinforce adhesion. In our previous study, the leakage pressure of simple fibrin glue sealant was 345 ± 36.4 $mmH_2O$ under a similar experimental system [16]. In this study, leakage pressure increased markedly to 816 ± 162 $mmH_2O$ after combining fibrin glue with atelocollagen sheet, significantly higher than with PGA sheets and more than double that with fibrin glue sealant only. Miyamoto et al. reported atelocollagen sheet to be a more effective material to prevent air

Please cite this article in press as: Goto Y et al. Application of atelocollagen sheet for sellar reconstruction. J Clin Neurosci (2016), http://dx.doi.org/10.1016/j.jocn.2015.08.039

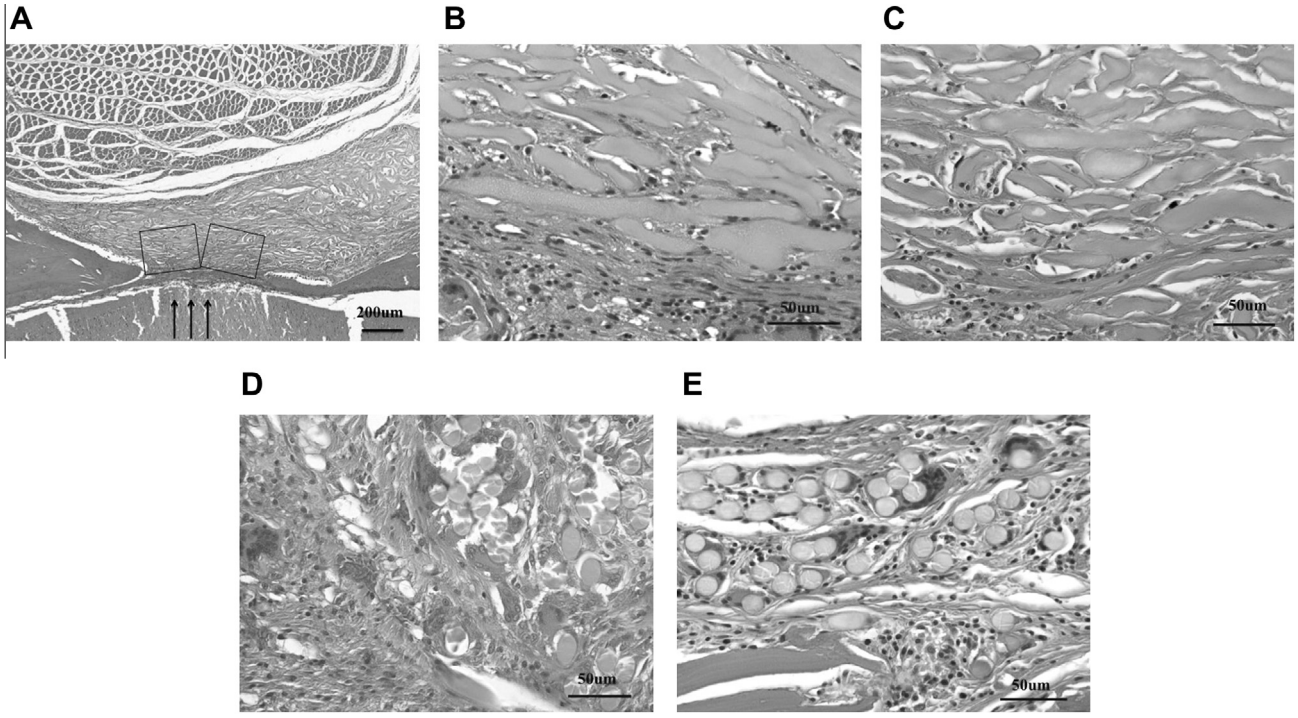


**Fig. 5.** Hematoxylin and eosin stained sections. (A) Low-power photomicrograph showing coronal section of the application site at 5 weeks in the atelocollagen sheet and fibrin glue group (CLG group). Swollen collagen fibers are apparent in the epidural space. Two boxes showed selected sequential microscopic fields. (B) The CLG group at 2 weeks shows atelocollagen sheet in the epidural space with inflammatory cell infiltration. (C) At 5 weeks, the number of inflammatory cells has decreased. (D) The polyglycolic acid and fibrin glue group (PGA group) at 2 weeks also shows inflammatory cell infiltration. (E) At 5 weeks, the number of inflammatory cells has not decreased, unlike in the CLG group, and multinucleated giant cells are seen around the PGA fibers. Black arrow = dura mater.

leakage after pulmonary surgery than other materials, such as sponge-like atelocollagen fibrin agent and PGA sheets [17]. These results support our clinical impression that atelocollagen with fibrin glue sealant achieves strong adhesion. In this experiment we used fibrin glue sealant, Bolheal. Bolheal contains 80 mg/ml of fibrinogen in Solution A and 250 units/ml of thrombin in Solution B. Tisseel (Baxter Healthcare, Westlake Village, CA, USA), the most common fibrin agent worldwide, contains 90 mg/ml of fibrinogen and 500 units/ml of thrombin. Although we have not directly compared experimental results, Tisseel results are likely to be comparable to those achieved with Bolheal.

Next, we investigated inflammatory changes around the remaining atelocollagen and PGA sheets. Both materials remained at the site after 5 weeks, whereas fat tissue and fibrin glue alone were completely absorbed by 2 weeks. Cell counts were similar in the CLG and PGA groups at 2 weeks, but were significantly decreased only in the CLG group at 5 weeks. This indicates that atelocollagen offer reduced antigenicity compared to PGA. Indeed, this atelocollagen sheet does not contain any proteins other than atelocollagen, completely removing the antigenic determinant of telopeptides. In contrast, PGA sheet contains PGA, a homopolymer with a molecular weight of $10^4$. In a reported case where resected visceral pleura of a patient with recurrent lung cancer was repaired with atelocollagen sheet, little infiltration of inflammatory cells was seen around the atelocollagen sheet and no microscopic differences between areas with or without atelocollagen sheet were identified, similar to our results [17]. Likewise in an animal study, Mizuno et al. described an atelocollagen sheet placed in the spinal epidural space of rabbits which showed less inflammatory reaction than a microfibrillar collagen hemostat, and fibrous tissue but no remaining atelocollagen fibers seen at 4 weeks after the procedure [18]. Atelocollagen has favorable biocompatibility due to this reduced antigenicity.

The implanted fat tissues were absorbed in all rats at only 2 weeks. Due to the propensity to shrink, autologous fat should be applied only for filling dead space after tumor resection, not as a scaffold for sellar reconstruction after TSS.

We had expected the atelocollagen sheet to promote bone regeneration in the cranial defect area, on the assumption that effective bone regeneration might be achieved via an atelocollagen scaffold [19]. However, atelocollagen fibers remained without absorption, but insufficient bone regeneration was achieved to lead to closure of the fenestrated skull area within 5 weeks. We consider that this may be a result of the poor trabecular structure and slower revascularization of cortical bone in the skull [19]. To evaluate the bone regenerative potential of atelocollagen sheet, further studies with longer observation periods are needed. In summery, these two experimental studies show that atelocollagen sheet and fibrin glue sealant has sufficient pressure resistance and favorable stability for the sellar reconstruction.

## 5. Conclusion

The combination of atelocollagen sheet and fibrin glue sealant has sufficient pressure resistance and favorable stability with minimal inflammatory changes for sellar reconstruction. In addition, these sheets are easy to handle in the surgical field because of the fabric-like structure. Atelocollagen sheets appear to offer an effective material for sellar reconstruction to prevent CSF leakage.

## Conflicts of Interest/Disclosures

The authors appreciate the donation of fibrin glue sealant (Bolheal) and the chamber system from Mr. Kenichiro Hirahashi at Kaketsuken Co., and Integran from Nippon Zoki Pharmaceutical.

No financial support was received from Kaketsuken Co., or Nippon Zoki Pharmaceutical.

## References

[1] Berker M, Aghayev K, Yucel T, et al. Management of cerebrospinal fluid leak during endoscopic pituitary surgery. Auris Nasus Larynx 2013;40:373–8.
[2] Cappabianca P, Cavallo LM, Valente V, et al. Sellar repair with fibrin sealant and collagen fleece after endoscopic endonasal transsphenoidal surgery. Surg Neurol 2004;62:227–33 [discussion 233].
[3] Cappabianca P, de Notaris M. The evolution of modern endoscopic endonasal skull base surgery and reconstruction techniques. World Neurosurg. 2014;82: e67–9.
[4] Cavallo LM, Messina A, Esposito F, et al. Skull base reconstruction in the extended endoscopic transsphenoidal approach for suprasellar lesions. J Neurosurg 2007;107:713–20.
[5] De Divitiis E, Cavallo LM, Cappabianca P, et al. Extended endoscopic endonasal transsphenoidal approach for the removal of suprasellar tumors: part 2. Neurosurgery 2007;60:46–58 [discussion 58–9].
[6] Hida K, Yamaguchi S, Seki T, et al. Nonsuture dural repair using polyglycolic acid mesh and fibrin glue: clinical application to spinal surgery. Surg Neurol 2006;65:136–42 [discussion 142–3].
[7] Leng LZ, Brown S, Anand VK, et al. Gasket-seal watertight closure in minimal-access endoscopic cranial base surgery. Neurosurgery 2008;62:ONSE342–43 [discussion ONSE343].
[8] Oshino S, Saitoh Y, Yoshimine T. Withstand pressure of a simple fibrin glue sealant: experimental study of mimicked sellar reconstruction in extended trans-sphenoidal surgery. World Neurosurg 2010;73:701–4.
[9] Tabaee A, Anand VK, Brown SM, et al. Algorithm for reconstruction after endoscopic pituitary and skull base surgery. Laryngoscope 2007;117:1133–7.
[10] Seda L, Camara RB, Cukiert A, et al. Sellar floor reconstruction after transsphenoidal surgery using fibrin glue without grafting or implants: technical note. Surg Neurol 2006;66:46–9 [discussion 49].
[11] Yano S, Tsuiki H, Kudo M, et al. Sellar repair with resorbable polyglactin acid sheet and fibrin glue in endoscopic endonasal transsphenoidal surgery. Surg Neurol 2007;67:59–64 [discussion 64].
[12] Neville L, Egan RA. Frequency and amplitude of elevation of cerebrospinal fluid resting pressure by the Valsalva maneuver. Can J Opthalmol 2005;40:775–7.
[13] Han CY, Backous DD. Basic principles of cerebrospinal fluid metabolism and intracranial pressure homeostasis. Otolaryngol Clin North Am 2005;38:569–76.
[14] Nakamura T, Suzuki K, Mochizuki T, et al. An evaluation of the surgical morbidity of polyglycolic acid felt in pulmonary resections. Surg Today 2010;40:734–7.
[15] Wax MK, Ramadan HH, Oritz O, et al. Contemporary management of cerebrospinal fluid rhinorrhea. Otolaryngol Head Neck Surg 1997;116:442–9.
[16] Saitoh Y, Oshino S. Enhancement of withstanding pressure of fibrin sealant by modified mixing ratio of fibrin sealant components for skull base reconstruction—technical note. Neurol Med Chir (Tokyo) 2013;53:65–8.
[17] Miyamoto H, Sakao Y, Sakuraba M, et al. The effects of sheet-type absorbable topical collagen hemostat used to prevent pulmonary fistula after lung surgery. Ann Thorac Cardiovasc Surg 2010;16:16–20.
[18] Mizuno K, Ikeda T, Ikoma K, et al. Evaluation of resorption and biocompatibility of collagen hemostat in the spinal epidural space. Spine J 2014;14:2141–9.
[19] Martin RB. Types of bone tissue. In: Martin RB, Burr DB, Sharkey NA, editor. Skeltal tissue mechanics. New York: Springer-Verlag, Inc; 1998. p. 32–34.

Please cite this article in press as: Goto Y et al. Application of atelocollagen sheet for sellar reconstruction. J Clin Neurosci (2016), http://dx.doi.org/10.1016/j.jocn.2015.08.039