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A Newly Designed Anal Fistula Plug: Clinicopathological Study in an Experimental Iatrogenic Fistula Model

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We report on a clinicopathologic study in an animal model of treatment with a new bioabsorbable polymer plug (BAPP). Over a 2-week period, 6 porcine models, which each had 4 anal fistulae, were created using Blake drains. The pigs were divided into 2 groups: the BAPP-treatment group (n = 12 fistulae) and the control group (n = 12 fistulae). Two weeks later, the pigs were humanely killed, and the perianal sites were excised and examined with gross and pathologic studies. Each fistula in the BAPP group was completely cured. In the pathologic study, the treatment sites had little disarray, few defects in the muscular layer, and small numbers of inflammatory cells. The control group had a significantly greater number of inflammatory cells and microabscesses than the BAPP group. The newly developed BAPP reduced the infection and induced good healing in anal fistulae. The BAPP may be a useful new device for the clinical treatment of anal fistulae.

Key words: Anal fistula – Anal fistula plug – Bioabsorbable polymer – Wound healing

Treatment of an anal fistula, which is difficult, can result in troublesome relapses and anal dysfunction.[1,2] Conventional treatments for anal fistulae include coring-out, lay-open, ligation of intersphincteric fistula tract procedures, and advancement flap procedures. These treatments may sometimes induce fecal incontinence because of the effect of the interventional approach on the sphincter muscle.[3–7] It is necessary for treatments of anal fistulae to prevent contamination and reduce the rates of infection and inflammation in order to induce good healing in anal fistulae.[8] A variety of

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techniques, including fibrin glue[9,10] and biologic plugs,[11] have been designed and applied in the clinical setting in order to accomplish these treatment goals. The outcomes of the use of these methods result in a wide variety of efficacy,[11] and the biologic materials used might cause zoonotic infections that go undetected. In order to resolve these problems, we recently developed a bioabsorbable polymer plug (BAPP) that is made completely of synthetic material. Thus, in this clinicopathologic study, we constructed anal fistula models using large animals and investigated the effects of BAPP treatment.

Materials and Methods

BAPP

The BAPP was composed of a 50:50 copolymer of polylactic acid and polycaprolactone, which was designed to degrade and be completely absorbed in about 8 weeks. It had a long conical shape, the greatest dimension of which was 5 mm (Fig. 1). It included an absorbable suture at the top of the cone, which was designed to make it easy to pull the BAPP into the fistula. The BAPP had a sponge-like texture, so that it could fit into the complicated fistula shape. The BAPP had an air porosity of 95% or more in order to allow easy penetration of the cells. Our laboratory developed the BAPP in collaboration with Gunze Ltd (Kyoto, Japan), but the invention is not yet commercially available.

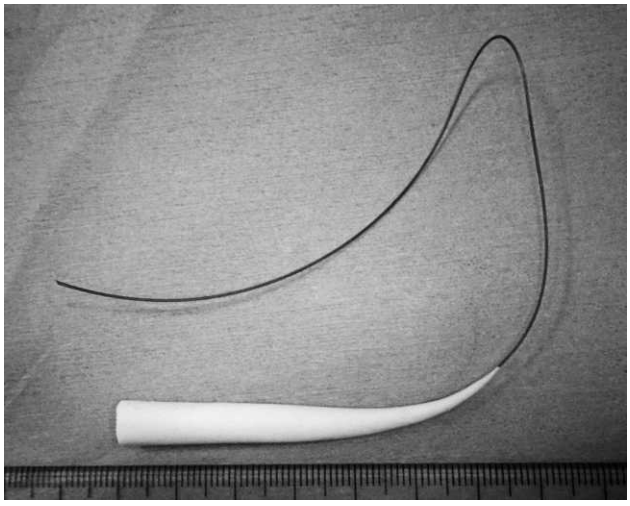

Fig. 1 Bioabsorbable polymer plug.

Animal operation

The experiment, which was performed on pigs, complied with the National Institutes of Health Guidelines and the Animal Research Protocol of Saitama Medical University (approval No. 690). We used 6 pigs, aged 1 to 2 years, with body weights of 20 to 30 kg. All of the pigs were fasted for 12 hours before the surgery. After premedicating the pigs with intramuscular ketamine hydrochloride (10 mg/kg), sevoflurane inhalation (2%–3%) and general anesthesia were maintained with mechanical ventilation. The animals were immobilized in the lithotomy position.

Construction of the anal fistula animal model

We constructed the anal fistula animal model shown in Fig. 2 by reference to the method developed by Buchanan *et al*[12] and Han *et al*.[13] BLAKE Silicone Drains (19 Fr; Ethicon Inc, Cornelia, Georgia) were introduced from the anal canal to the perianal skin at the 2-, 5-, 8-, and 11-o'clock positions (Fig. 2a). Four weeks after that, the drains were removed, and we confirmed construction of the fistulae (Fig. 2b). Twenty-four fistulae were constructed in order to create an anal fistula model. These fistulae were divided into the following 2 groups: the BAPP-treatment group (n = 12) and the no-treatment group (n = 12).

BAPP-treatment group

While under the same general anesthesia as that used in the first operation, the pigs (BAPP-treatment group, n = 12) were immobilized in the lithotomy position. The fistulae were irrigated with 50 mL saline. A pean was inserted from the secondary opening into the primary opening (Fig. 3a). A pull braid of the BAPP was grasped by the pean and pulled toward the secondary opening (Fig. 3b). We stopped pulling it out when we felt resistance, and the BAPP was cut in the excess end and fixed to the skin with an absorbable suture (Fig. 3c). The 2 sites of the treated fistulae were then configured in order to be placed in the diagonal site.

Control group

While under the same general anesthesia as that used in the first operation, the animals (control group, n = 12) were immobilized in the lithotomy position. The fistulae were irrigated with 50 mL saline. A pean was inserted from the secondary opening into the primary opening. The natural

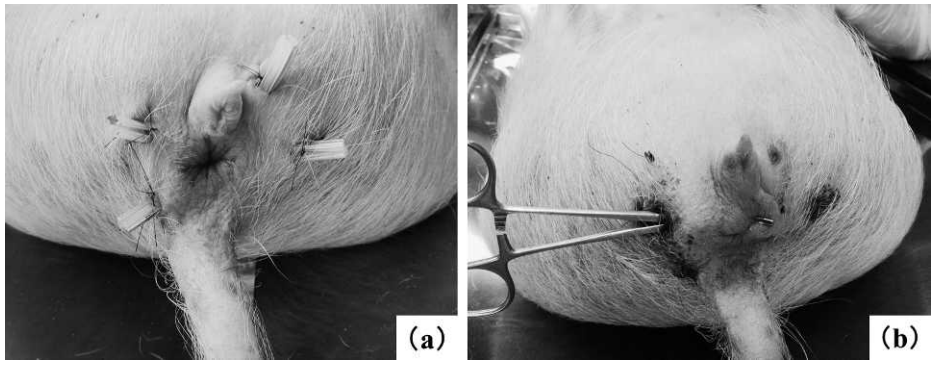

Fig. 2 Design of the anal fistula animal model. (a) The BLAKE drains were inserted from the anal canal into the perianal skin. (b) Confirmation of the construction of the anal fistula by a pean.

courses of the fistulae not treated with BAPP in this group were then followed.

Accordingly, each pig had 4 fistulae that were allocated to 2 sites with BAPP treatment and 2 sites without treatment. After treatment, the pigs were fed the same diet that they were fed preoperatively.

Evaluation

Two weeks after treatment, the perianal sites with the fistulae were excised en bloc. The specimens that were obtained were fixed in a 10% formaldehyde solution, sectioned in a line through the primary and secondary openings, embedded in paraffin wax, and sliced into 6-μm sections for microscopy. The sections that contained the maximum length of the fistulae were microscopically evaluated with hematoxylin and eosin (H&E), Elastica van Gieson stain, or immunohistochemical staining. Microabscesses stained with H&E in the section were counted at ×100.

Macrophages were immunohistochemically stained with a monoclonal anti-macrophage antigen (Biomeda Corp, Foster City, California) and counted in order to obtain an index of inflammation and foreign body reaction. Macrophage cells were counted in 5 areas, which were evenly selected at the section of fistula, and the number of macrophage cells per millimeter square was calculated.

Statistical analysis

The values of the numerical data were expressed as median and ranges. Statistical analyses were performed using the JMP 8.0 software (SAS Institute Inc, Cary, North Carolina). Comparisons between the 2 groups were analyzed by the Fisher exact test or the Mann-Whitney test. Probability values less than 0.05 were considered significant.

Results

It was easy to place the BAPPs; so, each BAPP was placed in each of the lesions within 1 minute. All operated animals had a normal oral intake of food and normal stool and survived until they were killed humanely.

The macroscopic examinations showed that all lesions in the BAPP-treated group were cured and

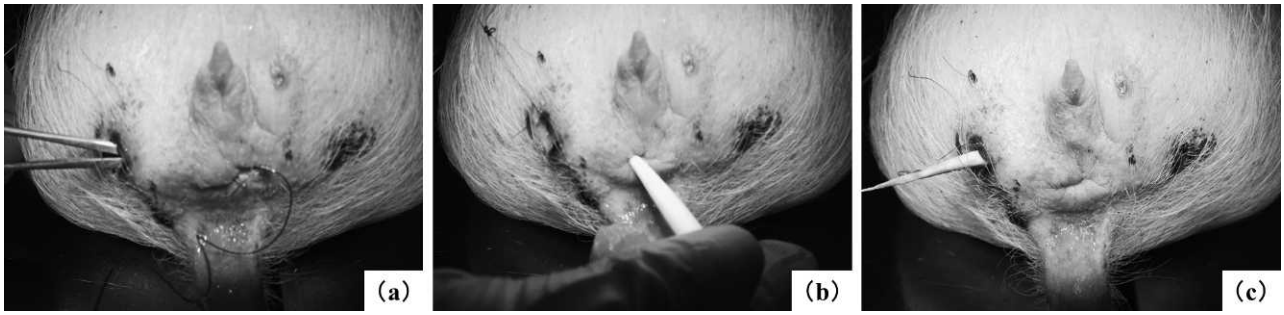

Fig. 3 Procedure of BAPP placement. (a) A pean was inserted from the secondary opening into the primary opening. (b) A pull braid was pulled toward the secondary opening. (c) The BAPP was fixed to the skin with an absorbable suture.

indistinguishable from the surrounding tissue, except for a small indentation in the anal mucosa (Fig. 4a), while 5 remaining fistulae (41.7%) in the untreated controls were found. Thus, there were significant differences in the rates of healing between the groups ($P < 0.05$).

The histologic study showed that there were no traces of the BAPPs and a small number of inflammatory cells in the treated group (Fig. 5a). The muscular layer had no ruptures. Minimal connective tissue was found between the sphincter muscles (Fig. 6a). The number of microabscesses was 1.25 ± 0.96, and the number of macrophages was 678.1 ± 285 (Table 1).

The control group had 5 residual fistulae [RF; 5 of 12 (41.7%)] and 7 spontaneously repaired [SR; 7 of 12 (58.3%)] fistulae. The number of microabscesses was 3.83 ± 1.64, and the number of macrophages was 1153 ± 379.1 in the control group (Table 1). These numbers were significantly larger than the numbers for the BAPP group ($P < 0.05$ and $P < 0.001$, respectively).

Residual fistulae

The RF were completely open and had thickened walls with feculence (Fig. 4b). The histologic study showed that the RF contained no cells (Fig. 5b and 6b). A large number of inflammatory cells, including macrophages, were found around the fistulae (Fig. 7). The number of microabscesses was 5.0 ± 1.58, and the number of macrophages was 1300 ± 415.2 around the RF (Table 1). The number of microabscesses and macrophages was significantly increased compared with the BAPP-treated group ($P < 0.001$ and $P < 0.05$, respectively).

Spontaneously repaired fistulae

Gross examinations showed that the fistulae were closed, but granulomatous mucosae were found at the site of the primary opening and at the site of the secondary opening (Fig. 4c). Histologic findings at the fistulae showed that there was a mass of connective tissue across the sphincter muscles (Fig. 5c and 6c). The number of microabscesses was 3.0 ± 1.15, and the number of macrophages was 1049 ± 343.4 around the SR fistulae (Table 1). The number of microabscesses and macrophages was significantly increased compared with the BAPP-treated group ($P < 0.005$).

Discussion

The treatments of anal fistulae have 2 major problems. The first is incontinence due to the invasive procedure, and the other is relapse of the fistula or infection.

In 2006, Johnson and his colleagues reported on a new treatment of anal fistulae using SurgiSIS, which involves a procedure that is less invasive and results in less relapse.[14] The SurgiSIS is an acellular matrix that is composed of porcine small intestinal submucosa, SIS which is an infection-resistant material. The material is degraded and replaced by host tissue over 3 months. However, according to later reports, the patient cure rate using the SurgiSIS is inconsistent and ranges from 24% to 92%.[11,15–20] The use of the SurgiSIS exhibited a few problems for anal fistula treatments. One of the major problems was that the degraded time was slow. The slowness in the degradation induced continuous foreign body reactions and a delay of fistula healing. Our BAPP was designed to degrade earlier than SIS, and it falls

Fig. 4 Posttreatment specimens of anal fistulae. (a) Specimen from the BAPP group. (b) Specimen from a residual fistula in the control group. (c) Specimen from a spontaneously repaired fistula in the control group.

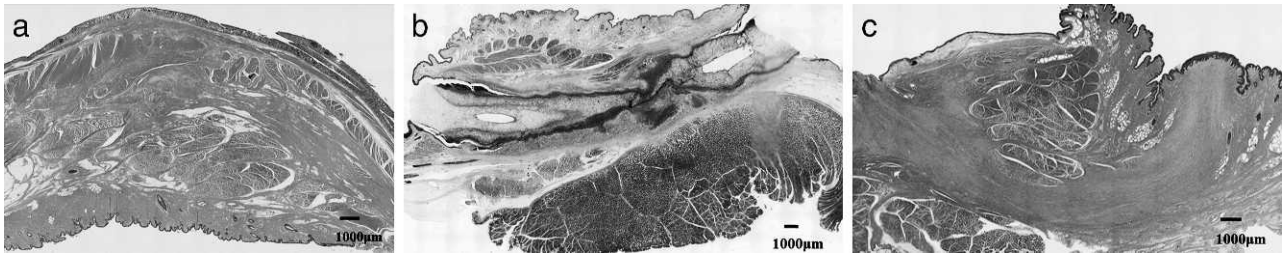

Fig. 5 Posttreatment specimens of anal fistulae stained with H&E and visualized at ×20. (a) Specimen from the BAPP group. (b) Specimen from a residual fistula in the control group. (c) Specimen from a spontaneously repaired fistula in the control group.

out before completely absorbed. Furthermore, the SurgiSIS may have a risk of zoonosis because it is a xenograft that is derived from pigs.

We newly developed the BAPP in order to prevent these problems. This material is artificially synthesized, thus it has no risk of zoonosis. In addition, modifying the structure or composition can make it possible to manage the time required for degradation. It can be used in contaminated fields, such as in bile duct replacement.[21] Because the BAPP has a pull braid, it can be moved through a long and thin fistula with guide wire.

We designed the anal fistula model using pigs. A large animal model is essential for this study when considering the necessity of the pathologic evaluations and the reproducibility in humans.

A rate of 58.3% of the fistulae in the control group was spontaneously repaired. We assume that one of the reasons for the differences in the repairs in the control group is the varied routes of the fistulae.

Two weeks after treatment, the BAPP did not remain in all of the fistulae. For 2 weeks, while the BAPP was degrading, a piece of the BAPP was hydrolyzed and another piece was defecated. We think that an advantage of the BAPP is that it prevents contaminated matter from entering into the fistula from the primary opening. It is necessary that no residual foreign bodies that are used as materials and that remain in the body be completely absorbable.

In this study, we evaluated the effects of the use of the BAPP on the cure rate and the pathologic findings. Significant differences were found between the BAPP-treated group (100%) and the control group (58.3%). There have been some reports on the treatments of anal fistulae using similar materials, but the reports did not include details on the healing mechanism. Therefore, we investigated the pathologic differences in the healing processes between the BAPP group and the control group. Significant differences between them were confirmed by the pathologic counts of microabscesses and macrophage cells. The results showed that treatment with the BAPP prevented the entry of contaminated matter into the fistulae and reduced the levels of inflammation and infection at an early state. Moreover, the significant differences between the BAPP and SR groups were confirmed in the numbers of microabscesses and macrophage cells. Compared with the BAPP group, the SR group had a lot of inflammatory cells and microabscesses in the healing connective tissue that

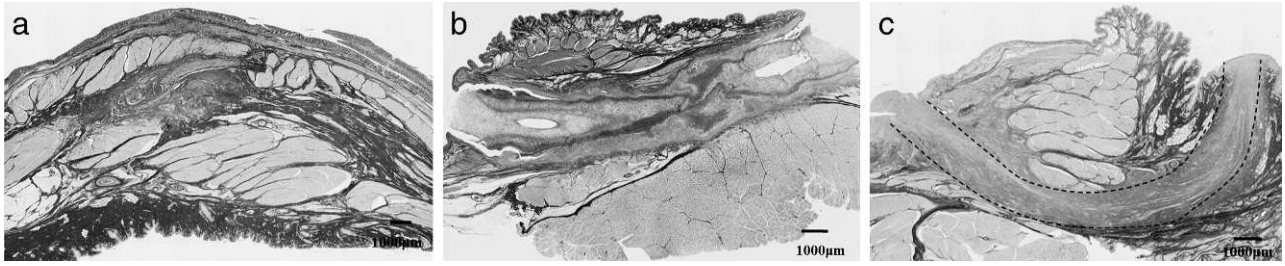

Fig. 6 Posttreatment specimens of anal fistulae stained with Elastica van Gieson and visualized at ×20. (a) Specimen from the BAPP group. (b) Specimen from a residual fistulae in the control group. (c) Specimen from a spontaneously repaired fistula in the control group. The dashed lines indicate the outline of the fistula.

Table 1 Counting of macrophages and microabscesses in the BAPP group and the control group[a]

	Treated group (n=12)	Non-Treated group (n=12)	
		Residual fistula(n=5)	Spontaneous repair (n=7)
Abscess	1.25 ± 0.965	5.0±1.58	3.0±1.15
		*	*
		*	3.83±1.64
Macrophages	678.1± 285.6	1300±415.2	1049±343.4
		*	*
		*	1153.1±379.173

*$P < 0.001$.

[a]Control group included 2 subgroups: residual fistula group and spontaneous repair group.

replaced the fistulae. This finding suggests that infection and inflammation had not been completely restored, which may contribute to the frequent relapses of anal fistulae. A prolonged infection, which the BAPP could prevent, may cause the recurrence of anal fistulae.

We are currently developing comparative experiments of the BAPP, SurgiSIS, and the newly developed GORE BIO-A Fistula Plug (W.L. Gore & Associates Inc, Newark, Delaware).[22] Therefore, we are planning a clinical trial using the BAPP with the exact protocol. The BAPP can be modified in order to suit wide varieties of shape, thus we are planning to use the BAPP for the treatment of gastrointestinal fistulae, biliary fistulae, and bronchial fistulae.

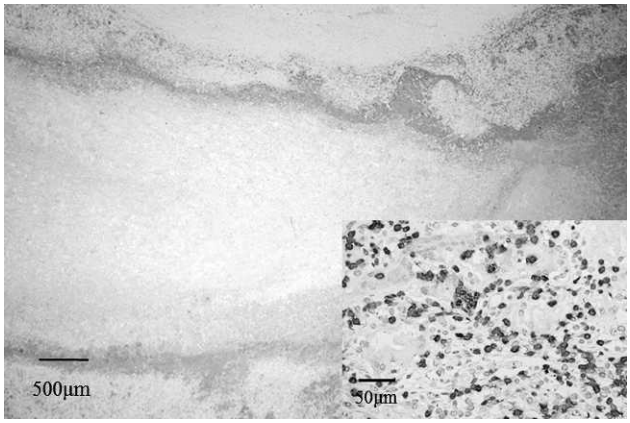

Fig. 7 Immunohistochemical staining with the anti-macrophage antigen. Many macrophages are shown around the fistula.

Conclusion

The BAPP that we developed reduced the levels of infection and inflammation in fistulae and induced the favorable healing of anal fistulae. Therefore, these findings suggest that the use of the BAPP could cure anal fistulae at high rates.

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