

Enhanced intestinal anastomotic healing with gelatin hydrogel incorporating basic fibroblast growth factor[†]

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Abstract

Anastomotic leakage is a common complication of intestinal surgery. In an attempt to resolve this issue, a promising approach is enhancement of anastomotic wound healing. A method for controlled release of basic fibroblast growth factor (bFGF) using a gelatin hydrogel was developed with the objective of investigating the effects of this technology on intestinal anastomotic healing. The small intestine of Wistar rats was cut, end-to-end anastomosis was performed and rats were divided into three groups: bFGF group (anastomosis wrapped with a hydrogel sheet incorporating bFGF), PBS group (wrapped with a sheet incorporating phosphate-buffered saline solution) and NT group (no additional treatment). Degradation profiles of gelatin hydrogels in vivo and histological examinations were performed using gelatin hydrogels with various water contents and bFGF concentrations to define the optimal bFGF dose and hydrogel biodegradability. The anastomotic wound healing process was evaluated by histological examinations, adhesion-related score and bursting pressure. The optimal water content of the hydrogel and bFGF dose was determined as 96% and 30 µg per sheet, respectively. Application of bFGF significantly enhanced neovascularization, fibroblast infiltration and collagen production around the anastomotic site when compared with the other groups. Bursting pressure was significantly increased in the bFGF group. No significant difference was observed in the adhesion-related score among the groups and no anastomotic obstruction and leakage were observed. Therefore controlled release of bFGF enhanced healing of an intestinal anastomosis during the early postoperative period and is a promising method to suppress anastomotic leakage. Copyright © 2013 John Wiley & Sons, Ltd.

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1. Introduction

Anastomotic leakage is one of the most common complications of intestinal surgery and is a major factor contributing to postoperative morbidity and mortality. Anastomotic leakage leads to potentially life-threatening complications, additional surgical procedures, increased

length of hospital stay, increased cost and long-term disability (Thompson *et al.*, 2006). Although various methods have been proposed to reduce the incidence of anastomotic failure, including preoperative bowel preparation, antibiotic prophylaxis, parenteral nutrition, usage of stapling instruments, omentopexy and careful surgical technique, the leak rate after a low anterior resection still remains high, ranging from 3% to 21% (Ho and Ashour, 2010; Kameoka *et al.*, 2005; Lee *et al.*, 2008; Madbouly *et al.*, 2010; Okajima *et al.*, 2007; Vignali *et al.*, 1997). Therefore, other methods for increasing the stability of an anastomosis are required.

One of the most important factors contributing to the stability of an anastomosis is the promotion of wound healing

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associated with local vascularization at the anastomotic site (Enestvedt *et al.*, 2006; Ho and Ashour, 2010; Inoue *et al.*, 2005; Kameoka *et al.*, 2005; Lee *et al.*, 2008; Madbouly *et al.*, 2010; Okajima *et al.*, 2007). Therapies using growth factors, such as basic fibroblast growth factor (bFGF), vascular endothelial growth factor, hepatocyte growth factor, epidermal growth factor, platelet-derived growth factor, insulin-like growth factor-I and transforming growth factors alpha and beta, are reported to be promising for the promotion of wound healing (Lynch *et al.*, 1989; Kiba *et al.*, 2003; Yoshida *et al.*, 2004; Demirdögen *et al.*, 2010). Among these, bFGF is an angiogenic factor that can promote the growth of fibroblasts, capillary endothelial cells and epithelial cells (Gospodarowicz, 1974; Matsumoto and Tabata, 2006). Several studies have demonstrated that bFGF serves as a key factor to accelerate wound healing by inducing vascularization and fibroblast proliferation (Okumura *et al.*, 1996; Nakajima *et al.*, 2004; Kawai and Suzuki, 2005; Matsumoto and Tabata, 2006).

We have developed a method for the controlled release of bFGF using a gelatin hydrogel (Tabata *et al.*, 1994; Tabata, 2003, 2008) and reported that they are able to achieve continuous bFGF release and consequently induce the regeneration of tissues, including blood vessels, bone, skin, nerves, and fat tissues (Tabata, 2003, 2008; Kawai and Suzuki, 2005; Aimoto *et al.*, 2007; Katsuno *et al.*, 2011). In the present study, the optimal bFGF dose and hydrogel biodegradability *in vivo* were determined first and then the effects of a gelatin hydrogel incorporating bFGF on wound healing at an intestinal anastomosis were further investigated.

2. Materials and methods

2.1. Preparation of gelatin hydrogel sheets incorporating bFGF

Gelatin with an isoelectric point (IEP) of 5.0 (MW = 100 000), prepared through an alkaline process from bovine bone collagen, was kindly supplied by Nitta Gelatin Co. (Osaka, Japan). Hydrogel sheets were prepared by chemical crosslinking of gelatin. Briefly, an aqueous solution of 3 wt% gelatin, containing various amounts of glutaraldehyde, was cast into a polytetrafluoroethylene mould (80 × 80 × 26.5 mm). Crosslinking was allowed to proceed for 12 h at 4°C, and the resulting hydrogel sheets were immersed in a 50 mM glycine aqueous solution at 37°C for 1 h in order to chemically block the residual aldehyde groups; this was followed by rinsing twice with double-distilled water at 37°C and freeze-drying (see Figure 1a,b). The water content of gelatin hydrogels A–E was calculated from their weight before and after hydration in a phosphate-buffered saline solution (PBS), pH 7.0, for 24 h at 37°C and expressed as the weight ratio of water in each hydrogel to that of the completely hydrated hydrogel (Table 1).

An aqueous solution of bFGF (IEP = 9.6, 10 mg/ml, MW = 17 000) was supplied by Kaken Pharmaceutical

(Tokyo, Japan). The bFGF solution (30 µl, containing 0.1, 3, 10, 30, and 100 µg bFGF) was dropped onto a freeze-dried hydrogel sheet (5 × 20 × 1 mm), which was then left to stand at 4°C for 12 h for bFGF impregnation. A similar procedure using bFGF-free PBS was performed to obtain gelatin hydrogel sheets incorporating PBS.

2.2. Preparation of the animals

Albino male Wistar rats (Shimizu Laboratory Animal Supply, Kyoto, Japan) with a median weight of 218 g (range 190–230 g) were used for the animal experiments. The study protocol was approved by the Animal Care and Use Committee of Kyoto University. All surgical procedures were performed using a surgical microscope under general anaesthesia by an intraperitoneal injection of pentobarbital (64.8 mg/kg) after the rats were fasted for 12 h.

To demonstrate significance, a sample of $n > 3$ is necessary. In consideration the time and cost required for the animal experiments, a sample of $n = 6$ was used in this study.

2.3. Degradation profiles of hydrogels incorporating bFGF *in vivo*

In order to examine the degradation profiles of the gelatin hydrogels, gelatin hydrogels with different water contents were implanted into the abdominal cavity of the rats. After shaving, a midline laparotomy measuring 3 cm was performed under sterile conditions. The bFGF-free hydrogels A–E or hydrogel B (incorporating 30 µg bFGF) were implanted into the lower abdominal cavity of the rats ($n = 6$ for each time-point in each group). The abdominal cavity was closed in two layers with 3–0 Vicryl® sutures (Ethicon Inc.). Feeding was resumed at 24 h after surgery. At different intervals after implantation, the rats were killed, the gelatin hydrogels were removed along with the tissues attached around the hydrogels. The dry weight of the hydrogels was measured to estimate their *in vivo* degradation.

2.4. Experimental design and operative procedure

2.4.1. Determination of the optimal concentration of bFGF incorporated in the hydrogel sheets

To examine the effects of gelatin hydrogel sheets incorporating bFGF on wound healing at an intestinal anastomosis, preliminary experiments were performed for the control, PBS and 0.1, 3, 10, 30 and 100 µg bFGF groups 7 days postoperatively. A previous study demonstrated that bFGF-promoted wound healing could be induced by bFGF release for 2 weeks (Tabata *et al.*, 1994); thus, hydrogels that degraded over a course of 2 weeks (hydrogel B; see Section 3.2.) were used in this preliminary experiment. After shaving, a midline laparotomy

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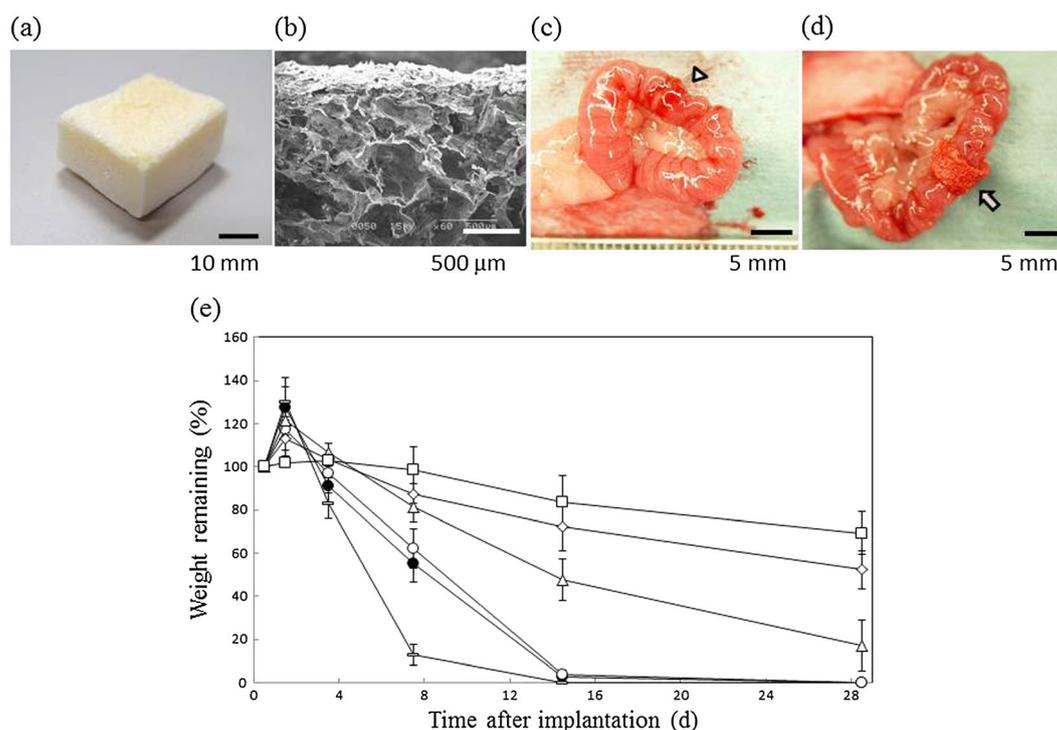


Figure 1. Photograph (a) and scanning electron microscope image of a freeze-dried gelatin hydrogel (b). Surgical procedure with the placement of a gelatin hydrogel sheet incorporating basic fibroblast growth factor (bFGF). The cut intestine was carefully anastomosed (c). The anastomosed site was wrapped with the hydrogel incorporating bFGF (d). The arrowhead indicates the anastomosed site and the arrow indicates the hydrogel sheet. (e) *In vivo* degradation profiles of gelatin hydrogel sheets: 450 mg of bFGF-free wet gelatin hydrogel A (solid line), 370 mg of bFGF-free wet gelatin hydrogel B (open circles) and incorporating 30 μ g bFGF (closed circles), 310 mg of bFGF-free wet gelatin hydrogel C (triangles), 250 mg of bFGF-free wet gelatin hydrogel D (diamonds), and 180 mg of bFGF-free wet gelatin hydrogel E (squares)

Table 1. Preparation conditions of gelatin hydrogels (A–E) with different water contents

Gelatin hydrogel	Concentration of gelatin solution (wt%)	Glutaraldehyde concentration (wt%)	Water content (wt%)
A	3	0.06	98.1
B	3	0.62	96
C	3	1.25	95.1
D	3	3.13	94.3
E	3	6.26	90.8

measuring 3 cm was performed under sterile conditions. The small intestine was divided at the intermediate point between the terminal ileum and the stomach. An end-to-end anastomosis of the small intestine was performed by continuous suture using 6–0 PDS® (Ethicon Inc., Somerville, NJ, USA). The rats were divided randomly into seven groups: (1–5) bFGF groups (anastomotic site wrapped with a hydrogel sheet incorporating 0.1, 3, 10, 30, or 100 μ g bFGF, respectively; $n = 6$); (6) PBS group (wrapped with a hydrogel sheet incorporating PBS; $n = 6$); and (7) NT group (no additional treatment; $n = 6$) (Figure 1c,d). The sheet was wrapped closely to contact anastomosis and both ends were fixed to the bowel wall with a single-knot suture of 6–0 PDS to release bFGF equally around the anastomotic site. The abdominal cavity was closed in two layers using 3–0 Vicryl sutures. Feeding was resumed at 24 h after surgery.

After each animal was killed, the anastomosed intestine and adherent organs were removed en bloc for histological examination. The specimen was fixed in a 4 wt% formaldehyde solution in PBS for 4 days and subsequently embedded in paraffin. Sections (5 μ m) were cut and stained with haematoxylin and eosin (H&E) and von Willebrand factor (vWF) reagent. The number of newly formed blood vessels and infiltrating fibroblasts around the site of the anastomosis was assessed 7 days postoperatively, as reported previously (Aimoto *et al.*, 2007; Nambu *et al.*, 2009; Radulescu *et al.*, 2011). The section was scanned systemically at $\times 20$ magnification and microvessel density was calculated by counting the number of lumens of newly formed blood vessels containing erythrocytes in each 1 mm² field at the site of the anastomosis. Counting was performed independently for three fields at both ends and the centre per section of the anastomosis. Fibroblasts were identified morphologically with H&E staining and calculated by counting in the same way. All specimens were examined in a blinded manner.

2.4.2. Determination of the optimal water content of the gelatin hydrogel

Next, to evaluate the optimal degradability of the gelatin hydrogel on the healing of an intestinal anastomosis, a competitive examination was performed for the control, PBS and bFGF groups using hydrogel sheets with various degrees of biodegradability 7 days postoperatively. Taking

into account the results of the preliminary experiment (see Section 3.3), 30 μ g bFGF was used for this experiment. The intestinal anastomosis was performed in the same way as described in Section 2.4.1. The rats were divided randomly into seven groups: (1–5) bFGF groups (anastomotic site wrapped with a hydrogel sheet A–E incorporating 30 μ g bFGF; $n = 6$), (6) PBS group (wrapped with a hydrogel sheet B incorporating PBS; $n = 6$) and (7) NT group (no additional treatment; $n = 6$).

The number of newly formed blood vessels and infiltrating fibroblasts around the site of the anastomosis was assessed 7 days postoperatively in the same way as described in Section 2.4.1.

2.4.3. Examination of the time-course of the effects of the controlled release of bFGF from a hydrogel sheet on intestinal anastomotic healing

Gelatin hydrogels with a water content of 96% (hydrogel B) incorporating 30 μ g bFGF were used for the following experiments (see Section 3.3). This study was designed to examine the time-course of the effects of the controlled release of bFGF from a hydrogel sheet on intestinal anastomotic healing (six animals for each time-point in each experimental group). The intestinal anastomosis experiment was performed in the same way as described in Section 2.4.1. The rats were divided randomly into three groups: bFGF group (anastomotic site wrapped with a gelatin hydrogel sheet incorporating bFGF; $n = 30$), PBS group (wrapped with a hydrogel sheet incorporating PBS; $n = 30$) and NT group (no additional treatment; $n = 30$). The abdominal cavity was closed and feeding was resumed 24 h after surgery.

2.5. Evaluation of wound healing

The number of newly formed blood vessels and infiltrating fibroblasts around the site of the anastomosis was assessed 1, 3, 7, 14, and 28 days postoperatively in the same way as described in Section 2.4.1.

2.5.1. Collagen content

The anastomotic sections were stained immunologically using collagen type I- and III-specific reagents. Collagen content at the anastomotic site was then evaluated by measuring the area of collagen type I and III staining 1, 3, 7, 14 and 28 days postoperatively. The sections were scanned systemically at $\times 20$ magnification, then the area stained with collagen type I and III at the anastomotic site was measured.

2.5.2. Thickness of granulation

The degree of granulation at the anastomotic site was evaluated by measuring the thickness of granulation formed at the anastomosed line at postoperative days 1, 3, 7, 14 and 28. The sections were stained with H&E and viewed

systemically at $\times 4$ magnification. Granulation tissue was identified morphologically as tissue containing collagen fibres, fibroblasts, microvessels and inflammatory cells at the centre line of the anastomotic site. The measurement was performed for three sections and the average was calculated.

2.5.3. Inflammatory cells infiltrating the anastomotic site

Inflammatory cells, including lymphocytes, plasma cells, and polymorphonuclear leucocytes cells, were evaluated at the anastomotic site using a modified 0–4 Ehrlich and Hunt numerical scale: 0 = no evidence, 1 = occasional evidence, 2 = light scattering, 3 = abundant evidence and 4 = confluent cells (Ehrlich *et al.*, 1973).

2.5.4. Intestinal obstruction

Intestinal obstruction was evaluated by the clinical condition of the rats, such as weight loss and abdominal distension, and dilatation of the oral side of the intestine was evaluated by measuring the inner perimeter of the oral/anal intestine at a distance of 1 cm from the anastomotic site.

2.5.5. Adhesion-related score

Adhesion around the anastomotic site was evaluated using an adhesion quantity score (0 = no adhesion, 1 = adhesion with 1 structure, 2 = adhesion with 2 structures and 3 = adhesion with 3 or more structures) and an adhesion quality score (0 = no adhesion, 1 = light adhesion, 2 = fixed adhesion and 3 = solid adhesion, only removable with damage) 1, 3, 7, 14 and 28 days postoperatively, as described previously (Hoepfner *et al.*, 2009).

2.6. Bursting pressure of the anastomosis

At postoperative days 1, 3 and 7, a 4-cm segment of intestine including the anastomosis with the hydrogel sheet and adherent organs was resected en bloc. A 3 mm catheter was placed in one side of the stump and both sides of the stump were ligated using a 3–0 silk suture to close the lumen. The catheter was connected to an infusion syringe and a manometer (Handy Manometer PG-100N; Copal Electronics, Tokyo, Japan). Each anastomosis was immersed in water and air was infused into the intestine at the rate of 2 ml/s. The bursting pressure of the anastomosis was defined as the intraluminal pressure at which air leakage was initially observed from the anastomosis.

2.7. Statistical analysis

All results are expressed as means \pm standard deviation. Data were assessed statistically using analysis of the variance for multiple comparisons, and the level of significance was set at $P < 0.05$.

3. Results

3.1. Animal condition

There was no significant difference in body weight changes after surgery among the three groups. No rat died or developed clinically relevant diseases because of anastomotic leakage or an intra-abdominal infection.

3.2. Degradation profiles of the hydrogels *in vivo*

Figure 1e shows the *in vivo* degradation profiles of gelatin hydrogel sheets A–E with a range of water contents. The remaining hydrogels were plotted against the period of implantation. All of the hydrogels degraded over time and those with higher water content degraded faster than those with lower water content. At 28 days after implantation, 69.3% of the hydrogel with a water content of 90.8% still remained, whereas the gelatin hydrogel with a water content of 96.0 % was almost completely degraded within 14 days. In view of these results, a gelatin hydrogel with a water content of 96% (hydrogel B) we used for the following experiments. The incorporation of bFGF into the gelatin hydrogel had no effect on the rate of degradation *in vivo* (cf. hydrogel B incorporating PBS, Figure 1e).

3.3. Optimal bFGF dose and biodegradability of the hydrogel for intestinal anastomotic healing

Figure 2 shows the number of newly formed blood vessels and infiltrating fibroblasts at the anastomotic site 7 days after surgery. The number of newly formed blood vessels and infiltrating fibroblasts was saturated at a bFGF dose of 30 μg per sheet. The number of newly formed blood vessels and infiltrating fibroblasts was significantly higher when hydrogels B or C were used. Therefore, gelatin hydrogels with a water content of 96% (hydrogel B) containing 30 μg bFGF were used in the following experiment.

3.4. Wound healing

3.4.1. Blood vessels regeneration and fibroblast infiltration

Figure 3a shows histological sections of the anastomotic site 7 days after surgery. Microvessels are observed in the tissue around the implanted hydrogel sheet. Figure 3b illustrates the number of regenerated blood vessels and infiltrating fibroblasts over time. The number of newly formed blood vessels was significantly higher in the bFGF group compared with the PBS and NT groups 3, 7 and 14 days postoperatively (at postoperative day 3: bFGF group

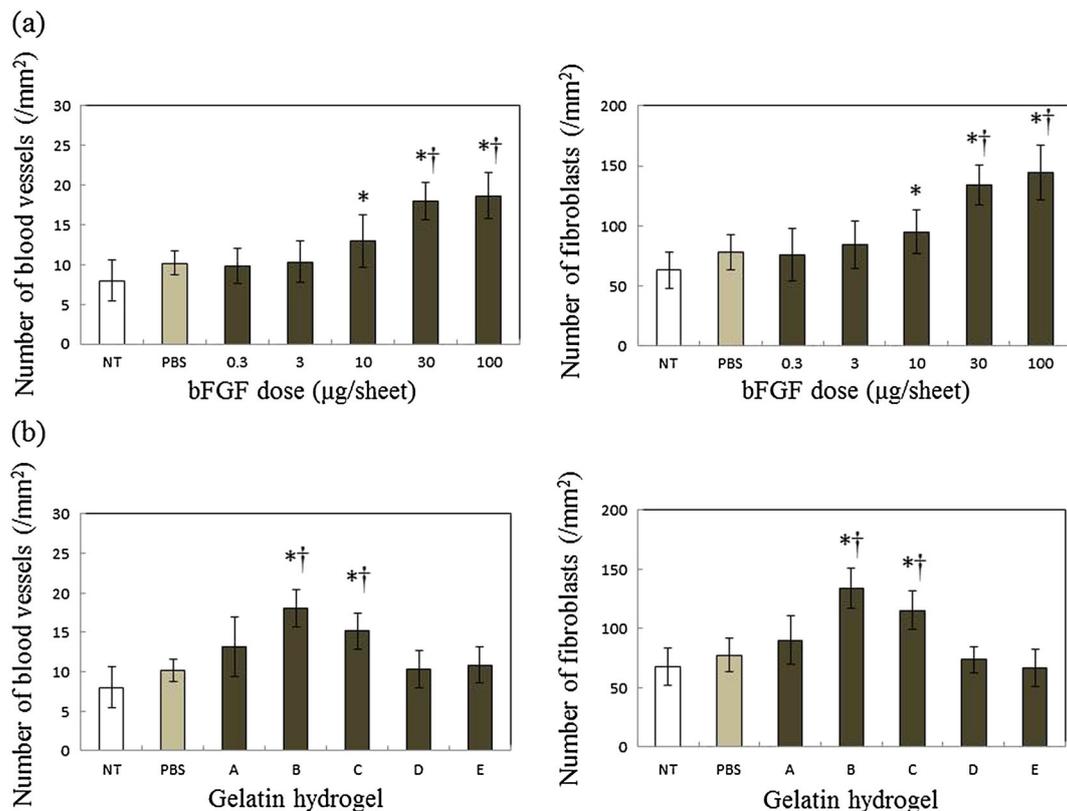


Figure 2. (a) The number of regenerated blood vessels and infiltrating fibroblasts 7 days after surgery using gelatin hydrogel B incorporating 0.1, 3, 10, 30 and 100 μg basic fibroblast growth factor (bFGF) or phosphate-buffered saline (PBS). (b) The number of regenerated blood vessels and infiltrating fibroblasts 7 days after surgery using gelatin hydrogel sheets A–E incorporating 30 μg bFGF and gelatin hydrogel sheet B incorporating PBS. * $P < 0.05$, significant vs. no treatment; † $P < 0.05$, significant vs. hydrogel incorporating PBS

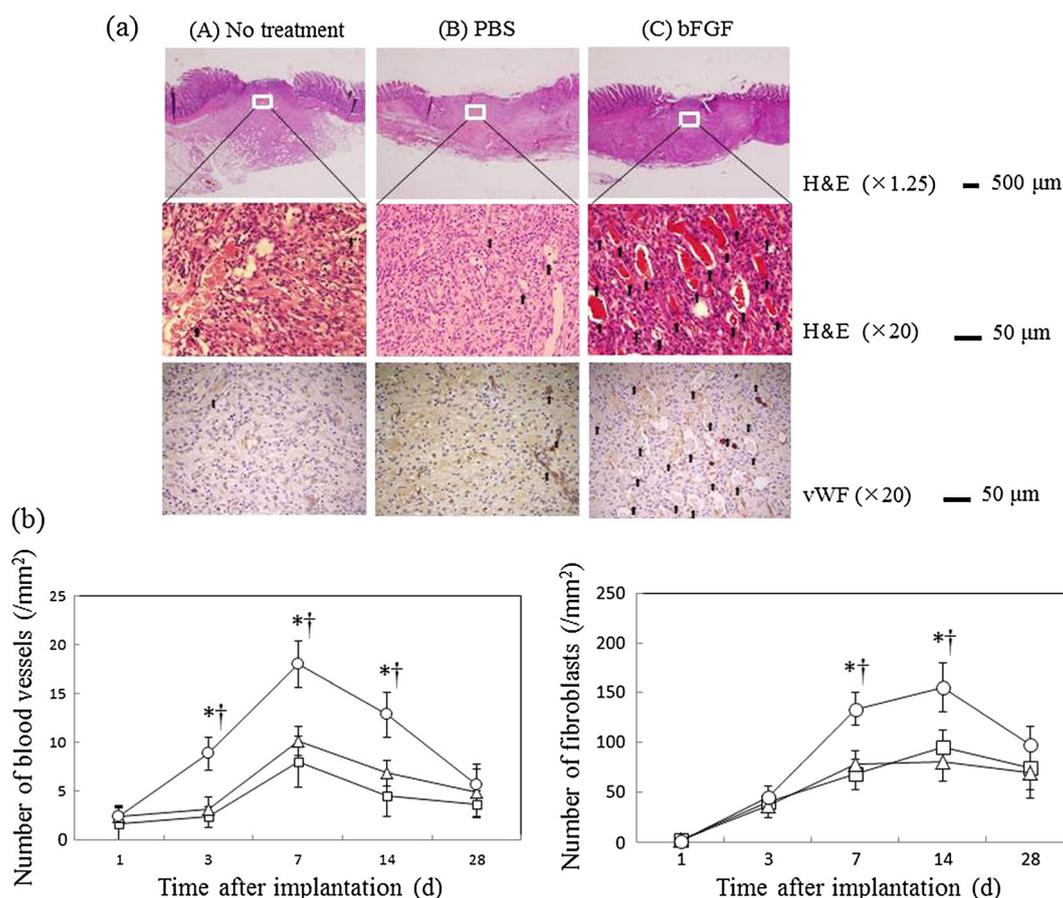


Figure 3. (a) Histological sections of the anastomosed site 7 days after surgery stained with haematoxylin and eosin (H&E) and von Willebrand factor (vWF): (A) no treatment; (B) hydrogel incorporating phosphate-buffered saline (PBS); (C) hydrogel incorporating basic fibroblast growth factor (bFGF). The arrows indicate newly formed blood vessels. (b) Time-course of the number of regenerated blood vessels and infiltrating fibroblasts at the anastomotic site. Wrapped with hydrogel incorporating bFGF (circles), wrapped with hydrogel incorporating PBS (triangles), and no additional treatment (squares). * $P < 0.05$, significant vs. no treatment. † $P < 0.05$, significant vs. hydrogel incorporating PBS.

$8.83 \pm 1.72/\text{mm}^3$; PBS group $3.17 \pm 1.17/\text{mm}^3$; NT group $2.33 \pm 1.03/\text{mm}^3$; $P < 0.05$). The number of infiltrating fibroblasts was also increased significantly by the application of hydrogel sheets incorporating bFGF compared with the other two groups 7 days and 14 days postoperatively (at postoperative day 7: bFGF group $133.8 \pm 16.8/\text{mm}^3$; PBS group $77.7 \pm 14.4/\text{mm}^3$; NT group $67.8 \pm 15.4/\text{mm}^3$; $P < 0.05$).

3.4.2. Collagen content

Figure 4a shows histological sections of the anastomotic site stained for collagen type I and III 7 days after surgery. Figure 4b illustrates the area of collagen type I and III staining over time. The area of both collagen type I and III staining was significantly greater in the bFGF group compared with the PBS and NT groups 3, 7 and 14 days postoperatively.

3.4.3. Granulation thickness

Figure 4c shows the thickness of granulation tissue. A significant increase in granulation thickness at the anastomotic site was observed 7 days and 14 days postoperatively for the bFGF-treated group (at postoperative day 7: bFGF

group $2503.2 \pm 445.2 \mu\text{m}$; PBS group $1819.6 \pm 331.8 \mu\text{m}$; NT group $1623.2 \pm 365.9 \mu\text{m}$; $P < 0.05$). However, the thickness decreased and no significant difference was observed among the groups 28 days postoperatively.

3.4.4. Intestinal obstruction

No intestinal obstruction was observed in any group over the study period.

3.4.5. Inflammatory cells infiltrating the anastomotic site

Figure 5a shows the levels of inflammatory cells at the anastomotic site. Although an inflammatory reaction was observed around the anastomotic site, there was no significant difference in the level of infiltration among the three groups over the study period.

3.4.6. Adhesion-related score

Figure 5b shows the adhesion-related score around the anastomotic site for each group. Although adhesion of fat or bowel segments was observed, there was no

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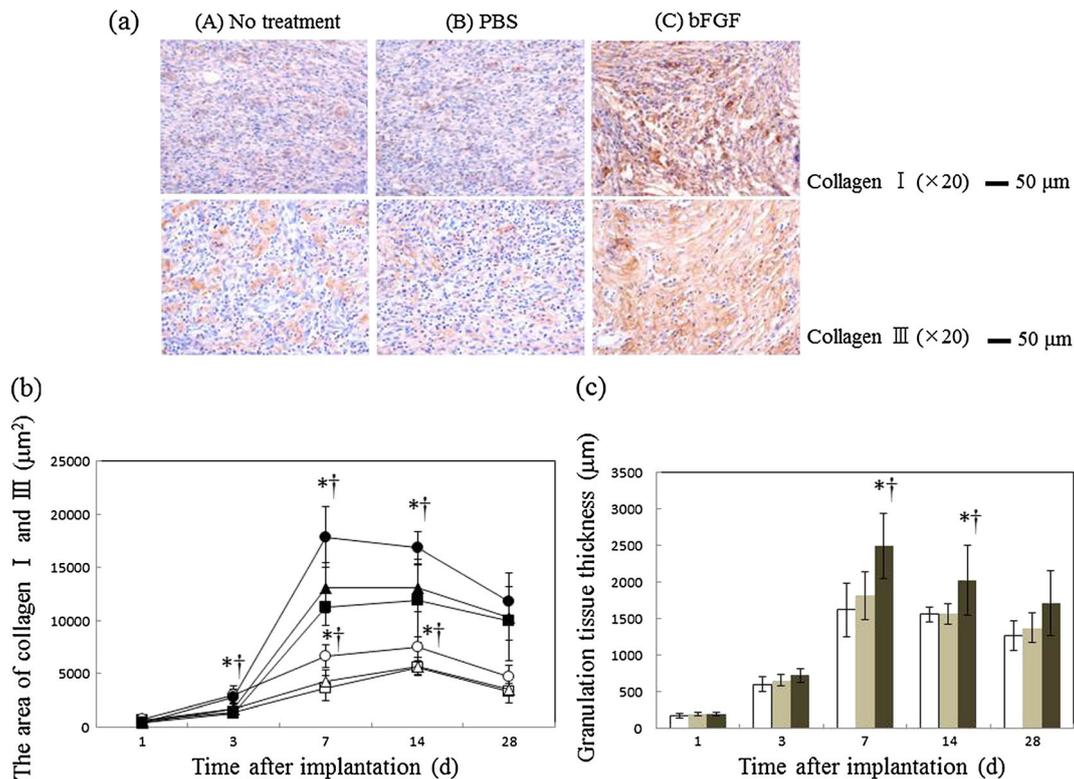


Figure 4. (a) Histological sections of the anastomosed site 7 days after surgery (collagen type I and III staining): (A) no treatment; (B) hydrogel incorporating phosphate-buffered saline (PBS); (C) hydrogel incorporating basic fibroblast growth factor (bFGF). (b) Time-course of the area of collagen I and III staining at the anastomotic site. Wrapped with hydrogel incorporating bFGF with collagen I (open circles) and III (closed circles) staining, wrapped with hydrogel incorporating PBS with collagen I (open triangles) and III (closed triangles) staining, and no additional treatment with collagen I (open squares) and III (closed squares) staining. * $P < 0.05$, significant vs. no treatment; † $P < 0.05$, significant vs. hydrogel incorporating PBS. (c) Thickness of granulation tissue at 1, 3, 7, 14 and 28 days after surgery. Wrapped with hydrogel incorporating bFGF (closed columns), wrapped with hydrogel incorporating PBS (tinted columns) and no additional treatment (open columns). * $P < 0.05$, significant vs. no treatment. † $P < 0.05$, significant vs. hydrogel incorporating PBS

significant difference in the adhesion quality or quantity scores among the three groups over the study period.

3.5. Bursting pressure of the anastomosis

Figure 6 shows the bursting pressure at the anastomotic site over time. At days 1 and 3, the bursting pressure was significantly increased by the application of hydrogel sheets (bFGF and PBS groups) compared with the NT group. Furthermore, at postoperative day 3, the bursting pressure was significantly increased by the application of hydrogel sheets incorporating bFGF compared with the other two groups (bFGF group 102.8 ± 20.6 mmHg; PBS group 69.2 ± 9.5 mmHg; NT group 33.3 ± 10.9 mmHg; $P < 0.05$). A high level of bursting pressure was reached in each group (> 800 mmHg) 7 days postoperatively.

4. Discussion

The present study clearly indicates that the controlled release of bFGF significantly enhances the bursting pressure of an anastomosis by increasing the formation

of new blood vessels, fibroblast infiltration and collagen production around the anastomotic site.

In the natural process of wound healing, numerous growth factors play a key role in angiogenesis and subsequent tissue repair (Robson *et al.*, 2001). It has been shown that a bFGF spray (Fiblast Spray®; Kaken Pharmaceutical, Tokyo, Japan) induces angiogenesis and granulation tissue formation and it is used in a clinical setting for the treatment of decubitus ulcers (Matsumoto and Tabata, 2006). However, when bFGF is applied in a solution, its effect is limited because of its short half-life period *in vivo*. Therefore, a drug delivery system is necessary when considering its usage in an intestinal anastomosis where additional administration after surgery is difficult.

A controlled release method of bFGF using a gelatin hydrogel has been developed, which is able to achieve continuous release of bioactive bFGF and, consequently, induce regeneration of tissues, including blood vessels, bone, skin, nerves and fat (Tabata, 2003, 2008; Kawai and Suzuki, 2005; Aimoto *et al.*, 2007; Katsuno *et al.*, 2011). Gelatin was selected for growth factor release as it is biodegradable, highly biocompatible and inert to protein drugs (Tabata, 2003). As the gelatin hydrogel degrades, immobilized bioactive bFGF molecules are

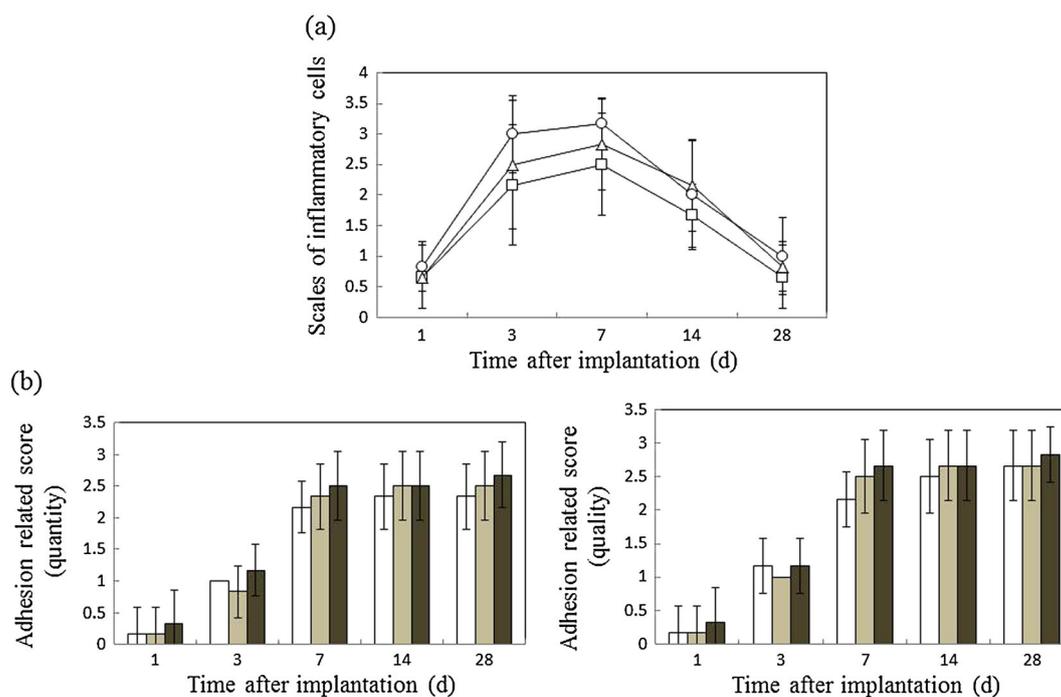


Figure 5. (a) The levels of inflammatory cells at the anastomotic site. Wrapped with hydrogel incorporating basic fibroblast growth factor (bFGF; circles), wrapped with hydrogel incorporating phosphate-buffered saline (PBS; triangles) and no additional treatment (squares). (b) Adhesion-related score (quality and quantity) around the anastomotic site at 1, 3, 7, 14, and 28 days after surgery. Wrapped with hydrogel incorporating bFGF (closed columns), wrapped with hydrogel incorporating PBS (tinted columns) and no additional treatment (open columns).

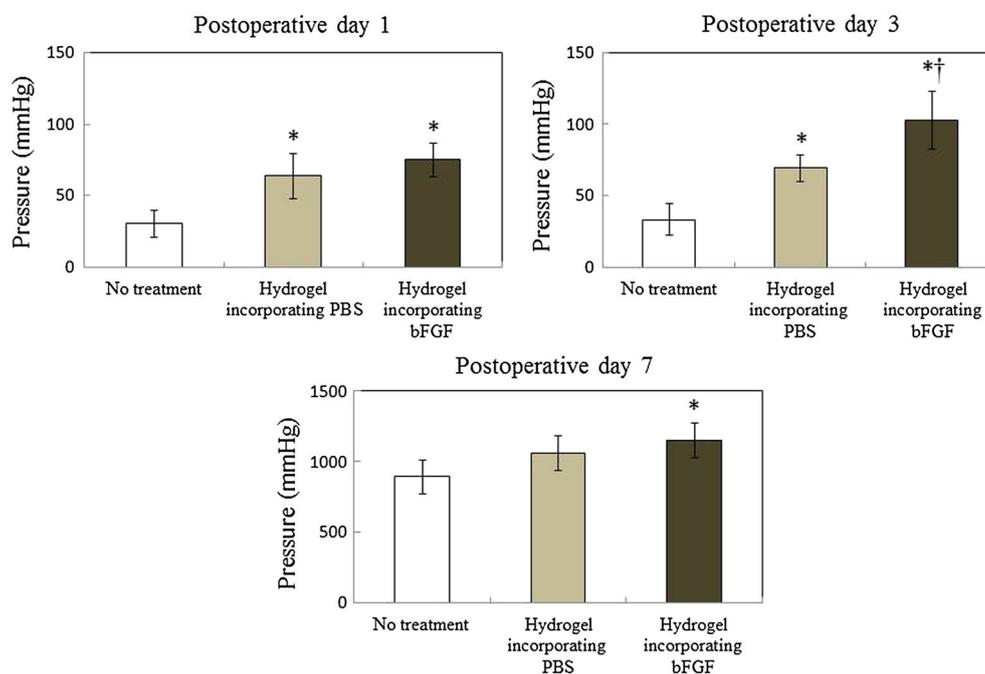


Figure 6. Bursting pressure of the anastomotic site 1, 3 and 7 days after surgery. * $P < 0.05$, significant vs. no treatment; † $P < 0.05$, significant vs. hydrogel incorporating phosphate-buffered saline (PBS).

released locally, together with gelatin fragments, over several weeks without increasing the systemic concentration of bFGF in mice (Tabata, 1999).

In a gastrointestinal anastomosis, angiogenesis is observed in wound granulation 3 days postoperatively,

which affects the subsequent anastomotic repair (Kitajima *et al.*, 1998; Robson *et al.*, 2001). Fibroblasts begin to infiltrate the wound as early as 2 days postoperatively, and the fibroblast density peaks between 7 and 14 days after injury (Hendricks and Mastboom,

1990; Robson *et al.*, 2001). It is known that anastomotic strength depends on the presence of collagenous networks produced by fibroblasts that infiltrate the site during the early stages after surgery (Hendricks and Mastboom, 1990; Thompson *et al.*, 2006; Aimoto *et al.*, 2007). Because anastomotic leakage usually occurs from 2 to 4 days after surgery (Morita *et al.*, 2004; Thompson *et al.*, 2006), it is beneficial to increase the mechanical strength of the anastomosis in order to resist the pressure increase caused by bowel movements during this period. Indeed, the results of this study indicate that bFGF release significantly increased the number of newly formed blood vessels around the site of the anastomosis 3, 7 and 14 days postoperatively, and the number of infiltrating fibroblasts was also increased 7 days and 14 days postoperatively. Subsequently, the production of a collagenous network at the anastomotic site was increased in the bFGF group at postoperative days 3, 7 and 14, which might enhance anastomotic strength during the early postoperative period.

The dose response curve of bFGF is typically bell-shaped as a result of the inhibition of cell differentiation and the downregulation of bFGF receptors at high doses of bFGF (Okumura *et al.*, 1996). In this study, the number of newly formed blood vessels and infiltrating fibroblasts saturated at a bFGF dose of 30 μg per sheet. With regard to the biodegradability of the hydrogel, it was found that an optimal period of 2 weeks for gelatin biodegradation supports the wound healing process during the early postoperative period.

Significantly elevated bursting pressure was observed on postoperative day 3 in the bFGF group, and the authors that it believe it is beneficial to enhance anastomotic strength during this early postoperative period before the anastomosis becomes sufficiently stabilized (cf. postoperative day 7, Figure 6). The gelatin hydrogel sheets remained around the anastomosis until their complete degradation. At postoperative days 1 and 3, the bursting pressure also increased significantly in the PBS group

compared with the NT group because the hydrogels act as a mechanical barrier.

Some authors advocate that growth factors can induce vascularization, including the formation of immature and non-functioning blood vessels (Yonemitsu, 2006). However, in the present study, no vascular malformations were observed histologically. The significant enhancement of normal granulation and the strength of the anastomosis likely indicates that the vascularization induced by bFGF was functional.

Another concern is that the enhancement of wound healing and granulation by bFGF may cause adhesion and intestinal obstruction by excessive granulation at the anastomotic site. In this study, although adhesion between the anastomotic site and the surrounding tissue was observed, there was no significant difference in the number of inflammatory cells or adhesion-related scores among the three groups. The thickness of granulation tissue at the anastomosis was significantly higher in the bFGF group than in the control group 7 days and 14 days postoperatively, but the difference disappeared 28 days postoperatively. No intestinal obstruction was observed over the study period.

In conclusion, this controlled bFGF release system is a simple and promising method for promoting wound healing, reinforcing an anastomosis and preventing anastomotic leakage. However, thorough safety evaluation is required before future clinical trials.

Conflict of interest

The authors have declared that there is no conflict of interest.

Acknowledgement

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