# Effects of Platelet-Rich Plasma on Intestinal Anastomotic Healing in Rats: PRP Concentration is a Key Factor

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**ABSTRACT**

**Introduction.** There have been few studies that examine the effects of platelet-rich plasma (PRP) on intestinal anastomotic healing. The applied preparation methods and PRP concentrations used in the few studies that have been carried out varied markedly.

Therefore, the positive effects of PRP on the anastomotic healing process remain unclear. The aim of this study is to examine the effects of different concentrations of PRP on intestinal anastomotic healing.

**Material and Methods.** From SD rat blood, three different concentrations of plasma were prepared: high-concentrated PRP (H-PRP: platelet count $5 \times 10^6/mm^3$), low-concentrated PRP (L-PRP: $2 \times 10^6/mm^3$), and platelet-poor plasma (PPP). Male SD rats underwent proximal jejunal anastomosis and central venous catheterization. Rats were divided into four groups (N=12 for each group): control, PPP, L-PRP, and H-PRP groups. Two types of PRP and PPP (0.21 ml) were applied to each anastomosis line, with the exception of the control group. Total parenteral nutrition (TPN) solutions were administered (151 kcal/kg/day). Five days after surgery, anastomotic bursting pressure (ABP) in situ and hydroxyproline concentration (HYP) in anastomotic tissue were evaluated.

**Results.** The ABP values of control, PPP, L-PRP, and H-PRP groups were 171±20, 174±23, 189±17, and 148±25 mmHg, respectively. The HYP values of each group were 516±130, 495±123, 629±120, and 407±143 μg/g dry tissue. Compared with the other groups, the L-PRP group exhibited a significant increase in both ABP and HYP, while

5    the H-PRP group exhibited a significant decrease in these two variables. As a result, L-PRP was considered to promote anastomotic wound healing, but H-PRP was considered to inhibit it. There was no significant difference between the PPP group and the control group.

**Conclusions.** PRP concentration plays a crucial role in the efficacy of PRP. PRP might

10    exert positive effects on intestinal anastomotic healing in a dose-dependent manner up to a certain level, but adverse effects occur when it is highly concentrated. The essential PRP action appears to be driven by the platelets themselves.

**INTRODUCTION**

Despite major advances in surgical techniques and perioperative management, the rates

of gastrointestinal anastomotic leakage have still not been reduced to a negligible level.

Anastomotic leakage has large negative impacts not only on mortality but also on

long-term survival [1][2][3]. Hence, innovative methods to avoid anastomotic leakage are

deemed necessary and are much anticipated.

There is a growing body of evidence to demonstrate that platelet concentrates such as

platelet-rich plasma (PRP) enhance wound healing in a variety of clinical fields on the

basis of the premise that higher growth factor content should promote better healing

[4][5][6][7][8]. Platelets contain high quantities of key growth factors, such as

platelet-derived growth factor (PDGF) and transforming growth factor-β1 (TGF-β1),

which regulate cell proliferation and matrix remodeling [9]. PRP is regarded as a storage

vehicle of growth factors. PRP application to gastrointestinal anastomosis is one of the

most useful methods to deliver concentrated amounts of growth factors throughout the

surgical site, and expected to accelerate the wound healing process. There have been only

two reports regarding the effect of PRP application on intestinal wound healing [10][11].

However, these two reports provided contrasting results. Yol et al. showed that PRP has a

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positive effect on wound healing in colonic anastomosis [10]. On the other hand, Fresno et al. indicated that PRP application only increases granulation tissue and fibrosis without improving jejunal anastomotic breaking strength [11]. Similarly, the effect of PRP remains a controversial subject in other research fields [12][13]. Behind the controversy is the fact that PRP preparation methods vary between studies, which results in significantly different platelet concentrations. Different platelet concentrations inevitably lead to different concentrations of various growth factors. Consequently, the platelet concentrations might be a crucial factor in determining whether PRP achieves the desired effects on wound healing. In addition, there is the possibility that the optimal concentration of PRP depends on the type of wound to which it is applied and adverse effects can be exerted when too high a concentration is used.

The aim of this study is to clarify the causal relationship between PRP concentration and its effect on intestinal anastomotic healing.

## MATERIAL AND MEHODS

### Animals

Seventy-seven male Sprague-Dawley rats (Nippon Clea, Tokyo, Japan), weighing 192 g

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(172-252 g), were used in the experiment. The animals were maintained at 21°C with a

12 h light/dark cycle and allowed free access to water and standard chow for 3 days. The

experimental protocol was carried out in a humane manner after receiving approval from

the Institutional Animal Experiment Committee of the University of Tsukuba, and in

5    accordance with the Regulations for Animal Experiments of our university and the

Fundamental Guidelines for Proper Conduct in Animal Experiments and Related

Activities in Academic Research Institutions under the Jurisdiction of the Ministry of

Education, Culture, Sports, Science, and Technology.

**Preparation of Platelet-Rich Plasma**

10    Ten milliliters of homologous fresh blood treated with 1 ml of ACD-A, anticoagulant

solution, was obtained in a sterile tube from the heart of each rat. Blood was centrifuged

immediately at 400 ×g and 22°C for 10 minutes. Blood was separated into three layers, red

blood cells at the bottom, acellular plasma in the supernatant, and a buffy coat layer in

between. The upper layer was transferred with a sterile pipette to another 10 ml centrifuge

15    tube without buffy coat and re-centrifuged at 800 ×g and 22°C for 10 minutes. About 0.5

ml of PRP (platelet count about $5.50 \times 10^8/mm^3$) was pipetted from the bottom of the tube

and about 1.0 ml of platelet-poor plasma (PPP) was harvested as a supernatant (Fig. 1A).

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The prepared PRP, PPP, and whole blood of the rats were subjected to platelet and white blood cell counting with a cell counter (MICROS abc LC-152, HORIBA, ltd., Kyoto, Japan), and PPP was added a little at a time to adjust PRP to different platelet concentrations: low-concentrated PRP (platelet count $2\times10^6/mm^3$) and high-concentrated

5    PRP (platelet count $5\times10^6/mm^3$). To produce a viscous coagulum gel that can be applied to the anastomosis, 180 μl of PRP was mixed with the "activator", a mixture of 180 units of bovine thrombin (Sigma-Aldrich, St. Louis, MO, USA) and 30 μl of 10% calcium chloride. Calcium chloride neutralizes the anticoagulant effect of citrate, and bovine thrombin initiates the clotting process. Likewise, PPP was activated by the above-mentioned process.

10    Finally, each of activated PRP and PPP was prepared as a total amount of 0.21 ml. The mixture, which clots within a few seconds, should be used immediately to allow the release of growth factors.

**Determination of PDGF-BB and TGF-β1 concentrations**

The activated PRP and PPP aliquots were incubated for 30 min at 37°C in a 5% $CO_2$

15    incubator (Auto-Flow incubator, NuAire, Inc., Plymouth, MN, USA). Samples were centrifuged for 10 min at 15500 ×g, and the supernatants were harvested. All supernatants were immediately stored at −80°C until further analysis. After being thawed,

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the samples were assayed for platelet-derived growth factor-BB (PDGF-BB) and transforming growth factor-β1 (TGF-β1) by enzyme-linked immunosorbent assay (ELISA) testing (R&D Systems, Minneapolis, MN, USA). The levels of PDGF BB and TGF-β1 are reported in picograms per milliliter.

5    **Operative procedure**

Prior to surgery, all rats were fasted overnight. They were anesthetized by intraperitoneal injection of pentobarbital sodium (Somnopentyl®, Kyoritsu Seiyaku, Tokyo, Japan) at a dose of 40 mg/kg of body weight. Under aseptic conditions, (1) jugular vein catheterization at a central venous position was carried out. The tip of the

10    catheter was a silicone tube of 0.5 mm inner diameter and 1.0 mm outer diameter (Fuji Systems Co., Tokyo, Japan), and the other part of the catheter was a plastic tube of 0.5 mm inner diameter and 1.0 mm outer diameter (Imamura Co., Chiba, Japan). The distal end of the catheter was tunneled subcutaneously and brought out in the cephalad portion of the interscapular area. The catheters were fixed to the skin using a harness attached to

15    a swivel assembly. (2) A laparotomy was performed through an upper midline incision measuring 3 cm. The proximal jejunum was divided 2.0 cm distal from the duodenum, and single-layer, end-to-end anastomosis was performed with 7-0 Proline sutures

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(Ethicon Inc., USA) in an inverted interrupted fashion. To ensure technical uniformity, all

anastomoses were sutured by only one surgeon. After that, the animals were divided into

four groups: the control group (n=12), in which neither PPP or PRP was applied to the

anastomosis line; the PPP group (n=12), in which activated PPP was applied to the

5    anastomosis line; the low-concentrated PRP (L-PRP) group (n=12), in which activated

low-concentrated PRP was applied to the anastomosis line; and the high-concentrated

PRP (H-PRP) group (n=12), in which activated high-concentrated PRP was applied to the

anastomosis line. In the PPP group, the L-PRP group, and the H-PRP group, the activated

PRP or PPP was applied as a film layer of around 8 mm width and 3 mm thickness

10   immediately after mixing with bovine thrombin and 10% calcium chloride (Fig. 1B). The

fascial layer of the abdominal wall was closed with a continuous suture using 2-0 Silk

(Ethicon Inc., USA), and the abdominal skin was closed in the same way. Following

these procedures, the rats were maintained in individual metabolic cages for 5 days.

**Nutrient solutions**

15   In all groups, nutrient solutions (Neoparen 2, Otsuka Pharmaceutical Co., Ltd., Tokyo,

Japan) were administered via the central venous catheter. Following surgical procedures,

rats were maintained in individual metabolic cages for 5 days. Identical solutions were

administered to the four groups. Continuous infusion using infusion pumps (SP-115, JMS

Co., Ltd., Tokyo, Japan) was initiated immediately after surgery. The full-dose energy

intake was 151 kcal/kg body weight per day, equal to an infusion rate of 9 ml/kg body

weight per hour. This target value corresponds to about 21 kcal/kg body weight per day

5    for humans [14], which is thought to be reasonable during the acute phase of surgical

stress [15]. Twenty-four hours postoperatively, half of the target calories had been

administered. The reason why the rats were fed with TPN instead of standard rat chow

and water ad libitum was to provide equivalent amounts of fluid volume and energy

intake, which allows for leveling of the nutritional backgrounds of all rats.

10    **Nutritional parameters**

Urinary output was monitored daily, and body weights were recorded. On postoperative

day 5, the rats were deeply anesthetized by an intraperitoneal injection of pentobarbital

sodium at a dose of 40 mg/kg of body weight, and the infusion of nutrients was stopped.

Blood was drawn from the inferior vena cava to determine the serum levels of total

15    protein, albumin, glucose, and blood urea nitrogen.

**Anastomotic bursting pressure**

After the animals were anesthetized, the abdomen was opened, and the anastomotic

bursting pressure (ABP) was measured in situ without interruption of the normal

mesenteric blood supply or adhesions to the anastomosis. A 16-gauge silicone rubber

catheter was inserted 1.5 cm from the oral side of the anastomosis to the distal side. Two

ligations were made, 1.5 cm proximal and distal from the anastomosis with 3-0 silk

5    sutures. Normal saline solution was continuously infused through the catheter via a

syringe pump at a rate of 1.0 ml/min. Intraluminal pressure was monitored continuously

via a transducer (BLPR®, World Precision Instruments Inc., Sarasota, FL, USA) and

recorded on a chart recorder (Power Lab®, AD Instruments, Tokyo, Japan). The ABP was

defined as the peak pressure attained just before rupture of the anastomosis.

10   **Collagen concentration**

Hydroxyproline is useful as an index of collagen concentration because it is an amino

acid unique to collagenous proteins [16]. After bursting of the anastomosis, 1 cm of the

anastomotic segment, that is, 0.5 cm proximal and distal from the anastomosis, was

excised and dissected free of mesenteric fat, intestine, and adherent omentum. Adhesions

15   to the surrounding tissue were not dense, and it was feasible to separate adhesions from

the anastomotic site using the blunt technique. The specimens were opened at the

mesenteric side, gently washed with saline solution, and cut into three pieces. A strip of

the specimen was used to determine the concentration of hydroxyproline as an index of

collagen concentration. The concentration of hydroxyproline was measured as described

by Reddy and Enwemeka [17]. The procedure is based on alkaline hydrolysis of the

tissue homogenate and subsequent determination of the free hydroxyproline in

5    hydrolysate. Chloramine-T was used to oxidize the free hydroxyproline for production of

a pyrrole. The addition of Ehrlich reagent resulted in the formation of a chromophore that

can be measured at 550 nm. Calculations were made to express the result as micrograms

of hydroxyproline per gram of dry tissue (µg/g dry tissue).

**Histopathological Analysis**

10    A strip of the anastomosis was immediately fixed in 10% phosphate-buffered

formaldehyde, and then embedded in paraffin. One sample was randomly selected from

each of the four groups. Histopathological sections (4 µm) were stained with

hematoxylin-eosin (HE) and Masson trichrome (MT). The degree of collagen deposition

was descriptively assessed in a blinded manner by two pathologists.

15    **Statistics**

All data are presented as the mean value ± standard deviation (SD). Comparisons among

the four groups were tested by non-repeated measures ANOVA. Multiple comparisons

were examined by Bonferroni test, and differences were considered statistically significant at $p < 0.05$.

**RESULTS**

5   **Number of platelets and quantification of growth factor levels for three different concentrations of platelets**

Blood samples obtained from SD male rats exhibited an original platelet count of $0.91 \pm 0.12 \times 10^6/\text{mm}^3$ and an original white blood cell count of $6.45 \pm 1.53 \times 10^3/\text{mm}^3$. PPP (n=5), L-PRP (n=5), and H-PRP (n=5) exhibited average platelet concentrations of $0.03 \pm$

10   $0.01$, $2.11 \pm 0.18$, and $5.07 \pm 0.19 \times 10^6/\text{mm}^3$, respectively. These platelet concentrations represented 0.03-, 2.33-, and 5.60-fold increases compared with the original platelet count. None of PPP, L-PRP, and H-PRP included white blood cells.

For PDGF-BB, $3439 \pm 2817$, $18462 \pm 4671$, and $49365 \pm 4299$ pg/ml were respectively determined in PPP, L-PRP, and H-PRP (Fig. 2). For TGF-β1, $5824 \pm 1647$, $101008 \pm$

15   $24584$, and $210453 \pm 37188$ pg/ml were respectively determined in PPP, L-PRP, and H-PRP (Fig. 3). Both PDGF-BB and TGF-β1 values of H-PRP and L-PRP were significantly higher than those of PPP ($p<0.01$). Both PDGF-BB and TGF-β1 values of

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H-PRP were significantly higher than those of L-PRP (p<0.01).

**Numbers of platelets in three different concentrations of platelets for surgical procedure**

Blood samples obtained from SD male rats exhibited an original platelet count of $0.93 \pm$ 0.12 $\times 10^6$/mm$^3$ and an original white blood cell count of $6.69 \pm 2.34 \times 10^3$/mm$^3$. PPP (n=12), L-PRP (n=12), and H-PRP (n=12) exhibited average platelet concentrations of $0.06 \pm 0.02$, $1.93 \pm 0.13$, and $5.15 \pm 0.30 \times 10^6$/mm$^3$, respectively. These platelet concentrations were equivalent to 0.06-, 2.08-, and 5.53-fold increases compared with the original platelet count. None of PPP, L-PRP, and H-PRP included white blood cells.

**Nutritional parameters**

The initial body weight and body weight change 5 days after operation were similar in the four groups (Table 1). Total infusion volume, total energy intake, and total urine volume did not differ between the four groups (Table 1). There were no significant differences between the four groups in serum levels of total protein, albumin, glucose, or blood urea nitrogen (Table 2). These results indicated that there was no crucial factor directly impacting on intestinal anastomotic healing other than the effects of PRP.

**Anastomotic bursting pressure (ABP)**

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There was no intraperitoneal abscess formation, and no anastomotic leakage was noted in any animal. All tested anastomoses burst exactly at the suture line. The ABP values of the control group, the PPP group, the L-PRP group, and the H-PRP group were 171 ± 20, 174 ± 23, 189 ± 17, and 148 ± 25 mmHg, respectively. In the L-PRP group, the ABP

5    values were significantly higher compared with those in the control group, the PPP group, and the H-PRP group (p<0.05). On the other hand, the ABP values of the H-PRP group were significantly lower than those of the other three groups (p<0.05) (Fig. 4). No significant difference was detected between the PPP group and the control group.

**Collagen concentration in jejunal anastomotic tissue**

10    As a surrogate marker of collagen, the hydroxyproline concentrations of the control group, the PPP group, the L-PRP group, and the H-PRP group were 515 ± 130, 495 ± 123, 629 ± 120, and 407 ± 143 µg/g dry tissue, respectively. In the L-PRP group, the hydroxyproline concentrations were significantly higher than those in the control group, the PPP group, and the H-PRP group (p<0.05). The hydroxyproline concentrations of the

15    H-PRP group were significantly lower than those of the other three groups (p<0.05) (Fig. 5). There was no significant difference between the PPP group and the control group.

**Histopathological examination in jejunal anastomotic tissue (Fig. 6)**

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In the L-PRP group, collagen deposition mainly in the serosal layer was most

pronounced, a clear sign of increased collagen production, compared with those of the

control group, the PPP group, and the H-PRP group. In contrast, the H-PRP group

exhibited the smallest collagen deposition among the four groups. There was little

5   difference between the control group and the PPP group.


**DISCUSSION**

Gastrointestinal wound healing is characterized by three phases of healing, namely, the

inflammation phase, the proliferation phase, and the maturation phase. At the early phase,

10  platelets play a key role in the wound healing process. After the formation of fibrin clots

that serve as a scaffolding for platelets, platelets act during the first 72 hours of the

inflammation phase. The initial and brief release of growth factors from platelets

mediates the entire process by controlling growth, differentiation, and cell metabolism

[18]. After that, macrophages take over the work of platelets, and initiate both wound

15  debridement and the production of growth factors that modulate fibroplasia and

angiogenesis. Because the anastomotic mechanical strength reaches the lowest value 3 or

4 days after gastrointestinal anastomosis, the anastomotic leakage most commonly occurs

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during this period [19]. From the above-mentioned viewpoint, the purpose of PRP application to gastrointestinal anastomosis is to accelerate the activation of fibroblasts and collagen formation by multiple viable growth factors released from platelets, which enables an increase in anastomotic strength at the earliest stage possible during the

5    inflammation phase.

In this study, PRP was prepared after two-step centrifugation of blood obtained from rats without any commercial kits. The advantage of this method is that both PRP and platelet-poor plasma (PPP) are easily available at the time, and the desired PRP concentration is fully adjustable by the addition of PPP. The concentration of each

10    growth factor released from PRP is predictable on the basis of PRP concentrations. There is a possibility that concentrations of growth factors in PRPs differ according to species, such as rat, goat, and human [20]. Therefore, we quantified the numbers of platelets and two representative growth factors (PDGF-BB, TGF-β1) in three different concentrations of platelets (PPP, L-PRP, H-PRP) obtained from rat, and verified the relationship

15    between numbers of platelets and concentrations of growth factors (Fig 2 and Fig 3). In our method, PRP excludes buffy coat completely. The influence of buffy coat including leukocytes is still unclear [21]. Some authors recommend the elimination of leukocytes

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[22], while several studies have shown that leukocytes in buffy coat play a part in the effects of PRP [23]. In our experiment, the buffy coat layer was eliminated because the aim of this study is to investigate effects of PRP simply depending on different platelet concentrations. By adding thrombin to PRP as an activator, PRP works via the degranulation of the α-granules, which leads to the release of the following 7 fundamental growth factors: the three isomers of platelet-derived growth factor (PDGF-AA, PDGF-BB, and PDGF-AB), two forms of transforming growth factor-β (TGF-β1 and TGF-β2), vascular endothelial growth factor (VEGF), and epidermal growth factor [24]. After this initial burst of PRP-related growth factors, platelets synthesize and secrete additional growth factors for the remainder of their life-span [10][11]. The addition of calcium chloride promotes the physiological clotting process, which leads to sound attachment of platelets to the anastomotic site and a sustained higher level of delivery of viable growth factors. Besides, clots also act as a suitable scaffold, where fibroblasts and endothelial cells can receive stimuli from several growth factors and proliferate. These growth factors are biologically active polypeptides, and most of them have key roles in every step of wound healing. Some reports demonstrate that the local application of PDGF-BB and/or TGF-β1 as a single agent enhances wound

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healing [9][25][26]. Moreover, it was reported that the local injection of VEGF into the muscularis propria accelerates colonic anastomotic healing and strengthens the anastomosis by increased angiogenesis [27]. The specific characteristic of PRP is that it acts as a combination of multiple growth factors, each of which exerts a unique influence

5    on the complex cascade of the wound healing process, and therefore should generate a synergistic effect unlike single recombinant growth factors that focus on a single function. As just described, the biological rationale for PRP application to various wounds seems to be theoretically sound, but its effects on intestinal anastomotic wound healing remain controversial [10][11]. To address this issue, we focused on the assumption that PRP has

10   a dose-specific effect on intestinal anastomoses, and thus PRP concentration affects the outcome.

In our study, the ABP values of proximal jejunal anastomoses in the L-PRP group, of which the PRP concentration corresponds to about a 2-fold increase over the original platelet concentration, were significantly higher than those of the other three groups

15   (increased by 11% compared with that of the control group). There was no significant difference in the ABP values between the PPP group and the control group. PPP contains serum proteins such as fibrin, fibronectin, and vitronectin, known to act as cell adhesion

molecules, but very few platelets [28]. These findings might provide an explanation for

the underlying mechanism of PRP action in intestinal anastomotic healing. The essential

PRP action appears to be driven by platelets themselves, but not some serum proteins. On

the other hand, the ABP values of the H-PRP group, of which the PRP concentration

corresponds to about a 5-fold increase over the original platelet concentration, were

significantly lower than those of the other three groups (decreased by 13.5% compared

with that of the control group). The ABP is a more reliable measure for evaluating early

postoperative anastomotic mechanical strength, especially within a week of the operation

[16][29]. Generally, the bursting pressure is considered to reflect the physiological strain

in the intestine more accurately than the breaking strength [16]. In addition, the bursting

pressure shows indirect collagen formation and reflects the balance between collagen

deposition and lysis [30]. Therefore, the ABP might be regarded as not only an integrated

measure of anastomotic wound healing but also the absolute outcome of gastrointestinal

anastomoses. Considering the physiological aspects of the ABP, we could conclude that

L-PRP application significantly promoted jejunal anastomotic healing, but H-PRP

application definitely impaired the wound healing process. Needless to say, the data on

collagen concentration at the anastomosis coincided with the ABP results and reinforced

them. Furthermore, self-explanatory histopathological findings supported the above-mentioned conclusion. In the L-PRP group, there was clear evidence of increased fibroblast density and collagen formation primarily in the serosal layer. In this experiment, the proximal jejunum was divided, and then single-layer, end-to-end

5    anastomosis was performed in an inverted interrupted fashion. This is also known as serosa-to-serosa anastomosis. The essence of this anastomotic technique is full-thickness intestinal anastomosis with serosal apposition, which places importance on union of the wound, especially in the serosal layer. In fact, the application of L-PRP increased the anastomotic mechanical strength by accelerating collagen formation in the serosal layer,

10    which should be central to wound healing. To the best of our knowledge, this paper is the first report demonstrating that PRP concentration plays a crucial role in the effect of PRP on intestinal anastomotic healing. Although PRP might exert positive effects in a dose-dependent manner up to a certain level, excessive PRP concentration results in serious adverse effects rather than no effect. Our results correlated with the findings of

15    several previous studies [24][31]. By evaluating the effect of different concentrations of PRP on osteoblast and fibroblast functions in vitro, Graziani et al. reported that maximal PRP concentrations used in their study, which varied between 4.2- and 5.5-fold increases

over the original platelet concentration, might not provide the optimal environment for the promotion of wound healing. Optimal results were observed at a platelet concentration of 2.5-fold, which was approximately half of the maximum concentration that could be obtained [24]. It has remained unclear how PRP highly concentrated above a certain level interferes with the normal wound healing process. However, one-dimensional thinking of "if some is good, more is better" is not recommended for PRP application to intestinal anastomoses. To achieve the desired effect of PRP on intestinal wound healing, the use of PRP at the optimal density is thought to be imperative. The present results provide a possible explanation for the existing criticism of the efficacy of PRP. Future studies should be designed with the understanding that the PRP concentration plays a crucial role in the efficacy of PRP.

Some limitations of our study need to be addressed. First, a potential weakness of our experiment is the use of homologous PRP, but not autologous PRP. Originally, the term PRP refers to an autologous concentration of platelets in a small volume of plasma. In small animal models such as rats, the blood volume is too small to produce autologous PRP. Inevitably, donor blood is commonly used. It has been pointed out that the use of donor animal blood platelets conveys a risk of imparting an overt immune reaction,

which could lead to false-negative results [31]. However, our study could remove such

doubts because of the demonstration of positive effects of PRP in the L-PRP group.

Second, we just investigated the overall biological properties of PRP but have yet to

evaluate all sorts of growth factors included in PRP individually in this study. In the next

stage, we need to identify the factor or factors that actually improve the anastomotic

strength and healing.

Finally, we propose the following vision for clinical practice. PRP can be easily

prepared from only 20-40 cc of autologous blood of the patient. After obtaining

autologous blood of the patient, the whole process takes no more than one hour. When

centrifugal machines are present in the operating room, we can proceed with the

preparation of PRP depending on the progress of the operation and accomplish the whole

process inside the operating room. Needless to say, quality control mechanisms are

always essential. First, PRP applied to the wound should be prepared at the optimal

concentration that allows for maximum enhancement of wound healing. Second, "fresh

PRP" needs to be used. "Fresh PRP" implies that each platelet is alive and active in PRP.

Otherwise, PRP cannot fully exert its positive effect on wound healing because platelets

are expected to synthesize and secrete additional growth factors for the remainder of their

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lifespan after the initial burst of PRP-related growth factors. In concrete terms, we think

that the clinical application of PRP can be targeted at patients who undergo

gastrointestinal anastomoses under unfavorable conditions for wound healing. In such

cases, leakage of intestinal anastomosis can occasionally occur, even though

5  gastrointestinal anastomosis is performed correctly and complies with the tension-free

construction of the suture, with adequate tissue perfusion. There should be a factor that

delays or impairs wound healing, but it is difficult to determine the specific cause of

anastomotic leak because the process of wound healing is very complex and depends on

many factors. Not surprisingly, specific individual countermeasures cannot be taken

10  because the specific cause cannot be identified. Therefore, we focus on PRP working as a

storage vehicle of growth factors. PRP is expected to coordinate the process of wound

healing and handle various dangerous situations prone to the occurrence of anastomotic

leak. PRP might achieve the desired effect to accelerate wound healing and ensure

adequate anastomotic mechanical strength earlier than normal, which would lead to the

15  prevention of anastomotic leak under adverse conditions. In the case of patients with

cancer, the risks of using PRP should be taken into full account. PRP application to an

anastomotic site is essentially a local therapy, not a systemic therapy (e.g., blood

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transfusion). In addition, the actual number of platelets administered as the modality of

PRP is extremely low compared with the total number of platelets given as the modality

of platelet transfusion. Therefore, it is unlikely that PRP application to an anastomotic

site stimulates remnant cancer cells in other remote regions. Generally, there seems to be

5    little likelihood that PRP application worsens clinical outcome after cancer operation.

However, there are some alarming pathological conditions. In particular, in the case of

subclinical peritoneal metastasis, PRP application might pose a risk of local recurrence in

the area surrounding the anastomotic site by stimulating cancer cells with a variety of

growth factors derived from PRP. In such cases, PRP application might hasten cancer

10   recurrence as well.

**CONCLUSION**

PRP might exert positive effects on intestinal anastomotic healing in a dose-dependent

manner up to a certain level, but adverse effects occur at a high concentration. The

essential PRP action might be driven by the platelets themselves, but not by some serum

15   proteins. Further investigations are needed to clarify the optimal PRP concentration that

allows for maximum enhancement of anastomotic wound healing.

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**Figure legends**

**Fig 1.** Actual preparation of PRP and application of PRP to the jejunal anastomotic site.

**A**: The arrow indicates PRP and the arrowhead indicates platelet-poor plasma. **B**: The

arrow indicates activated PRP covering the anastomotic site.

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**Fig 2.** The determination of PDGF-BB concentrations in three different concentrations of

platelets.

PPP: platelet-poor plasma, L-PRP: low-concentrated platelet-rich plasma, H-PRP: high-

concentrated platelet-rich plasma. Each column represents the mean value ± standard

10  deviation of PDGF-BB in each concentration of platelets; *: *p*<0.01 versus the PPP, †:

*p*<0.01 versus the L-PRP (non-repeated measures ANOVA and Bonferroni's multiple

t-test).

**Fig 3.** The determination of TGF-β1 concentrations in three different concentrations of

platelets.

PPP: platelet-poor plasma, L-PRP: low-concentrated platelet-rich plasma, H-PRP: high-

concentrated platelet-rich plasma. Each column represents the mean value ± standard

deviation in each concentration of platelets; *: $p<0.01$ versus the PPP, †: $p<0.01$ versus

the L-PRP (non-repeated measures ANOVA and Bonferroni's multiple t-test).

**Fig 4.** Anastomotic bursting pressure 5 days postoperatively in the four groups.

Control: without application of PPP or PRP to the anastomosis line; PPP: with

application of PPP to the anastomosis line; L-PRP: with application of low-concentrated

PRP to the anastomosis line; H-PRP: with application of high-concentrated PRP to the

anastomosis line. Each column represents the mean value ± standard deviation of rats in

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the four groups; *: $p < 0.05$ versus the control, PPP, and H-PRP groups, †: $p<0.05$ versus

the control, PPP, and L-PRP groups (non-repeated measures ANOVA and Bonferroni's

multiple t-test).

5    **Fig 5.** The concentration of hydroxyproline in jejunal anastomosic tissue 5 days

postoperatively in the four groups.

Control: without application of PPP or PRP to the anastomosis line; PPP: with

application of PPP to the anastomosis line; L-PRP: with application of low-concentrated

PRP to the anastomosis line; H-PRP: with application of high-concentrated PRP to the

10    anastomosis line. Each column represents the mean value ± standard deviation of rats in

the four groups; *: $p < 0.05$ versus the control, PPP, and H-PRP groups, †: $p<0.05$ versus

the control, PPP, and L-PRP groups (non-repeated measures ANOVA and Bonferroni's

multiple t-test).

**Fig 6.** Typical histopathological appearance of the jejunal anastomosis 5 days postoperatively in the four groups.

5 **A1-A3**: Control without application of PPP or PRP to the anastomosis line; **B1-B3**: PPP with application of PPP to the anastomosis line; **C1-C3**: L-PRP with application of low-concentrated PRP to the anastomosis line; **D1-D3**: H-PRP with application of high-concentrated PRP to the anastomosis line. **A1-D1**: 40-fold magnified hematoxylin and eosin stain (HE 40×); **A2-D2**: 40-fold magnified Masson trichrome stain (MT 40×);

10 **A3-D3** 100-fold magnified Masson trichrome stain (MT 100×).

**Table 1.** Nutritional parameters in the four groups.

| | Control | PPP | L-PRP | H-PRP | |
|---|---|---|---|---|---|
| Initial body weight (g) | 195 ± 21 | 209 ± 19 | 199 ± 16 | 207 ± 15 | N.S |
| Body weight change (%) | 1.7 ± 2.3 | 3.0 ± 1.3 | 2.0 ± 2.1 | 2.8 ± 3.4 | N.S |
| Infusion volume (ml/5 days) | 190 ± 23 | 196 ± 21 | 191 ± 14 | 197 ± 14 | N.S |
| Energy intake (kcal/5 days) | 155 ± 19 | 161 ± 17 | 157 ± 11 | 162 ± 12 | N.S |
| Urine volume (ml/g/5 days) | 0.53 ± 0.06 | 0.54 ± 0.05 | 0.59 ± 0.05 | 0.50 ± 0.07 | N.S |

Control: without application of PPP or PRP to the anastomosis line; PPP: with application of PPP to the anastomosis line; L-PRP: with application of low-concentrated PRP to the anastomosis line; H-PRP: with application of high-concentrated PRP to the anastomosis line. All values represent the mean value ± standard deviation of rats in each of the four groups. Total infusion volume, total energy intake, and total urine volume did not differ between the four groups (non-repeated measures ANOVA). N.S.: no significance.

**Table 2.** Biochemical nutritional parameters in the four groups.

| | Control | PPP | L-PRP | H-PRP | |
|---|---|---|---|---|---|
| Total protein (g/dl) | 4.6 ± 0.5 | 4.5 ± 0.3 | 4.7 ± 0.3 | 4.8 ± 0.3 | N.S |
| Serum albumin (g/dl) | 2.5 ± 0.5 | 2.6 ± 0.3 | 2.5 ± 0.3 | 2.6 ± 0.3 | N.S |
| Glucose (mg/dl) | 183 ± 102 | 229 ± 221 | 175 ± 68 | 153 ± 41 | N.S |
| Blood urea nitrogen (mg/dl) | 12.0 ± 2.6 | 10.5 ± 5.3 | 13.3 ± 4.8 | 12.4 ± 2.7 | N.S |

Control: without application of PPP or PRP to the anastomosis line; PPP: with application of PPP to the anastomosis line; L-PRP: with application of low-concentrated PRP to the anastomosis line; H-PRP: with application of high-concentrated PRP to the anastomosis line. All values represent the mean value ± standard deviation of rats in each of the four groups. There were no significant differences in serum levels of total protein, albumin, glucose, and blood urea nitrogen between the four groups (non-repeated measures ANOVA). N.S.: no significance.
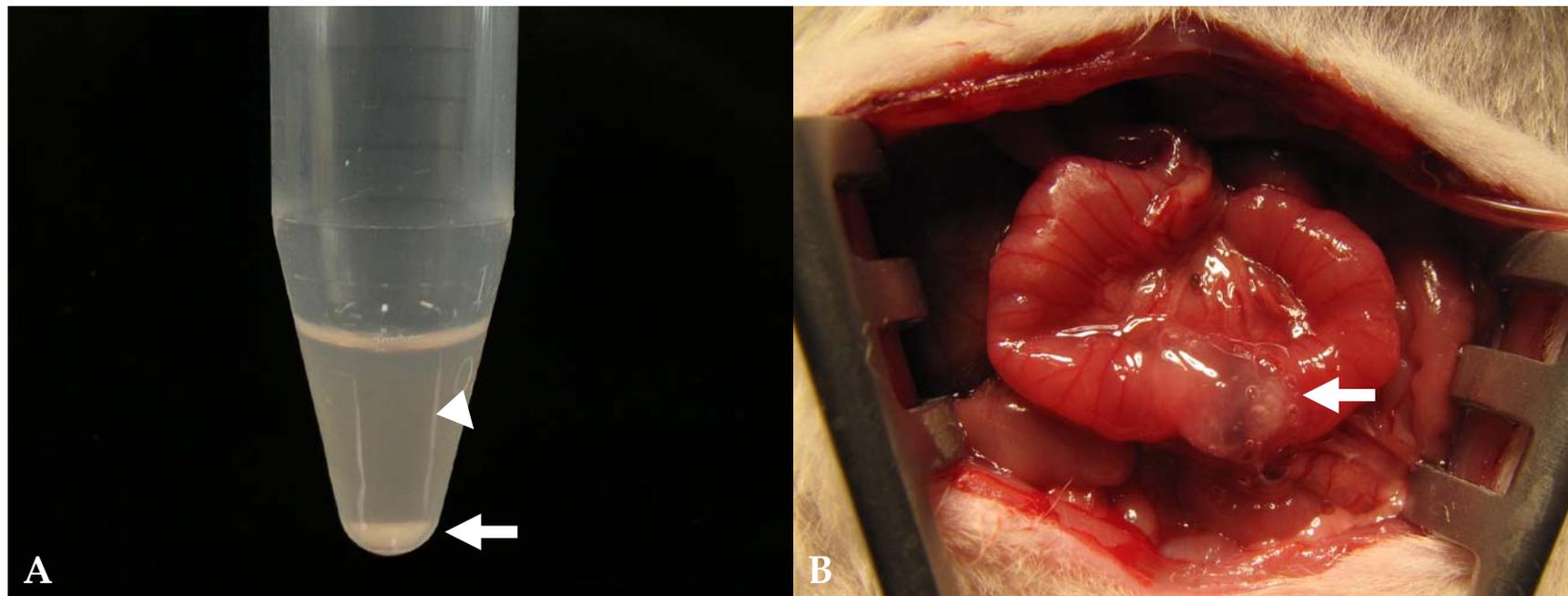
**Fig.1**

**Fig.2** The determination of PDGF-BB.
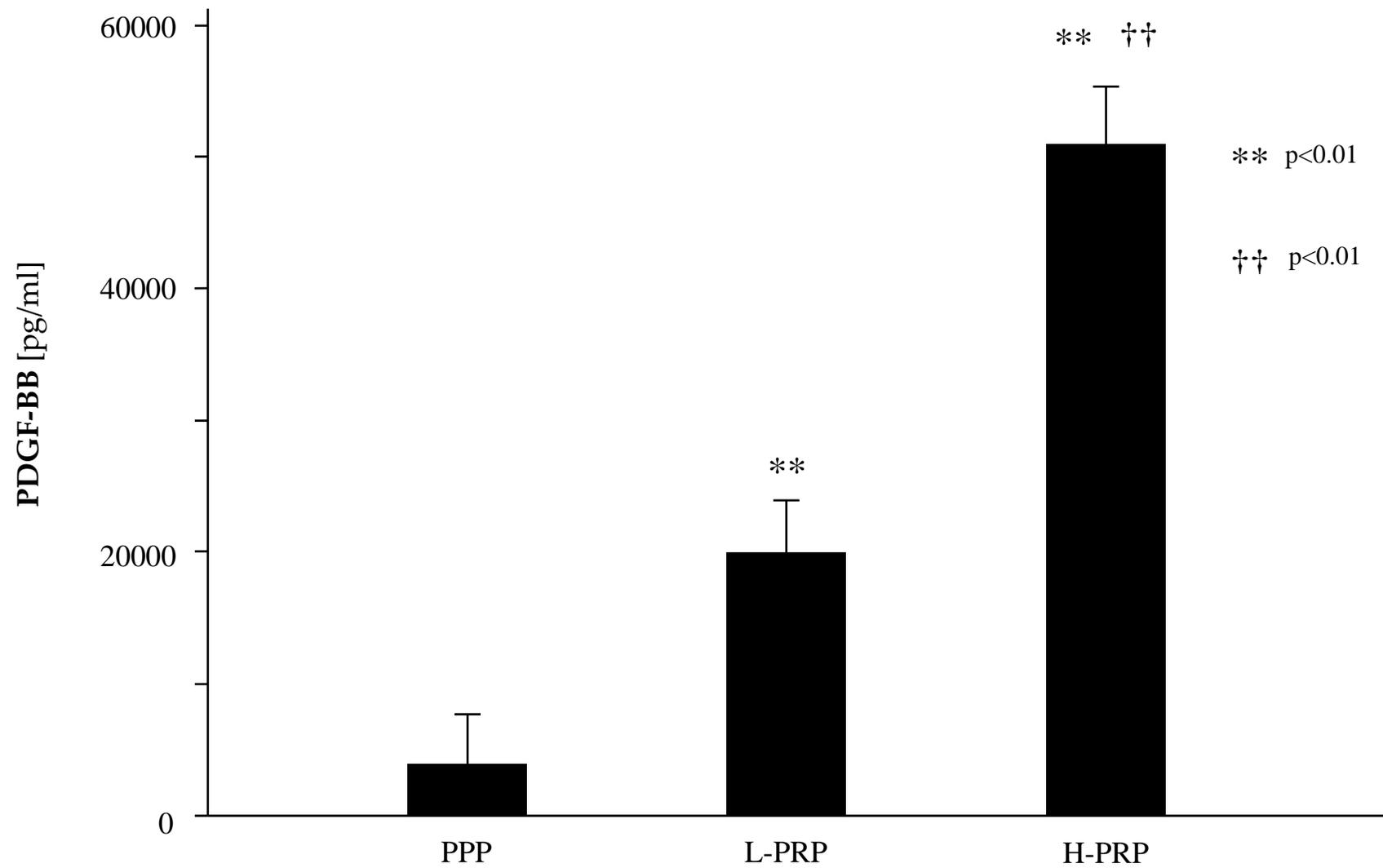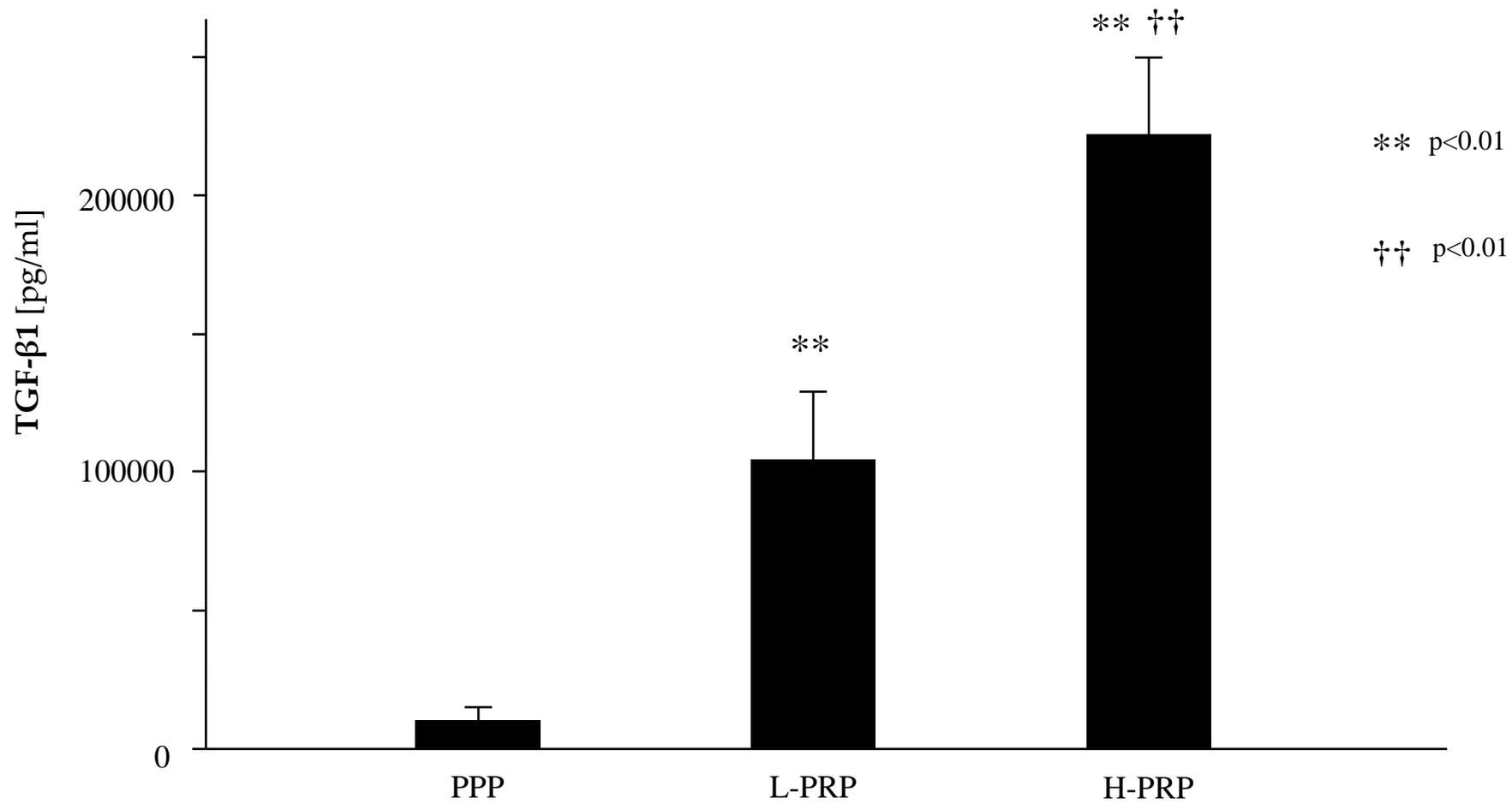
**Fig.3** The determination of TGF-β1.

**Fig.4** Anastomotic bursting pressure in the four groups
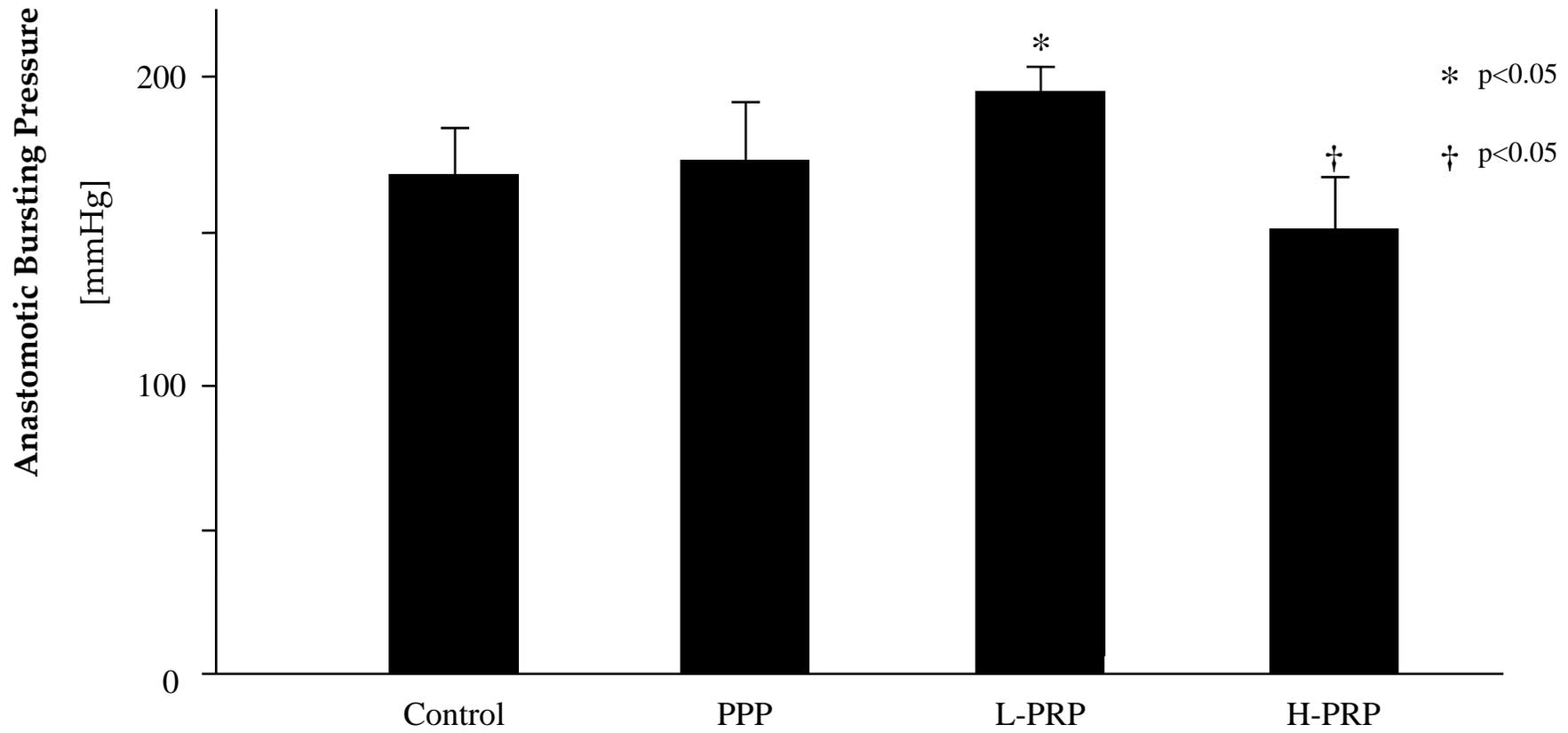
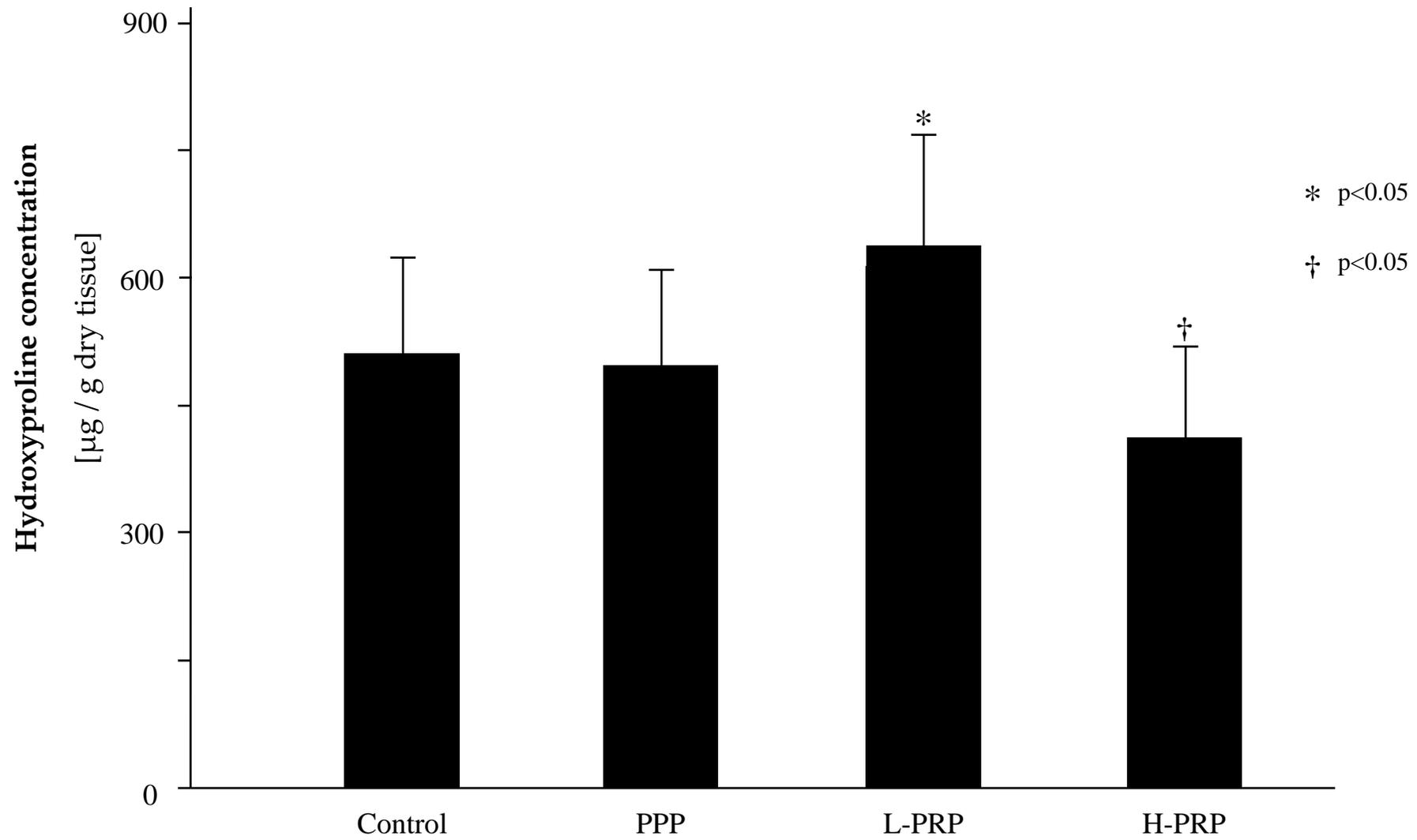**Fig.5** Concentration of hydroxyproline in jejunal anastomotic tissue in the four groups

**Fig.6** Histological finding of anastomosis