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# Protection of colonic anastomosis with platelet-rich plasma gel in the open abdomen

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ABSTRACT

*Background:* Although evidence for colonic anastomosis in the damage control abdomen continues to accumulate, anastomotic leak is common and associated with greater morbidity. The purposes of our study was to evaluate the effect of platelet-rich plasma (PRP) gel on the healing of colon anastomosis and anastomotic strength in the open abdomen.

*Methods:* PRP was prepared by enriching whole blood platelet concentration from healthy rat. In the rodent model, standard colonic anastomoses followed by closure of abdomen (Control; $n = 10$) and anastomoses followed by open abdomen (OA; $n = 10$) were compared to PRP-sealed anastomoses in open abdomen (OA + PRP; $n = 10$). One week after surgery, body weight, anastomotic bursting pressure, hydroxyproline concentration, and histology of anastomotic tissue were evaluated.

*Results:* All rats survived surgery and had no signs of anastomotic leakage. Compared with the control and PRP group, OA group exhibited a significant decrease in body weight, anastomotic bursting pressure, hydroxyproline concentration, and collagen deposition. No significant difference was detected in these variables between the PRP group and the control group.

*Conclusion:* PRP gel application prevented delayed anastomotic wound healing after open abdomen, which suggested that anastomotic sealing with PRP gel might improve outcome of colonic injuries in the setting of open abdomen.



## Introduction

Damage control surgery (DCS) techniques have been a widely accepted practice used in the management of the severely injured patients with abdominal trauma [1–4]. In damage-control procedure, destructive colon injuries are often initially resected and left in discontinuity at the initial operation. After resuscitation and physiological restoration, the patient is returned to the operating room for definitive repair of injuries. Although evidence for colonic anastomosis in the damage control abdomen continues to accumulate, the management paradigm remains far from optimal [5,6]. Anastomotic leak or dehiscence is a common complication and associated with significantly greater morbidity, including increased hospital stay and a decreased likelihood of fascial closure. Hence, innovative technical modifications to prevent anastomotic leakage in these populations are deemed necessary.

Platelet-rich plasma (PRP) gel, structurally similar to the natural fibrin clot [7], can be used as scaffolds for cells infiltration and assembly of vascular networks. Also, PRP gel can be used to deliver high quantities of key growth factors, such as platelet-derived growth factor AB (PDGF-AB), transforming growth factor β-1 (TGFβ-1) and vascular endothelial growth factor (VEGF), and thus recruit repairing cell to the site of tissue damage, which are essential to natural wound healing [8,9]. In fact, the topical use of platelet rich plasma gel has been shown to improve healing of colonic anastomosis in rat models of non-damage control setting [10]. However, few studies have specifically evaluated the effect on colon wound healing after open abdomen. This study was designed to examine the potential of PRP gel as part of treatment to promote wound healing in primary colonic anastomoses after the open abdomen.

## Materials and methods

### *Experimental animals*

Forty-two healthy male Sprague–Dawley rats (180–250 g, Jinling Hospital, Nanjing, China) were used in the experiments. Throughout the preoperative period, the animals were kept in a controlled environment (21 ± 2 °C, 50–60% humidity, 12-h light–dark cycle, lights on at 6 am) and allowed free access to standard chow and water. All animals were observed closely and weighed on days 1, 3, 5 and 7 after surgery. All the animal care and experimental protocols were reviewed and approved by Animal Investigation Ethics Committee of Jinling Hospital.

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*Preparation of platelet-rich plasma*

PRP was prepared by enriching whole blood platelet concentration using a two-step centrifugation procedure. Ten millilitres of whole blood was drawn from healthy rat ($n = 12$) through cardiac puncture into pre-chilled tubes containing ACD-A at a blood/ACD-A ratio of 9:1. Subsequently, each blood sample was centrifuged at $400 \times g$ for 10 min to obtain the three typical layers: red blood cells at the bottom, a 'buffy coat' layer in between and acellular plasma in the supernatant. Using a sterile pipette, the upper layer was transferred to another neutral tube along with the buffy coat and re-centrifuged at $800 \times g$ for 10 min. About 1 mL of PRP was collected from the bottom of the tube to yield the final PRP product. The final concentrations of platelet and growth factor in PRP were measured.

*Surgical procedures and experimental protocol*

Thirty rats were fasted overnight and anaesthetised by intraperitoneal injections of a ketamine (50 mg/kg body weight) and xylazine (5 mg/kg body weight) mixture. Under aseptic conditions, a 3-cm-long midline incision was performed on each rat. The descending colon was identified and exposed, and then 0.5-cm-long segment of the left colon was resected 3 cm proximal to the peritoneal reflection. Faecal contents were carefully removed with iodine gauze. The anastomosis was performed single layer end to end using continuous sutures of 6-0 polypropylene and a cylindrical needle. To ensure technical uniformity, all surgical procedures were performed by the same surgeon.

After anastomosis, the animals were randomly assigned to three groups of 10 animals each:

(1) Control group (Control)—standard colonic anastomosis followed by closure of abdomen;
(2) Open abdomen group (OA)—standard colonic anastomosis followed by open abdomen;
(3) Open abdomen + PRP group (OA + PRP)—standard colonic anastomosis sealed by PRP gel followed by open abdomen.

PRP gel was applied as a film layer of around 8 mm width and 3 mm thickness through a two-component system: the prepared PRP as one component and a thrombin/$Ca^{2+}$ composition as the other. The system used a double-syringe arrangement wherein the two components were mixed in situ immediately prior to dispensing to colonic anastomosis. The abdomen was closed with a running suture using 3/0 silk (Ethicon, Somerville, NJ, USA) in the control group and using aseptic polypropylene mesh (Budd Company, Troy, MI) in OA and OA + PRP group.

*Anastomotic strength*

To evaluate the integrity and strength of anastomosis, colonic bursting pressure of repaired bowel segment was measured in vivo. Using flexible atraumatic intestinal clamps, the repaired segment was isolated after insertion of insufflation tube, and submerged in 0.9% standard normal saline. The maximum pressure recorded immediately before visible bubbles as the bursting pressure. Meanwhile, the site of the rupture (within the anastomosis or not) was documented.

*Hydroxyproline concentrations*

The concentration of hydroxyproline was measured at day 7 after surgery using a procedure based on alkaline hydrolysis of the tissue homogenate and subsequent determination of the free hydroxyproline in hydrolysate [11].

*Histology*

The anastomosis was collected at day 7 after surgery and immediately fixed in 10% phosphate buffered formaldehyde, followed by dehydration in graded ethanol (70% to 100%), embedding in paraffin, serially section using a microtome (5 μm), and subsequent staining with either hematoxylin and eosin (HE) and Masson trichrome (MT). Inflammatory cell infiltration, blood vessel ingrowth, fibroblast ingrowth and collagen deposition in the anastomoses tissue were assessed and scored by a single pathologist blinded for the experimental protocol according to the method of Phillips et al. [12].

Histologic grading scale was as follows:

| | |
|---|---|
| No evidence | Score 0 |
| Occasional evidence | Score 1 |
| Light scattering | Score 2 |
| Abundant evidence | Score 3 |
| Confluent cells or fibres | Score 4 |

*Statistics*

Data are presented as means ± SEM unless otherwise noted. Continuous variables among groups were analysed by one-way analysis of variance (ANOVA), as appropriate. If a significant difference was found, post hoc analysis was performed using Fisher's least significant difference (LSD) test, unless stated otherwise. All statistical analyses were performed with IBM SPSS Statistics 18 (SPSS Inc., Chicago, IL, USA) and $p$ values $<0.05$ were considered statistically significant.

## Results

*General observations*

All rats survived surgery and had no signs of anastomotic leakage. Up to day 3, the animals lost their weight in all three groups (Table 1 and Fig. 1). Three days after operation, relative body weight in the OA group (81.78 ± 1.82%) was lower than that of the control and OA + PRP groups (89.84 ± 1.53%; $p = 0.002$ and 86.78 ± 1.67%; $p = 0.044$, respectively) (Fig. 1). And then all rats started to gain weight again, with a return to 99.82 ± 1.88% baseline in the OA group, which is lower than that in the OA + PRP group (109 ± 4.22%, $p = 0.048$) and in control group (110.07 ± 3.34%, $p = 0.036$) at day 7 (Fig. 1).

*Anastomotic bursting pressure*

The mean anastomotic bursting pressure in OA group (158.20 ± 5.08 mmHg) was significantly lower than those in the control group (184.80 ± 6.60 mmHg, $p = 0.006$) and OA + PRP group (177.20 ± 6.95 mmHg, $p = 0.041$) (Table 1 and Fig. 2). No significant difference was detected between the OA + PRP group and the control group ($p = 0.398$).

*Hydroxyproline concentrations*

The mean tissue hydroxyproline concentrations in anastomotic tissue showed the same trend as the mean anastomotic bursting pressure (Fig. 3). The mean hydroxyproline concentration in OA group (353.50 ± 6.75 μg/g dry tissue) was significantly lower than those in the control group (403.60 ± 8.55 μg/g dry tissue, $p < 0.001$) and OA + PRP group (399.70 ± 9.46 μg/g dry tissue, $p = 0.001$) (Table 1). No significant difference was detected between the OA + PRP group and the control group ($p = 0.74$).