The effects of stem cells and platelet-rich fibrin on colonic anastomosis:
An experimental study

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AIM - *Anastomotic leakage is among the most common complications following gastrointestinal surgery.*
MATERIAL AND METHODS - *This study aimed to determine the effects of stem cells and platelet-rich fibrin (PRF) on anastomotic healing. The study included 60 rats that were randomly divided into 3 groups, each with 2 subgroups. The study included the control group (no treatment post anastomosis), the PRF group (PRF administered following anastomosis), and the PRF + stem cell group (PRF + stem cells administered following anastomosis).*
Anastomosis was performed at the descending colon in all groups. Anastomosis bursting pressure was determined, and histopathological and angiographic examination were performed on postoperative D 7.
RESULTS - *Intraabdominal adhesion was significantly more common in the control group. Anastomosis bursting pressure was significantly higher and angiogenesis was significantly more common in the PRF + stem cell group ($P < 0.005$). Based on histopathologic examination, vascular proliferation and inflammation were significantly more common in the PRF + stem cell group than in the control group ($P < 0.005$).*
CONCLUSION : *In cases of risky gastrointestinal system anastomosis, PRF + stem cells might reduce the incidence of anastomotic healing.*

KEY WORDS: Anastomosis, Platelet-rich fibrin, Stem cell, Wound healing

Introduction

Colorectal surgery constitutes 10%-47% of all general surgical procedures.[1] Despite all advances in surgical technique, anastomotic leakage still occurs in 5%-69% of colorectal surgeries and the mortality rate is as high as 32% [2,3]. Mesenchymal stem cells (MSCs), which are produced by many tissues, can be multiplied many times for further use (5×10^4 - 2×10^5 stem cells are obtained from 1 g of fat tissue).[4] MSCs primarily originate from bone marrow and adipose tissue, and increase angiogenesis, local blood flow, and fibroblast and collagen synthesis in tissue, thusly contributing to wound healing.[4] Adipose-derived stem cells (ADSCs), a type of MSC that occurs via the separation of subcutaneous adipose tissue, have a positive effect on wound healing [4]. Platelet-rich fibrin (PRF) refers to the fibrin matrix structure obtained from natural blood tissue, and contains thrombocytes and leukocytes in its structure. PRF also contains growth factors. PRF accelerates and enhances healing [5].
To date, no study has examined the effect of PRF on colon anastomosis wound healing. The aim of the present study was to determine if administration of PRF + stem cells has a positive effect on wound healing in rats

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that underwent colon anastomosis. It was hypothesized that if PRF + stem cell therapy would have a positive effect on anastomosis healing and anastomotic leakage could be prevented, leading to significant reductions in morbidity and mortality rates.

Material and Method

The study protocol was approved by the XXXXXX Ethics Committee for Animal Experiments (no. DA 14/03). Rats were obtained from the XXXXXXX Turkey. The experiments were performed at XXXXXXX , Research Unit Laboratories. The study included 80 female Sprague-Dawley rats weighing 180-250 g (mean: 210 g). All animals were cared for under standard and optimal conditions.

Study Model

The rats were randomly divided into 3 groups of 20 each and were then randomly divided randomly into 2 subgroups (A and B) of 10 each, as follows: the control group, the PRF group, and the was PRF + stem cell group. Subgroups A underwent intraabdominal adhesion, followed by bursting pressure and histopathological assessment. Subgroups B underwent angiographic evaluation. All rats underwent laparotomy and colon anastomosis. The control group underwent colon anastomosis only. Postsurgery PRF and PRF + stem cells was applied roundly over the anastomose region in the RF and RF + stem cell groups, respectively.

All rats were euthanized on postoperative d 7. Those in subgroups A underwent relaparotomy after sacrification, and the anastomotic line was found and intraabdominal adhesion was assessed. Subsequently, a 60-mm area was resected with surrounding adherent tissues, with the anastomotic line in the middle. Bursting pressure was measured, histopathological assessment (hematoxylin-eosin, trichrome) was performed, and identification of labeled stem cells were determined on the anastomotic line. Rats in subgroups B were sacrificed via intracardiac 1/1 saline-diluted barium injection (Fig. 1). After sacrification and relaparotomy, a 60-mm area was resected with surrounding adherent tissues, with the anastomotic line in the middle, followed by freezing at -20 °C for 24 h. Next, direct radiographs were made and angiographic evaluation was performed. In the PRF group PRF was applied over the anastomotic line. In the PRF + stem cell group PRF + stem cells was applied over the colon anastomotic line. Intraabdominal adhesion was assessed according to Houston and Rotstein [6], and classified as mild, moderate, and severe (Fig. 2).

The segment from 3 cm distal to 3 cm proximal to the anastomosis was resected and inflated using saline solution via an infusion pump. Intraluminal pressure was observed on a monitor and the highest pressure recorded was considered bursting pressure [7] (Fig. 3).

Anastomosed colon segments were prepared by a pathologist blinded to the study groups. Tissue samples were assessed for mucosal reepithelialization, fibrosis, ischemic necrosis, vascular proliferation, and inflammation in the anastomosis. Labeled stem cells in the PRF + stem cell group were identified. Appropriate cross-sections 5 µm thick were prepared from tissues embedded in paraffin,

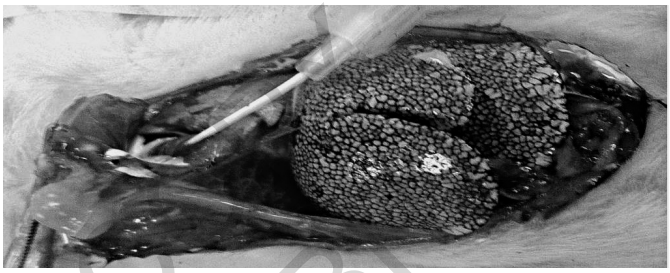

Fig. 1. Intracardiac barium injection.

Fig. 2. A. Mild adhesion. B. Moderate adhesion. C. Severe adhesion (cotton swab indicates the anastomosis sites).

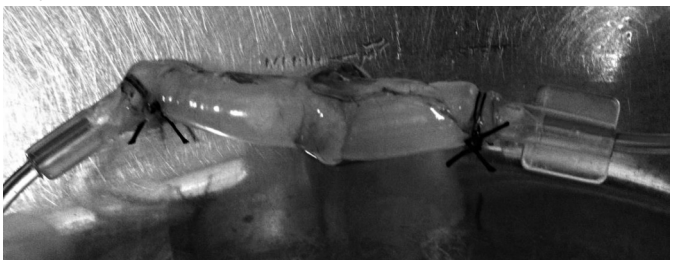

Fig. 3. Measurement of anastomosis bursting pressure.

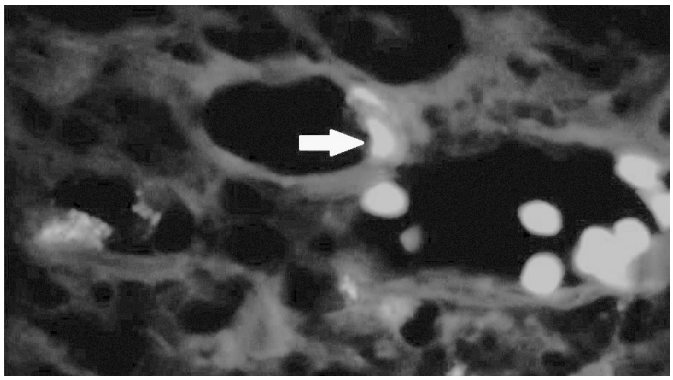

Fig. 4: ADSCs adjacent to villi and in the endothelium (stem cells indicated by yellow arrow).

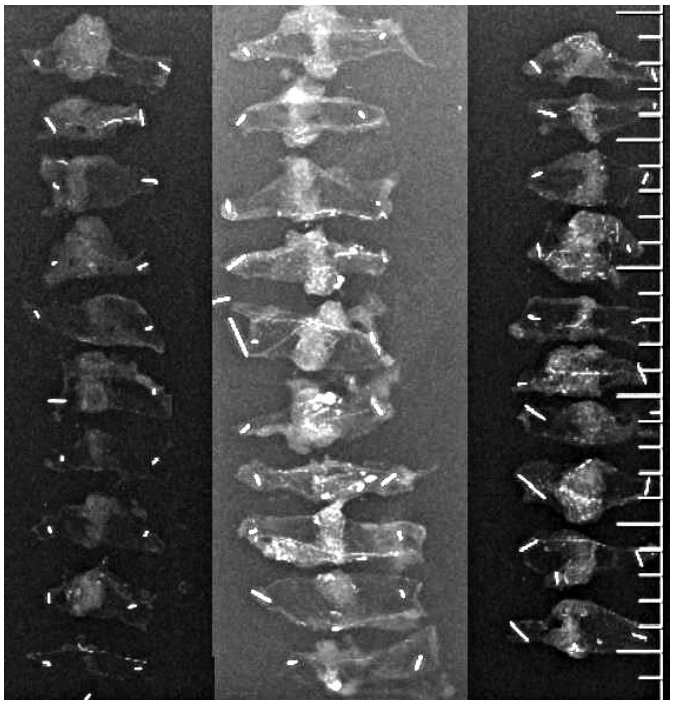

Fig. 5. Angiographic evaluation.

which were first stained with hematoxylin, and then with hematoxylin-eosin [8]. Both differentiation of stem cells in endothelial cells and the presence of stem cells adjacent to villi were determined (Fig. 4). Direct radiographs obtained for angiographic evaluation were scaled to 5 x 5 cm. Vascular structures in a 250 mm^2 area were enumerated on scaled graphs using Microsoft Paint [9] (Fig. 5).

Surgical Procedure

The rats underwent laparotomy via midline incision after being anesthetized via intraperitoneal injection. The descending colon was full-thickness transected. Then, single-layer continuous colon anastomosis was performed using a single 7/0 prolene suture. According to the groups, nothing, PRF, or PRF + stem cells was applied roundly over the anastomosis area. Following these procedures the abdomen was sutured and the surgery was completed. All animals were administered analgesic and provided with rat feed and water ad libitum post-surgery.

Collection of Adipose Tissue-Derived Stem Cells (ADSCS)

The same preoperative procedures with the other rats were performed on rats which will be used for stem cell and PRF collection. Approximately 20 cc of adipose tissue from inguinal fat pads was obtained from 4 rats (Fig. 6). The protocol published by Ogawa et al. [10] was

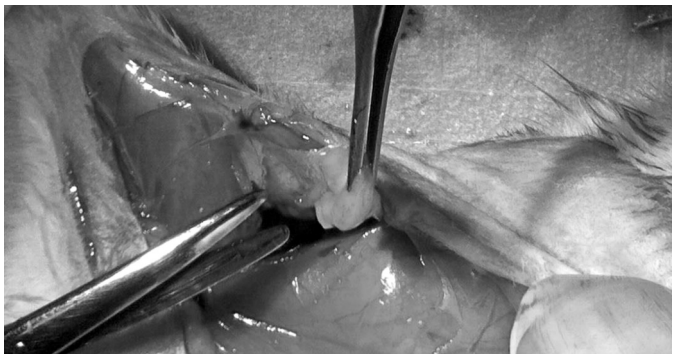

Fig. 6. Inguinal fat pad excision.

used for collection of ADSCs. To enumerate stem cells 0.1 mL of the fraction was stained with methylene blue. The stem cells obtained were mixed with phosphate-buffered saline (PBS) and suspended. The suspension obtained was mixed homogeneously with PRF. The quantity to be administered to each rat was determined to be 1.2 x 10^7 cells.

Preparation and Administration of Platelet-Rich Fibrin

In all, 16 rats were explorated via subcostal incision and the heart was exposed. The rats were sacrificed after collecting approximately 8 cc of blood, which was placed in tubes containing 0.109 mol L^{-1} sodium citrate. These tubes were centrifuged and platelet-rich plasma at the bottom was aspirated [11]. The platelet-rich plasma samples were mixed with 10% calcium chloride ($CaCl_2$) at a ratio of 1:0.15 to ensure platelet activation [12], and after waiting 10 min a ready-to-use material was obtained.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics for Windows v.20 (IBM Corp., Armonk, NY). Intraabdominal adhesion was analyzed using the chi-square test. The t-test was used to compare weight. Bursting pressure and angiogenesis results were analyzed via Tukey's test. Histopathological results were analyzed using Fisher's exact test. The level of statistical significance was set at $P < 0.05$.

Results

Assessment of Weight Loss

There weren't any significant differences in baseline weight and final weight between the 3 groups ($P = 0.306$) (Table I).

Table I - *Assessment of weight loss.*

Group		Number of rats(n)	Mean ± SD (g)	P
Control	Initial weight	10	245.9 ± 7	0.253
	Final weight	10	244.4 ± 6.7	
PRF	Initial weight	10	245.5 ± 6.1	0.039
	Final weight	10	243.9 ± 6.7	
PRF + stem cell	Initial weight	10	242 ± 6.1	0.525
	Final weight	10	242.7 ± 7.1	

Table II - *Assessment of intraabdominal adhesion.*

Severity of adhesion	Group			P
	Control (n = 10)	PRF (n = 10)	PRF +stem cell	
Mild	0	6 (30%)	11 (55%)	0.001
Moderate	7 (35%)	8 (40%)	6 (30%)	
Severe	13 (65%)	6 (30%)	3 (15%)	

Assessment of the Severity of Intraabdominal Adhesion

There was a significantly strong correlation between the type of procedure and adhesion ($P < 0.05$); the severe adhesion rate in the control group was 65%, versus 30% in the PRF group and 15% in the PRF + stem cell group. the mild adhesion rate was highest (55%) in the prf + stem cell group (Table II).

Assessment of Bursting Pressure And Angiogenesis

The difference in bursting pressure between groups was significant ($P < 0.05$). According to Tukey's test for paired comparisons, the burst pressure values in the PRF + stem cell group were significantly higher than in the control group ($P < 0.05$). In addition, the highest angiography values were noted in the PRF + stem cell group, whereas the lowest values were observed in the control group (Fig. 7).

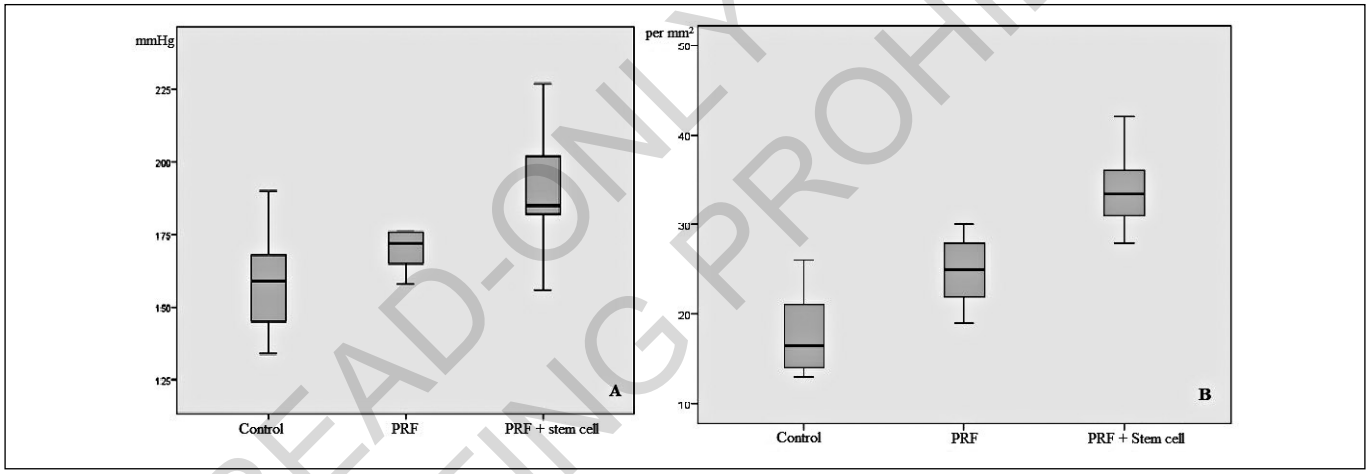

Fig. 7. Bursting pressure (A) and angiogenesis (B) results.

Table III - *Histopathological assessment.*

Parameter	Parameter Intensity	Group Control(n = 10)	PRF(n = 10)	PRF + stem cell(n = 10)	P
Reepithelization	High	6 (60%)	8 (80%)	10 (10%)	0.082
	Low	4 (40%)	2 (20%)	0 (0%)	
Fibrosis	Focal	4 (40%)	3 (30%)	0 (0%)	0.089
	Diffuse	6 (60%)	7 (70%)	10 (10%)	
Ischemic necrosis	High	6 (60%)	3 (30%)	1 (10%)	0.058
	Low	4 (40%)	7 (70%)	9 (90%)	
Muscular layer degeneration	Focal	7 (70%)	9 (90%)	10 (10%)	0.133
	Diffuse	3 (30%)	1 (10%)	0 (0%)	
Vascular proliferation	Mild	2 (20%)	0 (0%)	0 (0%)	0
	Moderate	6 (60%)	3 (30%)	0 (0%)	
	Severe	2 (20%)	7 (70%)	10 (10%)	
Inflammation	Moderate	4 (40%)	1 (10%)	0 (0%)	0.012
	Severe	6 (60%)	9 (90%)	10 (10%)	

Histopathological assessment

There weren't any significant differences in pathological parameters, other than vascular proliferation and fibrosis, between the groups. The severe vascular proliferation rate was 20% in the control group, versus 70% in the PRF group, and 100% in the PRF + stem cell group. In addition, the incidence of severe inflammation was 60% in the control group, as compared to 90% in the PRF group and 100% in the PRF + stem cell group (Table III).

Discussion

Studies have shown that the anastomosis leakage rate is 1.95%-14.16% following gastrointestinal surgery in immunosuppressed patients [13,14]. The present findings, especially the increase in bursting pressure, show that subserosal PRF + stem cell application increases anastomosis healing and reduces the anastomosis leakage rate in immunosuppressed rats.

After colorectal surgery the catabolic rate increases, leading to loss of body weight [15]. In the present study the difference in weight loss on postsurgery d 7 between the groups was not significant. On the other hand, even though it was not significant, there was a decrease in weight in the control group and an increase in weight in the PRF + stem cell group on postoperative d 7. We think that the increase in weight gain in the PRF + stem cell group was due to the anabolic effect of the stem cells, whereas weight loss is normally experienced during the postoperative period [16].

Adhesion after intraabdominal surgery leads to intestinal obstruction and intestinal ischemia [17]. In the present study application of stem cells partially prevented this increase in the severity of intestinal ischemia during the postsurgery period. Pascual et al. [18] performed colonic anastomosis using adipose-derived mesenchymal stem cells in biosutures, similarly to the present study, and observed that use of stem cells significantly reduced the adhesion formation rate.

This low adhesion rate during the early postsurgery period might be due to the immunomodulatory effect of stem cells, which suppresses in vitro proliferation of activated lymphocytes [19].

Bursting pressure is an indicator of anastomotic healing and the collagen concentration in the colon. Jong Han Yoo et al. [20] studied the effect of ADSCs on ischemic colonic anastomosis healing, reporting that bursting pressure and the collagen concentration are directly proportional.

Similarly, in the present study the highest bursting pressure was observed in the PRP + stem cell group. In addition, the highest vascularization values were noted in the PRF + stem cell group. According to these findings, we think that the observed increase in anastomotic stability might have been due to an increase in the amount of collagen, although the collagen concentration was not measured in the present study due to technical difficulties. Measuring the amount of collagen in new studies can provide more detailed information on this subject.

Histopathological assessment in the present study showed that there weren't any significant differences in pathological parameters, other than vascular proliferation and fibrosis, between the groups.

Furthermore, based on the present findings, we think that the cause of increased vascular proliferation in the PRF + stem cell group was due to an increase in the vascular endothelial growth factor level caused by the stem cells and the ability of stem cells to differentiate into such cell types as endothelial cells, as reported by Cazius et al. [4]

The present study has some limitations, including the lack of both measurement of the tissue collagen level and measurement of molecular parameters after stem cell application.

In addition, although the healing mechanisms in rats and humans are similar, experiments in rats may not be as effective at wound healing mechanism as in humans.

Conclusions

In conclusion, PRF + stem cell application over the anastomosis site following gastrointestinal surgery might prevent anastomosis leakage. In addition, mixing ADSCs with PRF facilitates use of the procedure.

If anastomotic leakage can be reduced thanks to the use of PRF + stem cells, which can be easily prepared in a timely fashion during surgery, the surgical success rate can increase, and significant decreases in the morbidity and mortality rates can be achieved.

Riassunto

È tra le complicazioni più comuni dopo un intervento chirurgico gastrointestinale.

Materiale e metodi: Questo studio è finalizzato a valutare gli effetti delle cellule staminali e della fibrina ricca di piastrine (PRF) sulla guarigione anastomotica.

La sperimentazione si è svolta su 60 ratti divisi a random in 3 gruppi, ciascuno con 2 sottogruppi: gruppo di controllo (nessun trattamento post anastomosi), il gruppo PRF (PRF apposta dopo anastomosi) e il gruppo PRF + cellule staminali (PRF + cellule staminali somministrate dopo anastomosi).

L'anastomosi è stata eseguita al colon discendente in tutti i gruppi. È stata determinata la pressione di scoppio dell'anastomosi ed è stato eseguito un esame istopatologico e angiografico nel 7° giorno postoperatorio.

Risultati: Le aderenze intraaddominale sono risultate significativamente più comuni nel gruppo di controllo.

La pressione di scoppio dell'anastomosi è risultata significativamente più elevata e l'angiogenesi significativamente più comune nel gruppo di cellule staminali PRF + (P <0,005). Sulla base dell'esame istopatologico, la proliferazione vascolare e l'infiammazione sono risultate significativamente più comuni nel gruppo con cellule staminali PRF + rispetto al gruppo di controllo (P <0,005). Conclusioni: In caso di anastomosi a rischio del sistema gastrointestinale, le cellule staminali PRF + potrebbero ridurre l'incidenza della deiscenza anastomotica.

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