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ARAŞTIRMA / RESEARCH

Efficacy of pure olive oil and PRF in the prevention of postoperative peritoneal adhesions

Postoperatif peritoneal adhezyonların önlenmesinde saf zeytinyağı ve PRF'nin etkinliği

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Abstract

Purpose: In this study, we applied platelet rich fibrin (PRF) and pure olive oil on the incision surfaces of rats. We aimed to examine whether PRF may be used safely to prevent peritoneal adhesions.

Materials and Methods: Fourty rats were divided into 4 groups (n=8). Eight rats, not included in the study groups, were used to obtain PRF material. Group 1 had no surgical procedure, Group 2 was operated without medication, Group 3 was operated and received 1cc olive oil, Group 4 was operated and received 1 cc PRF. After 21 days, cecum areas were examined histopathologically. Tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), intercellular adhesion molecule-1 (ICAM-1) and platelet-derived growth factor (PDGF) levels were measured in serum by ELISA.

Results: The adhesion scores and severity of fibrosis in Group 3 and 4 were significantly lower than Group 2. Plasma TNF-α value was significantly higher in Group 2 than Group 4. Plasma PDGF value was significantly higher in Group 2 than Group 3 and 4.

Discussion: PRF reduced intestinal adhesion by inhibiting the proliferation of fibroblasts and inflammatory cells, and promoting the proliferation of mesothelial cells. PRF has anti-inflammatory effect and prevented postop adhesions, based mainly on growth factors and cytokines in its content.

Keywords:. postoperatıve perıtoneal adhesıons, platelet rich fibrin, pure olive oil

Öz

Amaç: Bu çalışmada, adhezyon oluşması beklenen alanlara uygulanan trombositten zengin fibrin (PRF) ve saf zeytinyağının etkilerinin incelenmesi amaçlanmıştır. PRF'nin adezyona neden olup olmadığının ve PRF'nin batın içi operasyonlarda güvenle kullanıp kullanamayacağını araştırılmaktadır.

Gereç ve Yöntem: Toplamda 4 grup (n=8) olmak üzere 40 adet Wistar albino rat çalışma gruplarında kullanıldı. Çalışma gruplarına dahil edilmeyen 8 rattan PRF eldesi için yararlanıldı. Grup 1'e herhangi bir cerrahi işlem yapılmadı. Adezyon modelin uygulandığı Grup 2'de ilaç kullanılmadı. Adezyon modeli sonrası Grup 3'de 1 cc zeytinyağı ve Grup 4'de 1 cc PRF uygulandı. 21 gün sonra çekum alanları histopatolojik olarak incelendi. Serumda proinflamatuar sitokinlerden tümör nekroz faktör-α (TNF-α), interlökin-6(IL-6), hücreler arası adezyon molekülü-1(ICAM-1) ve platelet-türevli büyüme faktörü(PDGF) düzeyleri ELISA yöntemiyle ölçüldü.

Bulgular: Grup 3 ve 4'te adezyon skorları ve fibrozis şiddeti Grup 2'ye göre anlamlı olarak düşüktü. Plazma TNF-α değeri Grup 2'de Grup 4'e göre anlamlı olarak yüksekti. Plazma PDGF değeri Grup 2'de Grup 3 ve 4'e göre anlamlı olarak yüksekti. .

Sonuç: PRF, fibroblastların ve inflamatuar hücrelerin çoğalmasını engelleyerek ve mezotelyal hücrelerin çoğalmasını teşvik ederek bağırsak yapışmasını azaltabilir. PRF, içeriğindeki büyüme faktörleri ve sitokinlere bağlı olarak antiinflamatuar etkiye sahiptir ve postop adezyonları önler.

Anahtar kelimeler: Postoperatif peritoneal adezyon, trombositten zengin fibrin (PRF),saf zeytinyağı

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INTRODUCTION

Adhesions, intestinal obstructions, infertility, pelvic pain and subsequent surgical interventions are among the leading problems of surgeons after abdominal surgery today. Even the most delicate surgical techniques alone are insufficient to prevent adhesions [1]. Adhesion occurs in approximately 90% of patients who have undergone intraabdominal surgery and approximately 3% of these develop intestinal obstruction. In advanced adhesions, even death may occur[2]. Postoperative peritoneal adhesions cause approximately 15-20% of female infertility. Today, it has been reported that up to 75% of intestinal obstruction is due to adhesions due to previous surgeries, and this rate can increase to 93% after multiple operations[1,3].

Damage to the peritoneum and serosal surfaces due to chemical, thermal, foreign body reaction, infection, and traumatic factors initiates the sequence of events that result in adhesion formation. As a result of injury to the peritoneum or serosa, histamine and vasoactive kinins are released from mast cells, causing an increase in capillary permeability and serous accumulation[3]. Damage to the mesothelioma area brings close connective tissue into contact with peritoneal fluid, which increases the secretion of Leukotriene B4 and prostaglandin E2 (PGE2) in the peritoneal fluid, while inhibiting the tissue plasminogen activator (tPAA) activity[4]. While leukotriene B4 and PGE 2 increase stimulate adhesiogenesis, tPAA inhibition reduces fibrin breakdown; ultimately the balance changes in favor of adhesion formation. Peritoneal injury also causes thromboplastin (tissue factor) to be released, activating the coagulation cascade resulting in the formation of fibrin. If fibrin destruction is not sufficient, this fibrin provides matrix for adhesion formation[4].

Platelet-rich fibrin (PRF) is the fibrin network obtained by centrifugation of blood[5]. PRF contains dense granules of platelets, platelet-derived angiogenesis factor (PDAF) transforming growth factor (TGF)-β, epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), insulin-like growth factor-I (IGF-I), platelet-derived epidermal growth factor (PDEGF), and platelet factor-4 (PF-4). They are released from platelet granules promote angiogenesis, cell proliferation, differentiation and wound healing. PRF works synergistically with fibrinogen and cytokines[6]. Various cytokines in PRF enable cell proliferation and differentiation, also promoting the proliferation of mesothelial cells.

PRF functionally provides clot formation, supports tissue regeneration, acts as a natural fibrin scaffold containing growth factors and stem cells. PRF is obtained by centrifugation of whole blood. PRF spontaneously forms a fibrin matrix which collects growth factors (GF) at the coagulation site without any anticoagulant. The fibrin matrix is regulated by fibroblasts and collagen synthesis is initiated. Therefore, both the growth factors and fibroblasts in PRF serve synergistically to support collagenesis and tissue regeneration.

It has been shown that the fibrin matrix in PRF increases the efficacy of GFs secreted from platelets[7]. Thanks to its strong fibrin structure and persistent abundant release of GF, we hypothesized that PRF can prevent permanent adhesion by accelerating wound healing and reducing adhesion formation[8,9].

Pure olive oil has beneficial effects on wound healing thanks to its anti-inflammatory and tissue regeneration enhancing properties[10,11]. One of the most important features of pure olive oil, a high-viscosity liquid, is that it minimizes trauma during manipulation by creating lubrication on the serosal and peritoneal surfaces. It can be used to prevent abdominal adhesions due to its mechanical separator (hydroflotation) feature between traumatized surfaces[12]. Herein, we aimed to investigate and compare the effects of olive oil and PRF application in preventing adhesions that occur during the postoperative period.

MATERIALS AND METHODS

Animals

A total number of 40 Wistar albino rats, between 200-300 g, were used in this study. Housing was achieved in hygienic stainless steel cages with free access to food and tap water. Rats were kept at an ambient temperature of 22°C and 60±5% humidity with a 12-hr light/dark cycle throughout the experiment. All experiments were carried out in a strict compliance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals. The experimental procedures were performed in the

Practice and Research Laboratory of Kahramanmaraş Sütçü İmam University. This experimental study is approved by the Kahramanmaraş Sütçü Imam University Local Ethical Committee on Laboratory Animals (ethics committee file number, 2020/06; approval date, February 27, 2020)

Experimental design

A total of 40 rats were divided into 4 groups (n=8). Eight rats, not included in the study groups, were used to obtain PRF material. In previous studies, it was reported that autoantibody reactions have not been developed related to PRF obtained from the serum of other rats [7]. For this reason, we applied PRF material obtained from rats outside the study groups to our rats in Group 4, where PRF was applied, and we did not observe any reactions.

The experimental groups were as follows:

Group 1 (Control group): Group 1 had no surgical procedure.
Group 2 (Sham group): Group 2 was operated without medication.
Group 3 (Olive oil group): Group 3 was operated, the front surfaces of the cecum were coated with 1 cc olive oil after standard adhesion manipulation.
Group 4 (PRF group): Group 4 was operated, front surfaces of the cecum were coated with 1 cc PRF after standard adhesion manipulation.

21 days after the adhesion was formed, the abrased cecum tissues were examined with histopathological scoring macroscopic (Evans adhesion score) and microscopic (Zühlke's microscopic adhesion score) to observe the effect of the medications. Proinflammatory cytokines including tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), intercellular adhesion molecule-1 (ICAM-1) and platelet-derived growth factor (PDGF) levels were measured in serum by ELISA method.

Platelet rich fibrin (PRF) preparation

In this study, 8 rats outside our groups were selected as donors for the preparation of PRF and did not undergo any surgical procedure. An average of 6 cc (4.5 - 8 cc) of blood was obtained from these rats by intracardiac (inferior vena cava) puncture and placed in a sterile glass tube without anticoagulants. Since platelet activation and fibrin polymerization was triggered immediately in the absence of anti-coagulant, the tube was placed in a centrifuge previously adjusted to 3000 RPM (400 G) for 10 minutes. After centrifugation, a layer of erythrocytes collected at the bottom of the tube, acellular plasma poor from thrombocyte is formed at the top of the tube, and a platelet-rich fibrin, or PRF clot, is formed in the middle of the tube. PRF forms a complex three-dimensional fibrin matrix [12]. Approximately 1cc (0.8-

1.2 cc) of the intermediate layer (PRF gel) was taken and applied locally to the anterior surface of the intraperitoneal cecum.

Pure olive oil preparation

Pure olive oil is produced by the cold pressing method. Olive oil was sterilized by 0.45 nm porosity filtration into a sterile centrifuge tube. The pH value of olive oil is the same as the peritoneal dialysis fluid (pH 6.8) [13].

Intraperitoneal adhesion model in rats

A total of 32 rats in 4 groups were operated under anesthesia of ketamine 80 mg/kg and xylazine 10 mg/kg. As we experimented in our previous studies, intraperitoneal adhesion model was performed in all groups, except for Group 1 [13].

Rats were fixed in the supine position. The skin was disinfected with iodophor. We entered in the peritoneal cavity with a three-centimeter vertical midline incision. The cecum was found and carefully taken out of the abdomen. The surgeon's left hand was placed on the second finger of the surgeon, with the anterior surfaces of the intestine to be abrased on top, and the injury was caused by sterile dry gauze. The cecum was damaged by wiping with dry gauze to abrade the anterior serosal surface approximately 0.5 cm × 0.5 cm (standard adhesion pattern) until punctual bleeding foci appeared. After the abrasion created on cecum, it was replaced to the intraperitoneal space, and peritoneal abrasion was created on was the 2x2 cm parietal and visceral peritoneal area using a dry gauze. The incision was closed with 3/0 propylene using a continuous suture technique. Animals were fasted for 12 hours after surgery. Operations were performed by the same surgeon. 21 days after the incision closed, abrased cecum tissues were measured by histopathological scoring. Adhesion was evaluated in two ways: macroscopic (Evans adhesion score) and microscopic (Zühlke's microscopic adhesion score). Cecum tissue samples were taken for histopathological evaluation.

Macroscopic adhesion assessment

Adhesion scoring defined by Evans was used, in two ways according to adhesion strength and adhesion area. The scoring of adhesion area was calculated as follows: 0: No adhesion, 1: Adhesion in 25% of the area, 2: Adhesion in 50% of the area, 3: There is adhesion in the entire area. The scoring of adhesion strength was calculated as follows: 0: No adhesion, 1: Adhesion that separates spontaneously, 2: Adhesion separated by traction, 3: Decomposed by sharp dissections [14].

Histopathological evaluation

Histomorphological changes in intraperitoneal adhesions after PRF and olive oil application in rats were examined in 4 groups. The 2cm² front walls of the cecum and adhesions on this surface, if any, were fixed in formol from all rats. It was soaked in paraffin after dehydration. Sections of 5 mm were taken and stained with hematoxylin and eosin (H&E) and with Masson's trichrome. Zühlke's classification was used for histopathological grading. The grades of microscopic adhesion classification was calculated as follows: Grade 1: Weak connective tissue, rich cell, old and new fibrin, fibrins of thin reticle, Grade 2: Connective tissue with cells and capillary vessels, rare collagen fibers, Grade 3: Thicker connective tissue, rare cells, more vessels, rare elastin and smooth muscle fibers, Grade 4: Old thick granulation tissue, rare cells, difficult separation of serosal layers[15].

Enzyme-linked immunosorbent assay (ELISA)

After the cecum tissue samples were taken on the 21th day of experiment, an average of 6 cc (4.5 - 8 cc) blood was taken from each rat. Rat serum tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), intercellular adhesion molecule-1 (ICAM-1) and platelet-derived growth factor (PDGF) concentrations were measured using enzyme-linked immunosorbent assay (ELISA) kits (TNF-α, Catalog No.: E0764Ra; IL-6, Catalog No.: E0135Ra; ICAM-1, Catalog No.: E0418Ra; PDGF, Catalog No.: E069Ra; (Bioassay Technology Laboratory) according to the manufacturer's recommendations. All results are expressed as pg/mL of serum. Sensivity; TNF-α: 2,51 ng/L, IL-6: 0,052 ng/L, ICAM-1: 0,026 ng/mL, PDGF: 2,51 ng/L. Detection range; TNF-α: 5-1000 ng/L, IL-6: 0,1-40 ng/L, ICAM-1:0,05-20 ng/Ml, PDGF: 0,05-15 ng/mL.

Statistical analysis

The data were defined as the arithmetic average and standard deviation. To apply parametric tests, Shapiro Wilk test was used to determine whether the samples have a normal distribution and whether the variances are homogeneous. For multiple groups, analysis of variance test with post-hoc Tukey's test for significant difference was used for normally distributed data. Kruskal Wallis test was used for the analysis of none normally distributed data. Mann Whitney U test was used to compare the adhesion strength scores of the groups.

The p-values less than 0.05 were considered significant. The data were evaluated at a 95% confidence interval. The data were analyzed in SPSS 17.0 programme (IBM, IL, U.S.A.).

RESULTS

The groups were evaluated according to Evans' adhesion strength scoring and presented in Table-1a. We found that the adhesion strength scores of Group-3 and 4 were significantly lower than Group-2 ($p<0.001$). In the comparison of Group-3 with Group-4, we found no statistically significant difference was found in adhesion strength score (Table-1, Fig 1a-e).

Table-1. Adhesion strength scores of the groups

Groups		Evans Adhesion Strength Score			Total	Adhesion strength scores
	0	1	2	3		
Group 2	0 (%0)	0 (%0)	1(%12,5)	7 (%87,5)	8 (%100)	G3\G2 P < 0.001
Group 3	5 (%71,4)	2 (%28,6)	0(%0)	0 (%0)	7 (%100)	G3\G4 P > 0.05
Group 4	7 (%87,5)	1 (%12,5)	0(%0)	0 (%0)	8 (%100)	G4\G2 P < 0.001
Total	12 (%50)	4 (%16,6)	1(%4,16)	7 (%29.24)	24 (%100)	

Comparison of the adhesion strength scores of the groups with the Mann Whitney U test

The groups were evaluated according to Evans' adhesion area scoring and presented in Table-2. We found that the adhesion area scores of Group-3 and 4 were significantly lower than Group-2 ($p<0.001$).

In the comparison of Group-3 with Group-4, we found no significant difference in adhesion area score (Table-2, fig 1a-e).

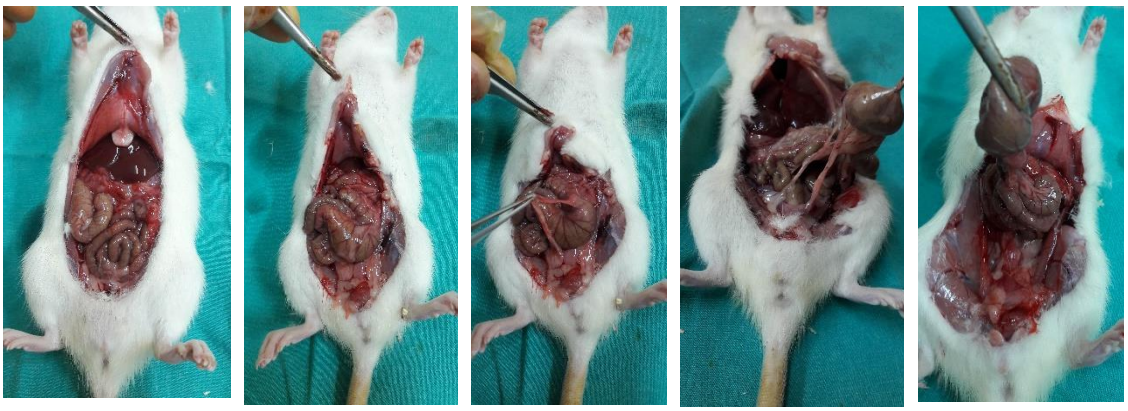


Figure 1a. (group 1, grade 0) Post-laparotomy, group 1, no adhesion
Figure 1b (grade 1) Adhesions separated spontaneously (grade-1) between the cecum and ileum (arrows)
Figure 1c (grade2) adhesions separated by traction between the cecum and ileum (arrow)
Figure 1d (grade3) Adhesions separated by sharp dissection between the cecum and intraabdominal organs (grade-3) (arrow)
Figure 1e (grade4-ileus) Severe adhesions (arrows) in the abdomen and ileus

Table 2. Adhesion area scores of the groups

Groups		Evans Adhesion Area Score			Total	Adhesion area scores	
	0	1	2	3			
Group 2	0 (%0)	0 (%0)	6(%75,0)	2 (%25,0)	8 (%100)	G3\G2	P < 0.001
Group 3	5 (%71,4)	2 (%28,6)	0(%0)	0 (%0)	7 (%100)	G3\G4	P > 0.05
Group 4	6 (%87,5)	2 (%12,5)	0(%0)	0 (%0)	8 (%100)	G4\G2	P > 0.05
Total	11 (%45,8)	5 (%20,8)	6(%25)	2 (%8,4)	24 (%100)		

Comparison of the adhesion area scores of the groups with the Mann Whitney U test

Cecum tissue samples were evaluated according to Zühlke's microscopic adhesion score classification, and presented in Table-3. We found that the microscopic adhesion scores of Group-3 and 4 were significantly lower than Group-2 ($p<0.005$). In the comparison of Group-3 with Group-4, we found no statistically significant difference in microscopic adhesion score (Table-3).

Table 3. Microscopic adhesion scores of the groups

Groups	Microscopic adhesion scores				Total	Microscopic adhesion scores	
	1	2	3	4			
Group 2	0(%0)	7(%87,5)	1(%12,5)	0(%0)	8	G3\G2	P=0.003(p <0,05)
Group 3	3(%42,8)	4(%57,2)	0(%0)	0(%0)	7	G4\G2	P=0.001 (p <0,05)
Group 4	6(%75)	2(%25)	0(%0)	0(%0)	8	G3\G4	P=0.398 (p >0,05)
Total	9(%39,2)	13(%56,5)	1(%4,3)	0(%0)	23		

Comparison of the microscopic adhesion scores of the groups with the Mann Whitney U test

There were significant differences in histopathological findings between the groups. Group 2, 3 and 4 had thick granulation tissue, less cell, more vessels compared to Group 2 ($p<0.05$). Groups 3 and 4 had loose connective tissue, more cells, abundant fibrin compared to Group 2 ($p <0.05$). Angiogenesis was higher in group 2 compared to other groups. The difference between

Group 3 and Group 4 was not significant (p> 0.05), but average fibrosis and inflammation scores in the PRF group were lower than Group 3. Pathological scores and findings were consistent with adhesion scores (fig 2, fig 3).

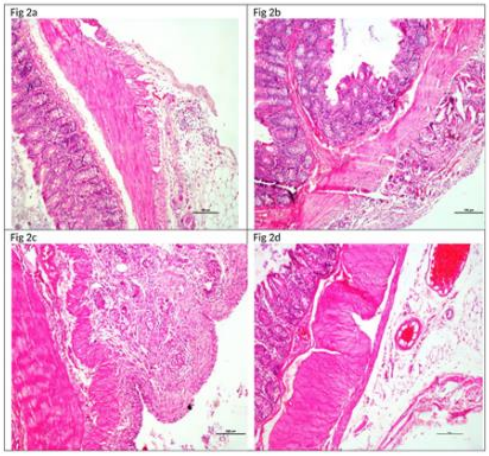


Figure 2: (Hematoxylin and Eosin) Figure 2a (grade 0)
Figure 2b (grade1) Weak connective tissue, rich cells, old and new fibrin, thin reticle fibrils Figure 2c (grade 2) Connective tissue with cells and capillary vessels, rare collagen fibers Figure 2d (grade 3) Thick connective tissue, rare cells, more vessels, rare elastin and smooth muscle fibers

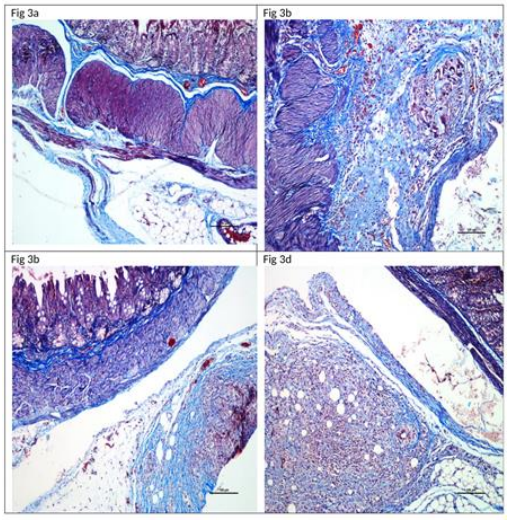
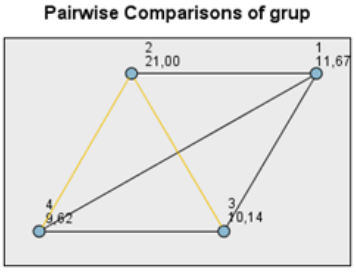


Figure 3: (Masson's trikrom) Figure 3a (grade 0) Figure 3b (grade1) Weak connective tissue, rich cells, old and new fibrin, thin reticle fibrils Figure 3c (grade 2) Connective tissue with cells and capillary vessels, rare collagen fibers Figure 3d (grade 3) Thick connective tissue, rare cells, more vessels, rare elastin and smooth muscle fibers

We found that the mean plasma PDGF, ICAM, IL6, TNF-α levels were significantly higher in Group 2. Plasma TNF-α was significantly higher in Group 2 than Group 4 (p <0.05). Plasma PDGF value was significantly higher in Group 2 compared to Group 3 and Group 4 (p<0.05). Plasma PDGF value in Group 3 was higher than Group 4. However, there was no statistically significant difference in plasma PDGF values between Group 3 and Group 4 (p = 0.896, p> 0.05). The statistical analysis of the plasma proinflammatory markers were presented in figure 4.



Each node shows the sample average rank of grup.

Sample 1-Sam...	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
4-3	,518	3,958	,131	,896	1,000
4-1	2,042	5,178	,394	,693	1,000
4-2	11,375	3,824	2,974	,003	,018
3-1	1,524	5,278	,289	,773	1,000
3-2	10,857	3,958	2,743	,006	,037
1-2	-9,333	5,178	-1,802	,071	,429

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is ,05.

Figure 4: Each node shows the sample average rank of grup.
Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same. Asymptotic significances (2-sided tests) are displayed. The significance level is, 05.

DISCUSSION

Intestinal adhesion is a common complication seen in postoperative surgery. It can lead to a number of adverse events such as intestinal obstruction, infertility, chronic abdominal pain and intestinal necrosis[16].

Various models have been developed to experimentally create peritoneal adhesion. These are abrasion, local peritoneal excision, ischemic damage, insertion of a foreign body into the peritoneal cavity, thermal damage and bacterial contamination[13]. In our study, we preferred the abrasion model as it mimics the physical trauma caused by laparotomies. Any manipulation performed by hand or surgical instruments during laparotomy is a mechanical trauma and it is known to be the most common cause of peritoneal adhesions[17]. Intestinal adhesion caused by injury to the cecum causes inflammation and fibrin formation. With intestinal serosa damage, large numbers of inflamed cells infiltrate the damaged tissue and thus amounts of mediators are secreted. They subsequently stimulate the proliferation of fibroblasts, secretion of collagen to form elastic fibers, which leads to adhesion. Trying to repair the damaged serosa also triggers adhesion formation[18]. A wide variety of treatment methods and therapeutic agents are available, especially to prevent postoperative intestinal adhesion[19]. Strategies to prevent adhesion are generally listed as minimally invasive surgical techniques, laparoscopic surgery and biomaterial-based methods[16,20].

Preparation protocol of PRP is simple and its cost is low. The fibrin matrix in PRF increases the efficiency of growth factors secreted from platelets[8]. PRF naturally contains fibrin for the clot scaffold and mesenchymal stem cells. PRF centrifugation is different from PRP, since it requires no anticoagulant, and also the rate of centrifugation is different. PRF releases platelet-related therapeutic granules for a longer time and at a slower rate than PRP. PRF has been first used in oral maxillofacial surgery[21]. Today, its application in both invasive and noninvasive aesthetic procedures is a significant achievement that indicates a bright future for surgical medicine.

PRF is a gel-like autologous biomaterial containing abundant GF within its three- dimensional fibrin structure. PRF was first used in dentistry as an inexpensive and easy-to-use method and stimulate tissue regeneration in oral surgery[22]. PRF is often preferred in plastic and reconstructive surgeries due to its beneficial effects in wound healing, maxillofacial surgery and even cosmetic surgery, as it contains high concentrations of cytokines and growth factors that promote proliferation, angiogenesis, differentiation, regeneration, and thus accelerate wound healing and tissue repair[23,24]. In our study, PRF decreased the frequency and severity of postoperative bowel adhesion. It increases natural regenerative processes. Previous studies have showed that for maintaining mesenchymal stem cells (MSC), fibrin is a successful culture for preserving the paracrine functions necessary to ensure regenerative effects [25]. PRF also protects other vital cells, including leukocytes. Analyzing the cellular content of the PRF clot shows that most of the leukocytes in the whole blood were present in the PRF after centrifugation[26]. Hence, PRF together with amounts of leukocytes, neutrophils and platelets reduces the risk of infection. Previous studies have investigated the regenerative effects of PRF. It is applied alone or in combination for the tissue trauma. Hence, it can promote tissue repair, accelerate cellular differentiation and proliferation[27]. Besides, PRF acts as a physical barrier to prevent intestinal adhesions. PRF prevents intestinal adhesion synergistically with these mechanisms.

MSC have important regenerative properties. Di Liddo et al detected in vitro multipotent stem cell markers in PRF and reported that some of the cells in the PRF defined the phenotypic properties of MSC[28]. Thus, PRF creates a local environment for stem cell source. Most importantly, it is suggested that the composition of PRF helps to prevent rapid proteolysis of growth factors, thus providing prolonged secretion [29]. The slow polymerization and remodeling of the fibrin matrix within the PRF effectively maintains growth factors and other critical cells. In our study, application of PRF to the surgical site effectively replaces the abundance of erythrocytes with fibrin, leukocytes, stem cells and platelet-derived growth factors. This accelerated wound healing and attracting MSC to the surgical area may have resulted in tissue regeneration and collagen remodeling.

PRF has the capacity to induce soft tissue regeneration. In addition, PRF is a reliable and cost-effective option to accelerate wound healing and increase the effectiveness of tissue repair after injury [30]. In our study, we found that PRF significantly reduced the severity of fibrosis and inflammation. We stated that PRF may reduce the proliferation of fibroblasts and local inflammation during wound healing. In response to PRF, plasma proinflammatory cytokines, in particular PDGF and TNF-α, were low, indicating that PRF provided tissue regeneration without increasing inflammation.

TNF is an important multifunctional proinflammatory cytokine and plays an important

role in homeostasis, host defense, inflammation and cancer. TNF is produced by macrophages, T and B cells, neutrophils, endothelial cells and fibroblasts. Prevention of infection is the most important task in the first days after injury and is achieved by the rapid (within a few hours after injury) production of pro-inflammatory cytokines, mainly IL-1 and TNF. This can lead to both activation of resident stromal and immune cells and removal of leukocytes, mostly neutrophils, from the circulation by subsequent activation of phagocytosis. TNF and IL-6 are the key proinflammatory cytokines involved in regeneration. However, excessive inflammation inhibits the regeneration process during this period. As a result, IL-1 and IFN that previously protected the from infection are now downregulated, while TNF and IL-6 are still expressed at higher levels [27]. IL-6 also serves as a signal by damaged tissues in non-infectious trauma (such as burns or post-surgical adhesion) to support the onset of the inflammatory response and facilitate the tissue repair. In the progress of adhesion-related inflammation, the expression of TNF-α and IL-6 increases [31,32]. In our study, we found that TNF-α and IL-6 were significantly higher in group 2, which indicates that inflammation was higher in group 2 compared to the other groups, and we found that PRF and olive oil in groups 3 and 4 prevented adhesion and decreased inflammation.

The other pro-inflammatory cytokine that we investigated in our study, PDGF, has a wide range of immunoregulatory functions. PDGF, first expressed from platelets, is a member of growth factors family. It performs cellular proliferation, chemotaxis and tissue remodelling in prenatal and postnatal period. In our study, we found that PDGF levels were significantly higher in group 2, indicating that inflammation was more in group 2. In our study, we also found that ICAM-1 level was the highest in group 2. ICAM-1 molecules initiate the migration of eosinophils, T lymphocytes and neutrophils.

ICAM-1, being an adhesion molecule and an important immunological parameter in the early stage of inflammation, has been used as an important marker in the early diagnosis of sepsis. It was first demonstrated by Seth et al in 1991 via serum immunoblotting. It is expressed by vascular endothelial cells and leukocytes. ICAM-1 levels are increased in inflammation, infection and cancer. Our results showed that ICAM-1 level was the highest in group 2, suggesting PRF and olive oil in groups 3 and 4 reduced inflammation and, thus, prevented the formation of adhesion [33].

In our study, we also used pure olive oil to prevent adhesions. There were two reasons in our preference for pure olive oil. First, the phenolic components in pure olive oil have been shown to represent beneficial effects due to their antiatherogenic, antioxidant, antineoplastic and anti-inflammatory potential. Second, it has a hydroflotation effect since it is a liquid with high viscosity. The anti-inflammatory effect of olive oil depends on its major components, erythrodiol, beta sitosterol, squalene and its minor phenolic components: oleuropein, tyrosol, hydroxytyrosol and caffeic acid [34].

The phenolic components in virgin olive oil had been stated to show anti-inflammatory effect by reducing thromboxane B2, leukotriene B4, COX1 and COX2 activity[11]. In our previous studies, we have also found olive oil showed similar effects after 21 days. this indicated that olive oil remains in the peritoneum without being absorbed and continues its hydroflotation effect.

In our present study, adhesion formation was significantly low in group 3, which received olive oil, compared to group 2. The scoring of adhesion was also lower in Group 4, which received PRF, compared to group 3 macroscopically. However, there was no statistically significant difference between group 3 and group 4. Therefore, one may conclude that PRF is not only a useful and safe therapeutic agent but also it is more effective than olive oil in terms preventing adhesions. The small number of rats in the groups are the major drawback of this study

In conclusion, PRF is a cost effective and reliable tool since it reduces intestinal adhesion by promoting the proliferation of mesothelial cells and inhibiting fibroblast infiltration. Thanks to the promising results obtained in this study, now further researches on animal models and clinical studies should be conducted to better understand the anti-adhesive and regenerative properties of PRF as well as to establish proper anti-adhesion prophylaxis algorithms.

Yazar Katkıları: Çalışma konsepti/Tasarımı: DAU, DAA, MS; Veri toplama: DAU, AGG, AEK; Veri analizi ve yorumlama: DAU, DAA; Yazı taslağı: DAU, DAA; İçeriğin eleştirel incelenmesi: DAU, DAA, AEK; Son onay ve sorumluluk: DAU, DAA, MS, AYB, AEK, AGG; Teknik ve malzeme desteği: MS, AYB, DAU; Süpervizyon: AEK, MS; Fon sağlama (mevcut ise): yok.
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REFERENCES

1. Ten Broek RP, Strik C, Issa Y, Bleichrodt RP, van Goor H. Adhesiolysis-related morbidity in abdominal surgery. .Ann Surg. 2013.258:98-106.
2. Cai T, Qiu J, Ji Y, Li W, Ding Z, Suo C et al. IL-17-producing ST2+ group 2 innate lymphoid cells play a pathogenic role in lung inflammation. J Allergy Clin Immunol. 2019.143:229-44.
3. Kırdak T, Uysal E, Korun N. Karın içi yapışıklıkların önlenmesinde metilprednizolonun farklı dozlarının etkinliğinin incelenmesi. Ulus Travma Acil Cerrahi Derg. 2008; 14:188-91.
4. Khan J, Chandler WL. Discordant partial thromboplastin time (PTT) vs Anti-Xa heparin activity. Am J Clin Pathol. 2019;151:424-32.
5. Dohan Ehrenfest DM, de Peppo GM, Doglioli P, Sammartino G. 25. Slowrelease of growth factors and thrombospondin-1 in Choukroun'splatelet-rich fibrin (PRF): A gold standard to achieve for all surgical platelet concentrates technologies. Growth Factors. 2009;27:63-9.
6. Yu P, Zhai Z, Jin X,Yang X, Qi Z. Clinical application of platelet-rich fibrin in plastic and reconstructive s urgery: A systematic review. Aesth Plast Surg. 2018;42:511-19.
7. Kaya F, Kismet K, Ozer H, V G Soylu, M E Duymus, Y A Akgun et al. Can platelet-rich plasma be used safely in intra-abdominal operations? Bratislavske Lekarske Listy. 2016; 117:525-29.
8. Altuntaş Z, Gündeşlioğlu AÖ, İnce B, Dadacı M, Savacı N. Platelet rıch plasma (Prp), platelet poor plasma (Ppp), platelet rıch fıbrıne (Prf) concepts, Theır bıologıcal roles on woung healıng and clınıcal applıcatıons in plastıc surgery. Turk Plast Surg 2014;22:49-53.
9. Shoshani O, Livne E, Armoni M, Shupak A, Berger J, Ramon Y et al. The effect of interleukin-8 on theviability of injectedadiposetissue in nude mice. Plast Reconstr Surg. 2005;115:853-9.
10. De la Puerta R., Martinez Dominguez E, Ruiz-Gutierrez V. Effect of minör components of virgin olive oil on topical anti-inflammatory assays.Z. Naturforsch. 2000;55:814-19.
11. Cicerale S, Lucas L, Keast R. Biological activities of phenolic compounds present in virgin olive oil. Int JMol Sci. 2010;11:458-79.
12. Tutar B, Ekincioglu E, Karaketir S, Berkiten G, Saltürk Z, Arkan ME et al. The impact of platelet-rich fibrin (PRF) on olfactory function and pain after septoplasty operations. Eur Arch Otorhinolaryngol. 2020;277:1115-20.
13. Ural DA, Sarıhan H, Saygın İ, Aykan DA, Ural A, İmamoglu M. Long-term outcomes of pure olive oil to prevent postoperative peritoneal adhesions in rats. J Surg Med. 2019; 3:218-22.
14. Aslan GI, Otgun I, Acer T, Tepeoglu M, Hicsonmez A. The effect of intraperitoneal n- acetylcysteine on postoperative adhesions in rat models. Ann Ital Chir. 2017;88:258-62.
15. Kurt N, Sıkar HE, Kaptanoğlu L, Küçük HF. Separate and synergistic effects of taurolidine and icodextrin in intra-abdominal adhesion prevention. Ulus Travma Acil Cerrahi Derg. 2017;23:377-82.
16. Charboneau AJ, Delaney JP, Beilman G. Fucoidans inhibit the formation of post-operative abdominal adhesions in a rat model. PLoS One. 2018;13:e0207797.
17. Nagae M, Ikeda T, Mikami Y, Hase H, Ozawa H, Matsuda K et al. Intervertebral disc regeneration using platelet-rich plasma and biodegradable gelatin hydrogel microspheres. Tissue Eng. 2007;13:147-58.
18. Hinoki A, Saito A, Kinoshita M, Yamamoto J, Saitoh D, Takeoka S. Polylactic acid nanosheets in prevention of postoperative intestinal adhesion and their effects on bacterial propagation in an experimental model. Br J Surg. 2016;103:692-700.
19. Montalvo-Javé EE, Mendoza-Barrera GE, García-Pineda MA, Jaime Limón ÁR, Montalvo- Arenas C, Castell Rodrıguez et al. Histological analysis of intra-abdominal adhesions treated with sodium hyaluronate and carboxymethylcellulose gel. J Invest Surg. 2016; 29:80-7.
20. Hu Z, Su Y, Xie P, Chen L, Ji J, Feng T et al. OCT angiography-based monitoring of neovascular regression on fibrovascular membrane after preoperative intravitreal conbercept injection. Graefes Arch Clin Exp Ophthalmol. 2019; 257:1611-19.
21. Garg AK. Autologous Blood Concentrates. Batavia (IL), Quintessence Publishing, 2018.
22. Yoshimura K, Sato K, Aoi N, Kurita M, Hirohi T, Harii K. Cell- assisted lipotransfer for cosmetic breast augmentation: supportiveuse of adipose- derived stem/stromal cells. AesthetPlastSurg. 2008. 32:48-55.
23. Miron VE. Microglia-driven regulation of oligodendrocyte lineage cells, myelination, and remyelination. J Leukoc Biol. 2017;101:1103-8.
24. Bilgen F, Ural A, Bekerecioglu M. Platelet-rich fibrin: An effective chronic wound healing accelerator. J Tissue Viability. 2021;30:616-20.
25. Kim I, Lee SK, Yoon JI, Da Eun Kim, Mihyung Kim, Hunjoo Ha et al. Fibrin glue improves the therapeutic

effect of MSCs by sustaining survival and paracrine function. Tissue Eng Part A. 2013;19:2373-81.
26. Ponte AL, Marais E, Gallay N, Langonne A, Delorme B, Herault O et al. The in vitro migration capacity of human bone marrow mesenchymal stem cells: comparison of chemokine and growth factor chemotactic activities. Stem Cells. 2007;25:1737-45.
27. Turabelidze A, Dipietro LA. Inflammation and wound healing. Endod. Top. 2012;24:26-38.
28. Di Liddo R, Bertalot T, Borean A, Pirola I, Argentoni A, Schrenk S et al. Leucocyte and platelet-rich fibrin: a carrier of autologous multipotent cells for regenerative medicine. J Cell Mol Med 2018;22:1840-54.
29. Lundquist R, Dziegiel MH, Agren MS. Bioactivity and stability of endogenous fibrogenic factors in platelet-rich fibrin. Wound Repair Regen. 2008;16:356-63.
30. Ding Y, Cui L, Zhao Q, Zhang W, Sun H, Zheng L. Platelet-rich fibrin accelerates skin wound healing in diabetic mice. Ann Plast Surg. 2017;79:e15-9.
31. Metzger CE, Narayanan A, Zawieja DC, Bloomfield SA. Inflammatory bowel disease in a rodent model alters osteocyteprotein levels controlling bone turnover. J Bone Miner Res. 2017;32:802-13.
32. 32- Wang J, Le K, Guo X, Yan F, Guo Y, Zhang T et al. Platelet-rich fibrin prevents postoperative intestinal adhesion. J Biomed Mater Res A. 2020;108:1077-85.
33. Ergüler G., Demir N., Demir R., Structural properties and functions of adhesion molecules. Turkiye Klinikleri J Med Sci. 2002;22:313-27.
34. De la Puerta R, Martinez Dominguez E, Ruiz-Gutierrez V. Effect of minor components of virgin olive oil on topical antiinflammatory assays. Z. Naturforsch. 2000;55:814-9.