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ORIGINAL ARTICLE – OPEN ACCESS

Effects of Triclosan-Impregnated Suture Materials on Colonic Anastomosis

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ABSTRACT

Objective: Symptomatic anastomotic leakage observed after colorectal surgery is one of the major complications. One of the factors affecting the anastomosis healing is the type of suture material used. This study aims to investigate the effects of the suture material polydioxanone (PDS, Ethicon), which is late absorbable and has a monofilament structure, and the suture material polyglactin 910 (Vicryl, Ethicon), which is absorbable and has a multifilament structure, on the healing of colonic anastomosis, and to compare the traditional forms of these sutures with their antibacterial effective triclosan-impregnated forms (PDS Plus, Ethicon and Vicryl Plus, Ethicon).

Materials and Methods: The rats were divided into four equal groups consisting of 10 subjects each: Group I: Vicryl; Group II: Vicryl Plus; Group III: PDS; and Group IV: PDS Plus. The presence of wound infection, whether the integrity of the abdominal wall was maintained, intra-abdominal adhesion scoring, the presence of intra-abdominal abscess, and whether the macroscopic integrity of anastomosis was maintained were evaluated.

Results: According to the results of this experimental study, while the highest ABP and hydroxyproline levels were observed in the PDS Plus group, the lowest values were observed in the Vicryl group. However, the difference was not statistically significant.

Conclusion: Slowly absorbable and monofilament PDS suture material causes less tissue reaction and inflammatory response compared to the Vicryl suture material that is absorbable in the colonic anastomosis line and multifilament.

Keywords: Polydioxanone, polyglactin 910, triclosan, colonic anastomosis, hydroxyproline

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INTRODUCTION

In a symptomatic anastomosis leak following colorectal surgery and intra-abdominal adhesion, morbidity and mortality are the main complications. Anastomotic leakage still poses a severe problem for surgeons. In the literature, while the anastomotic leakage rate determined clinically after elective colon surgery is 3%–4% (1–3), this rate is reported to be 11%–12% in rectal anastomosis (4, 5). The average rate of anastomotic leakage in colorectal surgery is 6.4% (6). Although wound healing in the gastrointestinal system (GIS) anastomosis resembles wound healing in the skin and contains the same stages, it also has some differences. Among these differences, the content of the GIS and the bacterial flora it has may affect anastomosis healing. While the mucosa from the layers of this tubular structure serves as a barrier for luminal bacteria, the submucosa is the most important layer in anastomosis healing due to the collagen it contains and vascular structures (7).

There are many local and systemic factors affecting anastomosis healing. One of these factors is the suture materials and the surgical technique used when performing anastomosis. In the early period (the first 2 days), the safety of anastomosis is ensured by the use of sutures or staplers. Since the suture materials used are perceived as foreign bodies, they cause an inflammatory reaction in the anastomosis area. The tissue reaction and inflammatory response developed due to sutures give rise to two significant results. First, the excessive inflammatory response reduces the durability of the tissue. This decrease also reduces the suture retention strength of the tissue. Second, there is evidence that infection is observed more frequently due to suture materials that cause an excessive tissue reaction (8). One of the most critical factors in the development of the surgical site infection (SSI) is bacterial colonization in the suture material. This colonization is observed especially in braided suture materials and around the knot (9). Microorganisms colonized in the suture pass from the tissue to the surgical site and form a biofilm. The formed biofilm layer develops a defense against antimicrobial agents and the immune system (10). Nowadays, polydioxanone and polyglactin 910 are among the most preferred suture materials for GIS anastomoses (11). The most recently published review article that evaluated the colorectal anastomosis technique demonstrated that absorbable sutures cause more tissue reaction and inflammatory response compared to nonabsorbable or slowly absorbable sutures. Furthermore, it was emphasized that multifilament (braided) sutures create more tissue damage than monofilament sutures and that bacteria stick to multifilament sutures more easily, and as a result, a

Table 1. Groups and their hydroxyproline levels and bursting pressure mmHg values and intra-abdominal adhesion scores

Groups	Bursting pressure		Hydroxyproline levels		Intra-abdominal Adhesion score	
	$\bar{X}$±SD	Med.±IQR	$\bar{X}$±SD	Med.±IQR	$\bar{X}$±SD	Med.±IQR
Group I Vicryl®	154.13±14.18	154.00±15.00	14.86±2.74	15.84±4.68	2.2±0.63[a]	2.00±2.00
Group II Vicryl Plus®	160.00±11.91	149.00±23.00	15.32±3.28	14.02±6.60	1.9±0.74	1.00±1.00
Group III PDS®	169.22±20.26	156.00±29.00	16.37±3.13	17.49±5.48	1.9±0.57[b]	2.00±1.00
Group IV PDS Plus®	179.78±19.58	175.00±17.00	16.43±2.93	16.20±3.77	1.1±0.74[c]	2.00±0.00
p	0.068		0.454		0.014	

a: Significance of the difference between Vicryl® and Vicryl Plus® groups *p=0.035; b: Significance of the difference between Vicryl® and PDS® groups *p=0.004; c: Significance of the difference between Vicryl® and PDS Plus® groups *p=0.018; SD: Standard deviation; Med.: Median; IQR: Interquartile range

biofilm layer is formed (11). To prevent SSI, the use of triclosan-impregnated or coated suture materials with antibacterial activity has been introduced. Triclosan (5-chloro-2-[2,4-dichlorophenoxy] phenol) has been widely used in human life as a broad-spectrum antiseptic for more than 30 years (12). Triclosan is an antibacterial and antifungal agent used in the production of various chemicals. It displays a bacteriostatic effect by inhibiting the fatty acid synthesis in bacteria (13). Triclosan-impregnated suture materials have been experimentally proved to affect *Staphylococcus (S.) aureus*, methicillin-resistant *S. aureus*, *S. epidermidis*, methicillin-resistant *S. epidermidis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, and *Klebsiella pneumonia* (14). This study aims to investigate the effects of the suture material polydioxanone, which is late absorbable and has a monofilament structure, and the suture material polyglactin 910, which is absorbable and has a multifilament structure, on the healing of colonic anastomosis, and to compare the traditional forms of these sutures with their triclosan-impregnated forms.

MATERIALS and METHODS

This experimental study was carried out at the Cumhuriyet University, Faculty of Medicine Experimental Animals Laboratory, Biochemistry Laboratory, and Veterinary Faculty Pathology Laboratory. To carry out this study, a permission was received from the Sivas Cumhuriyet University Faculty of Medicine Ethics Committee (B.30.2.CUM.0.01.00.00-50/60). Adult male Wistar albino rats, weighing 295–330 g (mean 312.625 g) and nourished under normal conditions at the Sivas Cumhuriyet University Experimental Animal Laboratory, were used. Rats were formed into four groups of 10 animals each (Table 1). After administering anesthesia by applying 5 mg/kg xylazine (Rhompun, Abdi Ibrahim, Istanbul) and 40 mg/kg ketamine hydrochloride (Ketalar, Eczacıbaşı, Istanbul), the surgical procedure was performed in the rats. During the surgical procedure, there was spontaneous respiration in all rats. Laparotomy was performed with a midline incision. The body temperature was kept between 36°C and 38°C. The rats were injected with 5 ml of Ringer's lactate solution to prevent the development of dehydration during the procedure.

Surgical Procedure

Laparotomy was performed with a midline incision. A 1-cm-long colon segment was resected by finding the left descending colon over 2–4 cm of the pelvic reflection. The proximal and distal colon were cleaned using the milking method. Anastomosis was performed again with suture materials (5/0) indicated in the groups, one by one and as a monolayer (eight sutures on average). Each subject's fascia and skin incision were closed with the anastomosis suture material.

Table 2. Nair's macroscopic adhesion classification in rats

Score	Description
1	Adherence
2	Single adherence between two organs or between an organ and the abdominal wall
3	Two adherences between organs or one organ and the abdominal wall
4	More than adherences between organs or a massive generalized adherence of the intestine with no adherence to the abdominal wall
5	Generalized adherences between organs and the abdominal wall or massive adherence among all organs

Analytical Procedures

Measurement of Anastomosis Bursting Pressure

A pressure transducer for the bursting pressure measurement (Transpac IV. Abbott, USA) and a monitor (Petaş KMA 800, Ankara, Turkey) were procured. A 2F feeding catheter was forwarded rectally. The colon was tied up with 2/0 silk from 2 cm below the anastomosis to include the catheter. The proximal part of the anastomosis was closed with a clamp, and a closed system was formed.

From the catheter placed in the rectum, the liquid was injected into the colon at a speed of 50 ml/hour by a perfusion pump, and the pressure was followed on the monitor. The value before the sudden decrease in pressure was recorded as the anastomosis bursting pressure.

Biochemical Evaluation

After the bursting pressure was measured, the colon was resected to include 2 cm distally and 2 cm proximally of the anastomosis. The resected colon segment was opened along its long axis, one

half of its segment was washed with bidistilled water, dried with drying paper, weighed, and divided into small pieces. By placing them into open beakers, they were dried in an oven for 72 hours at 100°C. The dried samples were then hydrolyzed in 6 M HCl for 18 hours at 110°C. The samples were washed with distilled water 3 times. The samples, from which acid was removed were dissolved again in 2 ml buffer (1.2% acetic acid, 12% sodium acetate, 5% citric acid, 3.4% sodium hydroxide, pH=6). Then, 0.5 ml chloramine-T was added to the 1 ml sample, and the samples were incubated at room temperature for 20 min. The 15.6% perchloric acid + 15.4% (dimethylamino) benzaldehyde mixture dissolved in 0.5 ml propanol was added. After incubating at 60°C for 15 min, its absorbance was read against blind at 550 nm in the spectrophotometer. The µg/mg tissue results were calculated by benefiting from the standard curve drawn using L-hydroxyproline (15).

Histopathological Evaluation

The resected colon segment was opened along its long axis, and one half of its segment was placed into 10% formaldehyde. After 24 hours of fixing, the segment was embedded in paraffin. Sections 4 microns thick were prepared from these tissues and stained with Hematoxylin–Eosin (H&E) dye. In routine H&E sections, inflammatory cell infiltration (polymorphonuclear leukocyte [PMNL], mononuclear cell [MNH], edema, neovascularization, collagen fiber, and fibroblast density were evaluated using the modified Ehrlich and Hunt scale (16).

Intra-abdominal Adhesions Evaluation

The adhesion formation was evaluated according to the Nair adhesion scoring system (Table 2) by a surgeon blinded to the study groups (17).

Statistical Analysis

The SPSS (Statistical Package for Social Sciences,. 15.0) program was used to evaluate the study data. The Kruskal–Wallis test was conducted to determine whether there was a difference between the groups' homogeneity of variance, while the Man–Whitney U test was used to compare the group differences with each other. A p-value <0.05 was considered as statistically significant.

RESULTS

Skin infection was detected in 1 rat in each group. In the groups that were sacrificed on the 7th postoperative day, there was partial anastomotic separation in 2 rats in the Vicryl group and 1 rat in each of the other groups. An intra-abdominal abscess was found in rats with anastomotic separation. In the statistical evaluation, there was no difference in terms of the anastomotic separation and abscess.

Anastomosis Bursting Pressures

Although the highest bursting pressure values were found in the PDS Plus group and then in the Vicryl Plus® group, no statistically significant difference was determined between the groups in terms of bursting pressures (Table 1) (p>0.005).

Biochemical Findings

The obtained hydroxyproline (OH-Proline) values were presented as microgram (µg/mg colon tissue) per milligram of the tissue. Tissue hydroxyproline values of the groups were presented as X±s-

Table 3. X±Standard deviation values of the histopathological parameters

Groups	PMNL*		MNH#		Edema§		Neovas.€		Coll. Dens.		Fib. Dens.	
	X̄±SD	Med.±IQR	X̄±SD	Med.±IQR	X̄±SD	Med.±IQR	X̄±SD	Med.±IQR	X̄±SD	Med.±IQR	X̄±SD	Med.±IQR
Vicryl®	2.17±0.41[a]	2.00±0.00	3.67±0.52	4.00±1.00	3.67±0.52[a]	2.00±0.00	2.17±0.41	3.00±0.00	2.67±0.52	3.00±1.00	2.33±0.52	3.00±0.00
Vicryl Plus®	1.43±0.53	1.00±0.00	2.67±1.03	3.00±1.00	2.17±0.75	4.00±1.00	2.33±0.52	2.00±0.00	2.83±0.41	3.00±0.00	2.83±0.75	3.00±0.00
PDS®	1.17±0.41[b]	1.00±1.00	2.33±0.52[b]	2.00±1.00	2.17±0.41[b]	2.00±1.00	2.67±0.52	3.00±1.00	3.17±0.41	3.00±0.00	3.00±0.63	2.00±1.00
PDS Plus®	0.83±0.75[c]	1.00±1.00	2.17±0.75[c]	2.00±1.00	2.00±0.00[c]	2.00±0.00	3.00±0.00[c]	2.00±1.00	3.33±0.52	3.00±1.00	3.00±0.63	3.00±0.0
p	0.009		0.016		0.002		0.023		0.094		0.227	

PMNL: Polymorphonuclear leukocyte; MNH: Mononuclear cell; Neovas.: Neovascularization; Coll. Dens: Collagen density; Fib. Dens.: Fibroblast density; a: Significance of the difference between Vicryl® and Vicryl Plus® groups *p=0.018, §: p=0.007; b: Significance of the difference between Vicryl® and PDS® groups *p=0.006; #: p=0.007; §: p=0.004; c: Significance of the difference between Vicryl® and PDS Plus® groups *p=0.007; #: p=0.007; §: p=0.002; ¤0 p=0.005; SD: Standard deviation; Med.: Median; IQR: Interquartile range

tandard deviation (SD) in Table 1. The highest OH-proline level was determined in the PDS Plus group, and the lowest level was determined in the Vicryl group. However, the difference between the groups was not statistically significant (Table 1) (p>0.05).

Histopathological Findings

In routine H&E sections, inflammatory cell infiltration (PMNL, MND), edema, neovascularization, collagen fiber, and fibroblast density were evaluated by using the modified Ehrlich and Hunt scale. Each parameter was scored within itself. The X±SD values of histopathological parameters were presented in Table 3. While the PMNL cell infiltration was statistically significantly higher in the Vicryl® group compared to the Vicryl Plus®, PDS®, and PDS Plus® groups, the MNH cell infiltration was statistically significantly higher in the Vicryl® group compared to the PDS® and PDS Plus® groups. Edema was found to be significantly more evident in the Vicryl® group compared to the Vicryl Plus® PDS® and PDS Plus® groups. Neovascularization was found to be statistically significantly higher in the PDS Plus® group compared to the Vicryl® group. Although the highest levels of collagen fiber and fibroblast density were observed in the PDS Plus® group and the lowest values were observed in the Vicryl® group, no statistically significant difference was determined between the groups in terms of these parameters.

Findings Regarding Intra-abdominal Adhesion

Intra-abdominal adhesion was evaluated using Nair's classification (17). The X±SD values of intra-abdominal adhesion scores are presented in Table 1. Intra-abdominal adhesion was determined to be significantly higher in the Vicryl group compared to the PDS®, PDS Plus®, and Vicryl Plus® groups.

DISCUSSION

Colonic anastomotic leakage is a potentially destructive complication. It has been observed that the clinically evident anastomotic separation increases perioperative mortality 3 times and the duration of hospitalization 2 times (18). Anastomosis wound healing displays similarities with the wound healing phases in other parts of the body. Wound healing begins with hemostasis (coagulation) provided by the fibrin plug formed by platelets after injury. Then, as a result of increased vascular permeability, inflammatory cells enter the injury area and initiate the inflammation phase. The first cells are neutrophils, and they are dominant at the beginning of this phase. The task of neutrophils is to clean the wound from the invading microorganisms (7).

As a result of proteinases, which are thought to be secreted by neutrophils, collagenolysis occurs at the early stages of anastomotic healing (19). Therefore, the power of anastomosis also naturally decreases (20). During this period, the safety of anastomosis is ensured by the suture or staplers used (8). The loss of power in anastomosis increases as a result of the prolongation or excessive development of the inflammatory phase. Therefore, the fact that the suture materials used in anastomosis cause the least tissue reaction, and inflammation gains importance in terms of anastomosis safety. One of the most critical factors that delay the healing of colonic anastomosis is the development of local sepsis in the anastomosis line (21).

In all intestinal anastomoses, including colorectal anastomoses, local infection may develop in the anastomosis line due to the contamination of intraluminal bacteria. Multifilament sutures cause more tissue damage in comparison with monofilament sutures, and bacteria adhere more easily to the cracks of multifilament sutures. Such a situation establishes a ground for infection (9, 22–24). In addition, multifilament suture's exposure to bacterial contamination may cause microabscesses and leakage in the anastomosis line (25). Dissolution (absorption) of braided absorbable suture materials used in anastomosis in a short time may cause weakening of the anastomosis tensile strength (22, 26). In addition, Dragovic M. et al. showed that the polypropylene suture material showed a lower bacterial adherence and superior clinical features compared to silk (27). In summary, according to the results of two review articles published in 1990 and 2013, absorbable and multifilament suture materials cause more tissue reaction, less anastomosis tensile strength, and more bacterial colonization in the suture compared to monofilament suture materials that cannot be absorbed or can be absorbed slowly (8, 11). Therefore, in the latest published review article, the authors recommend the use of monofilament suture, which cannot be absorbed, or can be absorbed slowly in colorectal anastomoses. Interestingly, studies carried out with PDS® are quite limited (24, 25).

In an experimental study comparing two synthetic late-absorbable monofilament (Maxon® and PDS®) suture materials, the effects of these sutures on the musculoaponeurotic incision, gastrostomy, and colon anastomosis were investigated, and similar results were obtained (23).

In this study, first the absorbable multifilament Vicryl® and slowly absorbable monofilament PDS® sutures and then Vicryl Plus® and PDS Plus® sutures, which are triclosan-impregnated forms of these sutures that have antibacterial activity, were compared. In the present study, no significant difference was determined between Vicryl and PDS in terms of anastomotic bursting pressure values and tissue OH-proline levels. However, inflammatory cell infiltration (PMNL, MND) in the anastomosis line and edema were significantly higher in the Vicryl® group than in the PDS® group. These results indicate that Vicryl® (absorbable multifilament) causes more tissue reaction and inflammatory response in the anastomosis line, as mentioned in the literature. Triclosan impregnation to these sutures further improves the results. In the present study, upon comparing all the groups, the highest bursting pressure and the OH-proline level were found in the PDS Plus® group, and the lowest values were observed in the Vicryl® group. However, there was no significant difference between the groups in terms of these parameters. In the histopathological evaluation, while the least inflammatory cell infiltration and edema were observed in the PDS Plus® group, they were the highest in the Vicryl® group. A significant difference was detected in terms of these parameters. The highest neovascularization, collagen, and fibroblast density were detected in the PDS Plus® group, while they were the lowest in the Vicryl® group. While there was a significant difference regarding neovascularization parameters, no significant difference was detected in terms of collagen and fibroblast density (26).

SSI is the most common cause of nosocomial infections in surgical patients, and it increases medical expenses by prolonging hospital stay. SSI, which is observed after colorectal surgery, often causes morbidity, and its incidence is above 20% (28). In a randomized controlled clinical study conducted on patients who underwent

elective colorectal surgery, the closure of the incision with triclosan-impregnated suture materials has been demonstrated to ensure reducing wound site infections and the cost (29). According to the results of the last published systemic review and meta-analysis including 17 randomized clinical studies, triclosan-impregnated suture materials have been demonstrated to be significantly effective in protection from infections after surgery (30). Postoperative intra-abdominal adhesions continue to be a severe medical problem. Extension of inflammation is a serious factor in the formation of adhesions. Absorbable and multifilament sutures have been shown to cause more tissue reaction and inflammation (8, 11).

CONCLUSION

In the present study, the weakest intra-abdominal adhesion was observed in the PDS Plus® group, while the strongest adhesion was observed in the Vicryl® group. The adhesion score in the Vicryl® group was significantly higher compared to the Vicryl Plus®, PDS®, and PDS Plus® groups. There was no statistically significant difference between the Vicryl Plus®, PDS®, and PDS Plus® groups. As a result, the slowly absorbable and monofilament PDS® suture material causes less tissue reaction and inflammatory response in the colonic anastomosis line compared to the absorbable and multifilament Vicryl® suture material. At the same time, impregnation of triclosan with antibacterial activity to these sutures further improves the results.

Ethics Committee Approval: To carry out this study, a permission was received from the Sivas Cumhuriyet University Faculty of Medicine Ethics Committee (date: 01.07.2010, number: B.30.2.CUM.0.01.00.00-50/60).

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Conflict of Interest: The authors have no conflict of interest to declare.

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