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Morphogenesis Characteristics of Experimental Intestinal Anastomoses Made by Microsurgical Technique

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Abstract

This article presents the results of morphologic examination of intestinal anastomoses (IAs) formed by a precision technique in an experiment. Processes of regeneration in the zone of bowel anastomoses made with the author's precision one-row continuous suture (POCS) (Ukraine patent UA 32940), the author's precision one-row interrupted suture (POIS) (Ukraine patent UA 119073) and a double-row Albert—Schmieden suture (DASS) on rabbits of butterfly breed (n=45) are reviewed. Histological examination on Days 1, 3, 5, 14 and 30 revealed differences in reparative processes depending on the type of anastomoses. The morphological pattern in the early term (Day 3) of reparation is caused by the predominance of lysis of collagen fibers; nevertheless, in the early term the quantitative evaluation of healing stages was higher in the two groups of precision anastomoses than in the DASS group (2.00±0.00 points vs. 1.33±0.33 points). Formation of granulation tissue and collagen started on Days 5-7 in the two groups of precision anastomoses versus Days 7-14 in the DASS group, and in the two groups the process reached Stage 5 of reparation (3.00±0.00 points), while in DASS group it reached only Stage 4 (1.67±0.33 points). There were differences in the quantitative evaluation of staging on Day 30. In Group 1 (POCS), it was 5.00±0.00 points in average while in Group 2 (POIS) it was insignificantly lower (4.67±0.33 points). Differences between healing stage criteria were statistically significant in the precision technique sutures versus DASS ($P<0.05$). We did not find a statistically significant difference in the healing process between the two types of anastomoses formed by the precision technique. In contrast to DASS, the precision technique sutures do not deform a bowel lumen. Thus, the use of microsurgical techniques is the preferred method for forming IA. (**International Journal of Biomedicine. 2019;9(3):242-246.**)

Key Words: intestinal sutures • intestinal anastomosis • precision technique • wound healing

Abbreviations

CFST, coarse fibrous scar tissue; **DASS**, double-row Albert—Schmieden suture; **IA**, intestinal anastomosis; **POCS**, precision one-row continuous suture; **POIS**, precision one-row interrupted suture.

Introduction

Currently, more than 300 techniques and methods for the application of intestinal anastomoses (IAs) are used in various surgical clinics. Their common goal is to provide a flexible, wide and functional IA that is not at risk of seam divergence, infection, and obstruction. Unfortunately, despite such a wide range of types and methods for forming anastomoses between different sections of the gastrointestinal tract, a flawless method of intestinal stitching and a perfect intestinal suture have not yet been proposed, and the failure of IA still remains an important problem of abdominal surgery and shows no significant tendency to decrease. The question of the logical choice of intestinal suture technique remains unresolved. Therefore, there continues to be a debatable question about the method for forming IA, and currently there is no consensus on the number of IA rows.[1,2]

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According to Cochrane, there are no advantages between different types of IA connected by suture elements.[3,4] Despite the fact that precision superimposed atraumatic sutures and symmetrical matching of layers of the intestinal wall reduced the incidence of IA failure in individual observation series, it is impossible to completely get rid of this terrible complication[3,5-7] because of the technical nature of the connection with suture elements or seamless methods that are not enough or too destructive for the intestinal wall.[8] At the same time, most researchers believe that reducing the number of complications can be achieved by improving the technique of IA formation and improving the quality of matching the same-name layers of the walls of hollow organs using new methods of intestinal sutures.[9,10] In this regard, it becomes obvious that there is a need for in-depth study (including histological and histochemical examinations) of patterns of regenerative morphogenesis with various methods of intestinal suture.[11]

Objective: to study the dynamics of reparative processes in the healing of IA formed in the experiment using microsurgical techniques.

Materials and Methods

The experiment was performed on 45 rabbits of butterfly breed, of both sexes, weighing 3-4 kg. Each animal was assigned an individual number. All interventions and the slaughter of animals were carried out in compliance with the requirements of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (ETS 123) (Strasbourg, 1986) and the Resolution of the First Ukrainian National Congress on bioethics (Kiev, 2001).

The animals were divided into 3 equal groups. In all series of experiments, small-bowel IAs were formed. In Group 1 (n=15), IA was formed using POCS with a Vicryl thread (Ethicon, USA) with a diameter of 0.070-0.099 mm (numbered 6/0), according to the author's method:[12] A continuous Vicryl thread, the first turn sewn on submucosal layers on both sides, then on the musculo-submucosal layers - the next turn, then again only the submucosal layers, etc. circularly. In the same time period, the mucosa and serous membranes are not stitched and are not captured into the suture. Thus, an accurate match of the layers of the stitched parts of the gastrointestinal tract is made and an adequate blood supply to the anastomosis zone is maintained.

In Group 2 (n=15), IA was formed using POIS with a Vicryl thread (Ethicon, USA) with a diameter of 0.070-0.099 mm (numbered 6/0), according to the author's method.[13] The layers are sutured as in the first method and an adequate blood supply to the anastomosis zone is also maintained.

In Group 3 (n=15), DASS was used, which, due to ease of development and speed of application, was most prevalent among a wide variety of methods of applying multi-row anastomoses.

The animals fasted for 12 hours before surgery. Anesthesia was administered intravenously—a 2% xylazine hydrochloride solution (INTERCHEMIE HOLLAND) at a dosage of 0.10 ml/kg + a 5% solution of ketamine at a dosage of 6-10 mg/kg body weight. In the postoperative period, daily clinical monitoring of the state of the animal and monitoring of the laparotomy wound was carried out; no treatment was given. From the first day after the operation, all the animals were active, fed independently, recovered the next day after the operation, and were removed from the experiment as scheduled on Days 1, 3, 5, 14 and 30 after the operation. A macroscopic assessment and histopathological study of intestinal sections with anastomoses, formed with the help of precision technology and DASS, was undertaken. The preparations were stained with hematoxylin and eosin, as well as Mallory, and light microscopy was performed. The stage of wound healing of the anastomosis zone was determined according to a scale from 1 to 5: Stage 1 - fibrinopurulent exudate; Stage 2 - granulation tissue <25%; Stage 3 - granulation tissue ≈ 25-75%; Stage 4 - granulation tissue >75% or the intestinal epithelium cells from intact intestinal glands and short villi <25%; Stage 5 - intestinal epithelium cells from intact intestinal glands and short villi >25%.[14]

Statistical analysis was performed using the statistical software «Statistica». (v10.0, StatSoft, USA) and Microsoft Excel 2007. Baseline characteristics were summarized as frequencies and percentages for categorical variables and as mean±SEM for continuous variables. The Mann-Whitney (U Test) was used to compare the differences between the two independent groups. A probability value of $P \leq 0.05$ was considered statistically significant.

Results and Discussion

A macroscopic assessment revealed that the area of IAs formed by precision seams in all animals was free of adhesions. The IA zone was a thin, barely perceptible circular strip less than 1mm thick, with moderate hyperemia during the first 3 days. On Day 5 after surgery, the IA zone could be determined by special marker ligatures and in palpation; the IA zone was determined as a section of thin circular infiltration of the intestinal wall layers. From the side of the mucous layer, the wound roller was no thicker than 1 mm and was uniform throughout the entire IA area. The areas of necrosis of the mucous membrane in the IA zone were not macroscopically determined. Starting from Day 7, the zone of precision IA could be determined only by the special marker ligatures.

In the macroscopic assessment of DASS in the entire series, the anastomosis zone was involved in a pronounced adhesion process with fibrin overlays, and in two cases in the infiltration; in one case it was an abscess wall. From the side of the mucous layer, the IA roller was pressed into the lumen by 3-4 mm, with areas of ulceration and necrotization between the ligatures. We found aigns of acute inflammation even on Day 7.

When evaluating the microscopic picture of IAs formed by precision technology in animals of Groups 1 and 2 during the first 3 days after the operation, we found edema and weak infiltration with neutrophils and lymphocytes in the muscular and submucosal layers. On serosa, thin fibrinous film was determined. Pierced channels were surrounded by unstructured eosinophilic masses with leukocytes. There were impaired blood supply and edema, more pronounced in the submucosal

and muscular layers. The described changes were less pronounced in Group 1. When staining according to Mallory in Groups 1 and 2, the predominance of the process of lysis of collagen fibers in the places of passage of ligature channels was traced; however, this process was less pronounced in animals of Group 1. Disorganization of the connective tissue was determined around the pierced channels: fragmentation of collagen fibers (Fig.1). On separate sections in their place we found unstructured basophilic masses. The wound gap was narrow, submucous layers were precisely matched. The revealed histological changes corresponded to Stage 2 (2.00 ±0.00 points) of wound healing of the anastomosing zone (Table 1).

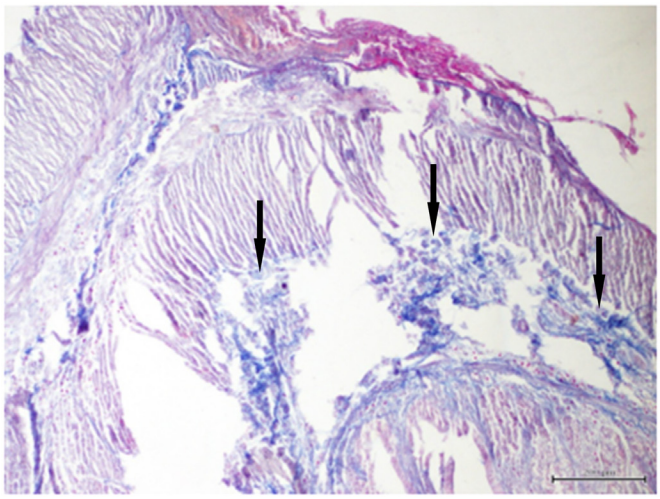

Fig.1. *The IA zone formed by POCS on Day 3 after surgery. Against the background of edema of the submucous and muscular mebranes, the areas of disorganization of the connective tissue in the form of fragmentation of collagen fibers are determined. Mallory staining. Magnification: 40x10.*

Table 1.

Stages of wound healing of the anastomosis zone at various periods of the experiment

Suture types/ Animal groups	Day of observation				
	Day 3	Day 5	Day 7	Day 14	Day 30
	Quantification of wound healing stages (points)				
POCS/Group 1	2.00±0.00	2.00±0.00	3.00±0.00	3.00±0.00	5.00±0.00
POIS/Group 2	2.00±0.00	2.00±0.00	3.00±0.00	3.00±0.00	4.67±0.33
DASS/Group 3	1.33±0.33	1.67±0.33	1.67±0.33	2.33±0.33	4.0±0.00

At the site of DASS formation, histopathological examination revealed a weak lymph granulocytic infiltration, severe impaired blood supply, hemorrhages in the submucosal layer and muscular membrane, and extensive foci of necrosis (Fig.2).

By Day 5 after surgery, we found moderate infiltration with lymphocytes and granulocytes of the intestinal wall in the area of precision anastomoses, and moderate intramuscular edema and vascular plethora. Delicate granulation tissue was formed. In Group 1, the adhesive zone was replete with capillary-type blood vessels surrounded by a delicate network of collagen fibers and inflammatory cells (formation of granulation tissue). In Group 2, the phenomena of collagenolysis increased. When staining according to Mallory, in Group 1, along with the process of collagenolysis, the process of collagen formation was activated: a network of thin interlacing collagen fibers was formed in the zone of formation of granulation tissue. In contrast, in Group 2 the predominance of the process of collagen destruction continued. A network of interlaced collagen fibers and capillaries was determined. The histopathological picture as a whole demonstrated Stage 2 of healing of the anastomosis line, both in Group 1 and Group 2 (2.00±0.00 points) (Table 1).

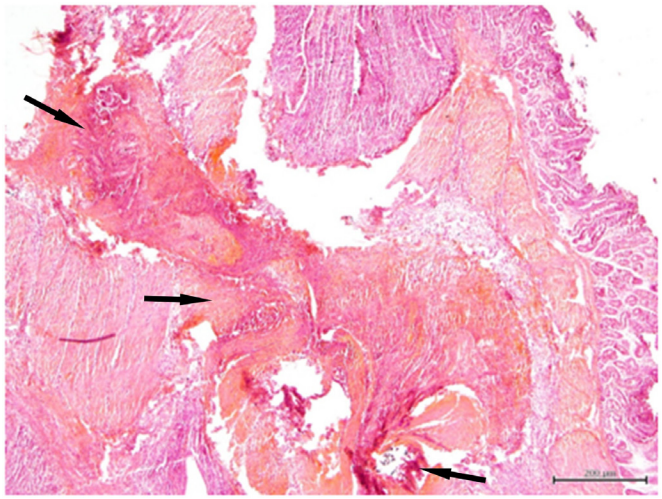

Fig.2. *The IA zone formed by DASS on Day 3 after surgery. On the background of a weak lymph granulocytic infiltration in the submucosal and muscle layers, areas of severe impaired blood supply, hemorrhages, and extensive foci of necrosis are revealed. Hematoxylin and eosin staining. Magnification: 40x10.*

In the DASS area, pronounced edema was noted in the edges of the formed IA, as well as an infiltration with leukocytes. Around suture material, we found necrotic detritus and extensive empty spaces (Fig.3).

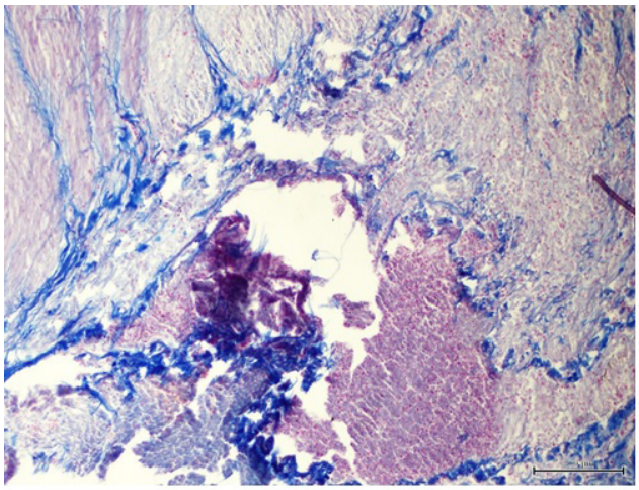

Fig.3. *The IA zone formed by DASS on Day 5 after surgery Many loose fibers around the suture material with a plot of purulent-necrotic detritus. Mallory staining. Magnification: 40x10.*

By Day 7 after surgery, the formation of granulation tissue continued in precision IAs. In the same time period, in the IA zone the volume of granulation tissue increased in Group 1. In Group 2, the wall of the anastomosis zone was sharply thickened due to edema and the formation of granulations with randomly alternating thick collagen bundles. Granulation tissue with a coarse network of collagen fibers of various thicknesses was determined. When staining according to Mallory, granulation tissue with a well-defined network of collagen fibers was determined around the suture material, which corresponded to Stage 3 of wound healing. The quantitative values of the evaluation of staging in animals of these two groups averaged 3.00±0.00 points (Table 1).

In animals of Group 3, with using DASS a pronounced edema in the intestinal wall and its thickening remained. In the muscle plate around the suture material, we found necrosis, pronounced swelling, and disordered bundles of collagen fibers. Around the suture material, in the necrotic detritus a partially destroyed, coarse network of collagen fibers was determined with Mallory staining. At a distance from the ligature channels, granulation tissue was detected, which corresponded to Stage 2 of wound healing (1.67±0.33 points) (Table 1).

After 14 days, in the muscle plate of the intestinal wall of precision IAs in animals of Groups 1 and 2, the single eosinophilic filaments of suture material with perifocal lympho-leukocyte and histiocytic infiltration were determined. On the rest stretch, there was a granulomatous inflammatory reaction with the presence of fibroblasts and collagen fibers in the peripheral parts of the granulomas. In Group 1, maturation of granulation tissue, formation of coarse fibrous connective tissue, and a decrease in the number of vessels were observed. The inflammatory reaction was of a perifocal nature, granulomas of resorption of foreign bodies were formed. In Group 2, the scar tissue was constructed from collagen fibers of various thicknesses. The wall of the intestine in the area of the anastomosis was unevenly thickened. Fibrous tissue with a coarse-fiber network of collagen fibers and a small number of vessels were determined by Mallory staining. The revealed features corresponded to Stage 3 wound healing (3.00±0.00 points) (Table 1).

In the same time period, animals of Group 3, with use of DASS, exhibited edema in the muscle and serous layers. Suture material was characterized by perifocal lymphohistiocytic infiltration and a small number of eosinophils. Mallory's staining around the suture filaments revealed an uneven network of bundles of collagen fibers of various thickness, which corresponded to the third stage of wound healing. However, the average quantitative assessment of staging (2.33±0.33 points) in this group of animals was lower than that of Groups 1 and 2 (Table 1).

On Day 30 after surgery, mature coarse fibrous scar tissue (CFST) was detected in the muscle layer of the zone of precision IAs. On serosa, fibrin masses with signs of organization were identified. The IA zone was epithelialized; a moderate amount of eosinophils was detected in the mucous plate. In the same time period, in the IA zones where the formed CFST was determined, certain features were revealed in animals of different groups. Thus, in Group 2, the CFST deformed the intestinal wall, and in animals of Group 1 the wall was not deformed. In the same time period, in the zone of one-row IAs in animals of both groups, the collagen bundles were more ordered, compared with Group 3. A network of intertwined collagen fibers was determined by Mallory staining. Scar tissue did not deform the intestinal wall. Such changes corresponded to Stage 5 of wound healing. In contrast to the previous periods of the experiment, on Day 30 after surgery, there were already differences in the quantitative assessment of staging. If in Group 1 it averaged 5.00±0.00 points, in Group 2 this indicator was slightly lower - 4.67±0.33 points (Table 1).

In the same time period, in the double-row suture DASS, in the muscle plate of the intestine, suture material was visualized with a perifocal fibroplastic reaction, moderately pronounced edema, and lymphocytic infiltration. The formation of cystic cavities was noted in the place of resorption of suture material. The anastomosis zone was epithelized, and its own mucosal plate was noticeably thinned. Scar tissue deformed the intestinal wall, and the location of collagen bundles had a more pronounced randomness, compared with Groups 1 and 2. In Mallory staining, a uniform network of collagen fibers was determined around the suture (Fig.4).Such changes corresponded to Stage 4 of wound healing.

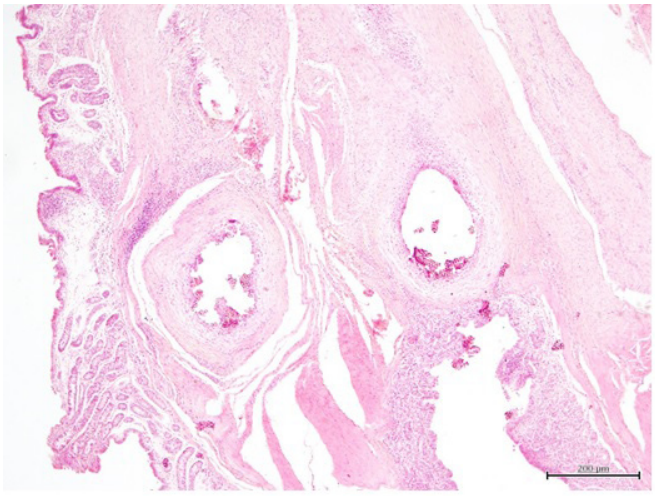

Fig. 4. *The IA zone formed by DASS on Day 30 after surgery. In the muscle plate of the intestine, suture material with a perifocal fibroplastic reaction, moderately pronounced edema, and lymphocytic infiltration. The formation of cystic cavities in the place of resorption of suture material. The anastomosis zone is epithelized, and its own mucosal plate is noticeably thinned. Hematoxylin and eosin staining. Magnification: 40x10.*

However, the average quantitative assessment of staging (4.00±0.00 points) in this group of animals was lower than that of Groups 1 and 2 (Table 1). When studying slices of precision IAs, the formation of pockets and slots in various serial terms was not noted.

Since the distribution of the obtained data of the healing stage does not correspond to the law of normal distribution, and the characteristic values are expressed in points, the non-parametric method (the Mann-Whitney U criterion)

was used in the statistical analysis. Based on the obtained U value (133.5000), the differences between the levels of the characteristic of the stage of healing of precision seams and DASS were statistically significant (P<0.05). There was no significant difference, however, between the healing of the two types of precision suture. Thus, the experimental results obtained prove that the use of microsurgical techniques is the preferred method for the IA formation. All of the above allows us to recommend the wide use of precision techniques for the formation of IAs using our improved techniques of surgery in a wide clinical surgical practice.

In conclusion, in the early stages (Day 3 after surgery) of IA healing, the morphological picture is due to the predominance of the processes of lysis of collagen fibers. The formation of granulation tissue and collagen in precision IA groups begins earlier (Days 5 to 7) than in the DASS group (Days 7 to 14) and reaches Stage 5 of wound healing, while double-row IA - only Stage 4. In the group of precision IAs, the formed anastomosis does not deform the lumen of the intestine, unlike in the DASS group.

Competing Interests

The authors declare that they have no competing interests.

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