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## Analysis of early morphological and functional perivulnar changes in the mucosa of the cecum after suturing with different surgical threads

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The correct choice of suture material is the key to successful treatment in abdominal surgery, particularly pediatric surgery. Given this, experimental studies on the effect of sutures made with different surgical threads are one of the most urgent surgery needs.

The **aim** – to conduct a comparative analysis of early morphological and functional changes in the perivulnar region of the mucosa of the rabbit cecum after suturing with polyglactin-910 and polycaprolactone modified with L-arginine.

**Materials and methods.** The study was conducted on 25 rabbits aged 8–10 months in compliance with all bio-ethical standards and recommendations. The control group consisted of 5 animals; the first experimental group, in which synthetic surgical polyfilamentous absorbable suture polyglactin-910 was used to close the wound defect, and the second experimental group, in which monofilamentous absorbable suture polycaprolactone modified with L-arginine was used to close the wound defect, consisted of 10 rabbits each. All stages of surgical access and sampling of intact cecal wall tissues were performed in the control group. The rabbits of the experimental groups underwent colotomy followed by suturing of the large intestine with appropriate suture material. Each experimental group was divided into two subgroups (5 rabbits in each), in which the condition of the perivulvar region was studied on days 3 and 7, respectively. The animals were not euthanised; during the second operation, tissues of the cecum were taken from the sutured area and the formed scar at the appropriate time. Histological, semi-thin serial sections, electron microscopic, morphometric, and statistical methods were used.

**Discussion.** The average thickness of the cecal mucosa after colotomy increased significantly on days 3–7 when using both surgical threads, but when using polycaprolactone modified with L-arginine, the above indicator was 63.06% and 22.46% lower, respectively, due to a decrease in hyperhydration of the connective tissue component of the mucosa, leukocyte infiltration in the perivulnar area and acceleration of reparative processes.

**Conclusions.** Polycaprolactone modified with L-arginine has a positive effect on the course of morphological and functional processes in the perivulnar region of the rabbit cecum's mucosa. In terms of its characteristics, it is not worse than polyglactin-910.

No conflict of interests was declared by the authors.

**Keywords:** laboratory animals, intestine, cecum, morphometry, polyglactin-910, polycaprolactone modified with L-arginine, haemomicrocirculatory bed, crypts.

**Аналіз ранніх морфофункціональних перивульнарних змін у слизовій оболонці сліпої кишки після накладання швів різними хірургічними нитками**

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У черевній хірургії, зокрема, дитячого віку, правильний вибір шовного матеріалу є запорукою успішного лікування. Зважаючи на це, експериментальні дослідження з вивчення впливу швів, накладених різними хірургічними нитками, є однією з нагальних потреб хірургії.

## Original articles. Abdominal surgery

**Мета** – провести порівняльний аналіз ранніх морфофункціональних змін у перивульнарній ділянці слизової ділянки сліпої кишки кролів при зшиванні поліглактином-910 та полікапролактоном, модифікованим L-аргініном.

**Матеріали та методи.** Дослідження проведено на 25 кролях віком 8–10 місяців із дотриманням усіх біоетичних норм і рекомендацій. Контрольна група налічувала 5 тварин; перша експериментальна група, у якій для зашивання ранового дефекту використовували синтетичну хірургічну поліфіламентну розсмоктувальну нитку поліглактин-910, та друга експериментальна група, у якій для зашивання ранового дефекту користувалися монофіламентною розсмоктувальною ниткою полікапролактоном, модифікованим L-аргініном, складалася з 10 кролів кожна. У тварин контрольної групи проведено усі етапи оперативного доступу та забір інтактних тканин стінки сліпої кишки. Кролям експериментальних груп виконано колотомію з подальшим ушиванням товстої кишки відповідним шовним матеріалом. Кожну експериментальну групу поділено на дві підгрупи (по 5 кролів у кожній), у яких вивчали стан перивульнарної ділянки на 3 та 7-му добу відповідно. Евтаназію тварин не проведено, під час повторної операції у визначені строки взято тканини товстої кишки з ділянки накладання швів і сформованого рубця. Застосовано гістологічний, напівтонких серійних зрізів, електронномікроскопічний, морфометричний, статистичний методи.

**Результати.** Показник середньої товщини слизової оболонки сліпої кишки після колотомії достовірно на 3–7-му добу зростав при використанні обох хірургічних ниток, але при застосуванні полікапролактону, модифікованого L-аргініном, вищезначений показник був відповідно на 63,06% та 22,46% меншим за рахунок зменшення процесів гіпергідратації сполучнотканинного компонента слизової оболонки, лейкоцитарної інфільтрації у перивульнарній ділянці та прискорення репаративних процесів.

**Висновки.** Полікапролактон, модифікований L-аргініном, позитивно впливає на перебіг морфофункціональних процесів у перивульнарній ділянці слизової оболонки сліпої кишки кролів та за своїми характеристиками не поступається поліглактину-910.

Автори заявляють про відсутність конфлікту інтересів.

**Ключові слова:** лабораторні тварини, кишечник, сліпа кишка, морфометрія, поліглактин-910, полікапролактон, модифікований L-аргініном, гемомікроциркуляторне русло, крипти.

## Introduction

The scientific achievements of modern medicine make it possible to introduce more and more new means and treatment methods into surgical practice. Currently, the effectiveness of surgical interventions depends not only on the surgeon's professionalism but also on the quality of material and technical support, particularly on the properties of the suture material [9,10,15]. In abdominal surgery, particularly in pediatric surgery, the correct choice of suture material is the key to successful and rapid wound healing [13]. In pediatric surgery, the need for surgical interventions on the large intestine arises in several pathological conditions: acute appendicitis, congenital malformations (stenosis and atresia of the rectum, dolichosigma, megacolon), adhesive disease, intestinal invasion, in particular, ileocecal invasion [4,8,14]. In view of this, experimental studies to investigate the effect of sutures applied with different surgical threads on the morphological and functional state of large intestine tissues are considered relevant, timely and promising, and the development of new modified surgical suture materials is one of the urgent needs of modern abdominal surgery [1,3,6]. There are various attempts to improve the quality of surgical suture materials: changing sterilisation methods, using other sources of biological raw materials, and adding chemicals. In particular, absorbable sutures modified with L-arginine have been proposed. L-arginine is a conditionally essential amino acid that is a cellular regulator of many vital body functions and is involved in regulating the tone of the smooth muscle component of the walls of blood vessels,

bronchi and intestines [2,11]. L-arginine's antihypoxic and reparative properties may be useful in abdominal surgery, as laparotomy causes a systemic inflammatory response, oxidative-nitrosative stress, and disorders of lipid and carbohydrate metabolism [5,12].

The **aim** of the study – to conduct a comparative analysis of early morphological and functional changes in the perivulnar region of the mucous membrane of the rabbit cecum after suturing with polyglactin-910 (PG-910) and polycaprolactone modified with L-arginine (PCL-MA).

## Materials and methods of the research

The study was conducted on 25 sexually mature outbred domestic rabbits of both sexes aged 8–10 months and weighing ( $2.62 \pm 0.21$ ) kg. Rabbits were chosen because of the similarity of the intestinal wall structure to that of humans. The diameter of the cecum is approximately the same as that of the corresponding intestine of an infant.

We divided animals into three groups: control 5 rabbits) and two experimental groups (10 animals in each). The control group was used to study the structural features and morphometric parameters of the cecum wall in rabbits under normal conditions. The rabbits of the first experimental group underwent colotomy followed by suturing of the large intestine with PG-910, and the second experimental group – with PCL-MA. Each experimental group was divided into two subgroups 5 rabbits in each), in which the condition of the perivulnar area and scar formation were studied on days 3 and 7, respectively.

In animals of the control group, all stages of surgical access to the large intestine and sampling of intact tissues of the cecum wall were performed. After surgical access, rabbits of the first experimental group underwent a colotomy performed with a scalpel through all layers of the cecum wall 4 cm in length; the wound edges were sutured with synthetic polyfilament absorbable suture PG-910. In the second experimental group, after surgical access, the animals underwent a similar colotomy, and the wound edges were sutured with a synthetic monofilament absorbable suture PCL-MA. An atraumatic needle with a 3-metric size thread was used for suturing.

The animals were not euthanised; during the second operation, large intestine tissue was taken from the sutured area at the appropriate time.

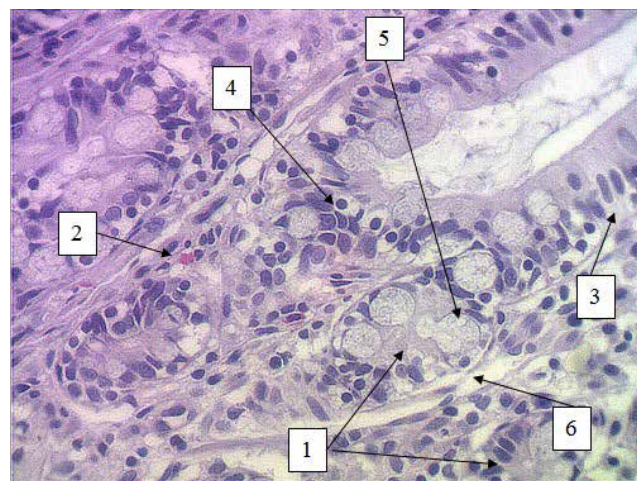
Before the experiment, the animals were prepared. For one day, the animals were not fed and kept in a «hungry period», and for 3 to 4 hours, rabbits were not allowed to drink. Before surgery, the animals were sedated by intramuscular injection of a Lytic cocktail (0.2 ml of 1% Sol. Dimedroli, 0.1 ml of 0.1% Sol. Atropinii Sul-fatis and 0.1 ml of 10% Sol. Nalbufini). 5.5–5.7 ml of 4% Ubistesini forte was used for local anaesthesia. After preoperative treatment and preparation of the surgical field, an upper-middle-lower midline laparotomy was performed. The colotomy was performed by opening the cecum wall, 3–4 cm long, between the omental taenia and free taenia, parallel to them and perpendicular to the haustra. After dissection, the cecum wall was sutured with a double-row suture. For the first row of sutures, continuous screw-in Schmiden suture was used. For the second, clean row of sutures, a Lambert's sero-serous interrupted suture was used.

To suture the surgical wound in the animals of the first experimental group, we used PG-910, a modern suture material that is bio inert, does not cause general toxic effects on the body and allergic reactions during degradation, is resistant to infection, is sufficiently reliable, atraumatic, does not cause an absorbing effect, has good surgical and technical characteristics, and therefore has been used in surgical practice for a long time [7].

In order to study and compare the effectiveness in animals of the second experimental group, the novel domestic surgical suture material PCL-MA was used.

PG-910 and PCL-MA are very similar in terms of their characteristics and resorption time, but PG-910 is a polyfilament suture, while PCL-MA is a monofilament suture.

The following methods were used to study the morphological and functional features of the mucous membrane of the cecum in the normal and the dynamics of



Notes: 1 – crypts; 2 – capillary; 3 – columnar epithelial cells with a brush border, 4 – columnar epithelial cells without a brush border; 5 – goblet cells; 6 – connective tissue crypt case.

**Fig. 1.** Cellular composition of the crypts of the cecum of rabbits in the control group. Paraffin section. Hematoxylin and eosin staining. Magnification: Lens  $\times 40$ , Eyepiece  $\times 10$

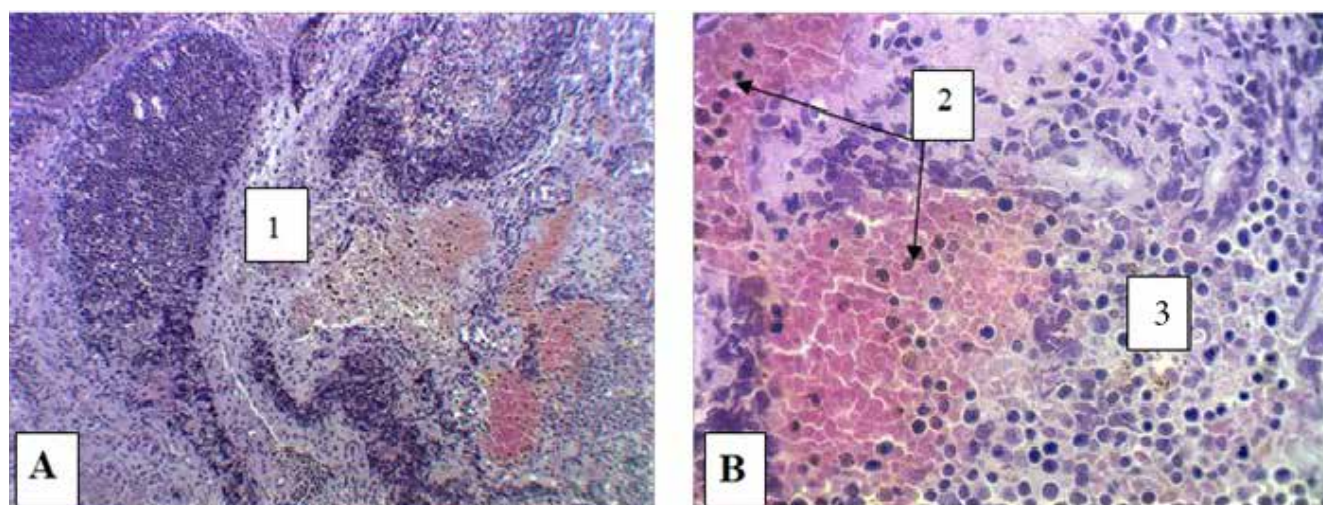
the experiment on days 3 and 7: histological – to determine the general morphology of the wall of the mucous membrane of the cecum; the method of semi-thin serial sections – to detail the histological structures of the intestinal wall; electron microscopy – to determine the processes of remodeling of ultrastructures in the dynamics of the experiment; reconstruction method – for visualization of the shape, size and relative position of the components of the cecum; morphometric method – for analysis of quantitative changes in angioarchitectonics and histocytotopography of structural elements; statistical method – to establish the reliability of the dynamics of quantitative changes in structural components.

The studies were conducted in compliance with the principles of bioethics, following the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986), Council of Europe Directive 2010/63/EU and the Law of Ukraine No. 3447-IV «On the Protection of Animals from Cruelty», on 25 mature outbred domestic rabbits of both sexes aged 8–10 months and weighing  $(2.62 \pm 0.21)$  kg. The animals were kept in the vivarium of Poltava State Medical University in accordance with sanitary and hygienic standards and rules. Surgical interventions were performed in the animal operating room of the Department of Anatomy with Clinical Anatomy and Operative Surgery of Poltava State Medical University.

## Results of the study

A comprehensive morphological study shows that the cecum wall in rabbits is formed of 4 layers: mucosa, sub-





Notes: 1 – perivulnar area at the border of the mucosa and submucosa; 2 – blood clots; 3 – leukocyte infiltrate.

**Fig. 2.** Morphological changes in the perivulnar area of the rabbit cecum wall on day 3 after suturing the wound defect with PG-910 surgical thread. Paraffin section. Hematoxylin and eosin staining. Magnification: A – Lens  $\times 10$ , Eyepiece  $\times 10$ ; B – Lens  $\times 100$ , Eyepiece  $\times 10$

mucosa, muscular layer and serosa. The average thickness of the mucous membrane of the cecum in rabbits was  $(133.74 \pm 5.03) \mu\text{m}$ . Circular folds and crypts form the relief of the rabbit cecum.

Crypts were visualized as a tubular ingrowth of the mucosal epithelial layer into its own lamina propria. The average diameter of the crypt was  $(20.48 \pm 1.63) \mu\text{m}$ , and its average depth was  $(79.64 \pm 11.01) \mu\text{m}$ . The cellular composition of the crypts of the mucous membrane of the cecum of rabbits was represented by columnar epithelial cells with a brush border, columnar epithelial cells without a brush border, goblet cells, and Paneth cells. They also included single endocrinocytes of the diffuse endocrine system associated with the mucosa and intraepithelial lymphocytes (Fig. 1).

Morphometrically, it was determined that the average number of columnar epithelial cells with a brush border in the crypts of the mucous membrane of the cecum was  $12.74 \pm 1.12$ , the average number of goblet cells is determined at the level of  $42.02 \pm 3.42$ , the average number of Paneth cells is  $11.67 \pm 1.84$ , and the average number of enteroendocrine cells is  $0.84 \pm 0.04$ .

Cellular elements of connective tissue and leukocyte cells were visualized in the mucous membrane of the rabbit cecum. The average number of fibroblasts was  $28.94 \pm 1.48$ , the average number of plasmacytes was  $0.78 \pm 0.04$ , the average number of macrophages was  $1.84 \pm 0.08$ , the average number of monocytes was  $0.68 \pm 0.04$ , the average number of lymphocytes was  $14.48 \pm 1.22$ , and the average number of smooth muscle cells was  $34.46 \pm 0.42$ .

The hemomicrocirculatory bed of the mucous membrane of the cecum in rabbits is formed by arterioles,

precapillary arterioles, capillaries, post capillary venules and venules.

Morphometrically, it was found that the average outer diameter of arterioles in rabbits of the control group was  $(15.71 \pm 0.96) \mu\text{m}$ , capillaries –  $(8.18 \pm 0.64) \mu\text{m}$ , venules –  $(16.66 \pm 1.12) \mu\text{m}$ . The mean values of the diameter of the lumens of the elements of the hemomicrocirculatory bed of the mucous membrane of the cecum of rabbits of the control group were: for arterioles –  $(10.52 \pm 0.78) \mu\text{m}$ , capillaries –  $(7.46 \pm 0.56) \mu\text{m}$ , venules –  $(11.77 \pm 0.92) \mu\text{m}$ . The average thickness of the vascular walls of arterioles was  $(6.56 \pm 0.46) \mu\text{m}$ , capillaries –  $(2.34 \pm 0.18) \mu\text{m}$ , venules –  $(7.27 \pm 0.84) \mu\text{m}$ .

After the experimental colotomy of the cecum, an inflammatory process of the perivulnar area was observed in the early stages. In animals on day 3 of observation, the intestinal wall mucosa was thickened due to the expansion of collagen fiber bundles of loose fibrous connective tissue, clearly defined hyper hydration, significant hemorrhages and leukocyte infiltration around the formed blood clots (Fig. 2).

The average thickness of the mucous membrane, both when using PG-910 sutures and PCL-MA sutures, increased significantly ( $p < 0.05$ ) compared to the control group. However, with the use of PCL-MA, the above indicator was 63.06% lower than with PG-910 ( $(261.45 \pm 8.76) \mu\text{m}$  and  $(414.59 \pm 7.16) \mu\text{m}$ , respectively) due to a decrease in hyperhydration of the connective tissue component of the mucous membrane and leukocyte infiltration in the perivulnar region (Fig. 3). The same trend was observed on day 7 of observation when the average thickness of the mucous membrane when using PG-910 suture was 22.46% significantly higher

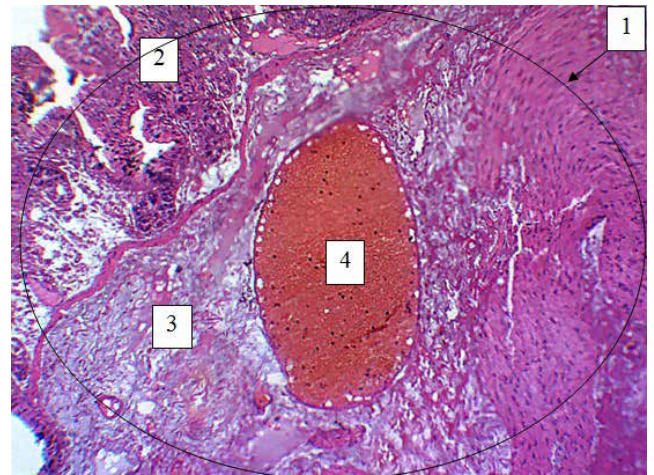
than when suturing the intestinal wall with PCL-MA ((276.41±8.78) μm and (214.21±8.12) μm, respectively).

The vessels of the hemomicrocirculatory bed also actively responded to the inflammatory process that occurred as a result of experimental colotomy (Fig. 4).

The arterioles of the mucosa and submucosa of the cecum in the area of the wound defect statistically significantly (at  $p < 0.05$ ) reacted with an expansion of the average outer diameters starting from day 3 of the experiment, but when using PCL-MA, the vasodilation of the arterioles was 21.42% less than when using PG-910 ((22.23±2.98) μm and (28.27±3.02) μm, respectively); after seven days of the experiment, the above indicator was statistically significantly lower by 26.92% ((19.02±2.82) μm and (26.03±2.12) μm, respectively). The capillaries in the mucous membrane already after three days of the experimental study reacted with vasoconstriction, but when using PCL-MA, the narrowing of the total diameter of the capillaries was 13.31% less than when using PG-910 ((5.79±0.87) μm and (5.11±0.63) μm, respectively); after seven days of the experiment, the above indicator was not statistically significantly different for both surgical threads. Venules in the area of the wound defect statistically significantly (at  $p < 0.05$ ) responded with an expansion of the average diameters on day 3 of the experiment, but when using PCL-MA, vasodilation of venules was 24.61% less compared to PG-910 ((22.61±2.12) μm and (29.99±2.84) μm, respectively). After seven days of the experiment, the above indicator was also statistically significantly lower by 21.39% compared with PG-910 ((21.02±1.94) μm and (26.74±2.08) μm, respectively). The results indicate that L-arginine, which enters the perivulnar area with PCL-MA suture, has a positive effect on the microhemodynamic processes in the area of the wound defect.

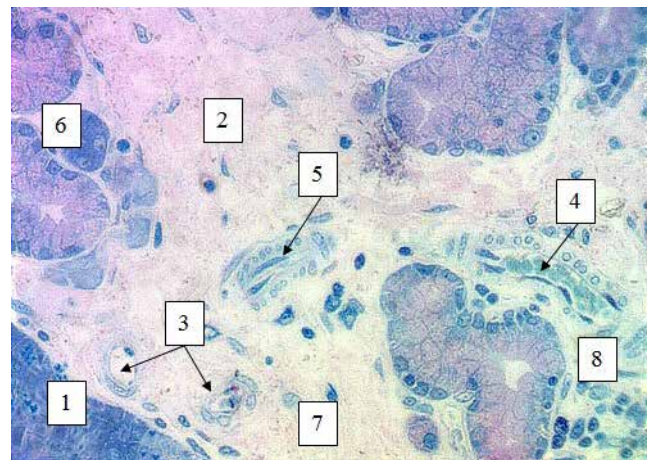
In the area of the wound defect on day 3 of the experimental study, mucosal crypts were located deeper relative to the submucosa compared to the control group (Fig. 5). But, compared to PG-910, when using PCL-MA, their depth was 15.84% less ((87.48±4.28) μm and (92.42±3.24) μm, respectively). A similar trend was observed on day 7 ((89.34±4.32) μm and (94.48±4.32) μm, respectively) when this depth was 17.59% less (Fig. 6).

Changes in the histotopography of the crypts of the mucosa of the cecum in the perivulnar region directly depend on quantitative changes in the number of its cellular components. Thus, on the 3rd day of the experimental study, the average number of columnar epithelial cells with a brush border decreased sharply, but when using PCL-MA, this indicator was 16.57% lower than when using PG-910 (3.12±0.24 and 3.74±0.24 respec-



**Notes:** 1 – perivulnar zone at the border of the mucosa and submucosa; 2 – mucosa; 3 – submucosa; 4 – implanted surgical thread.

**Fig. 3.** Morphological changes in the perivulnar zone of the rabbit cecum wall on day 3 after wound suturing with PCL-MA. Paraffin section. Hematoxylin and eosin staining. Magnification: Lens ×10, Eyepiece ×10



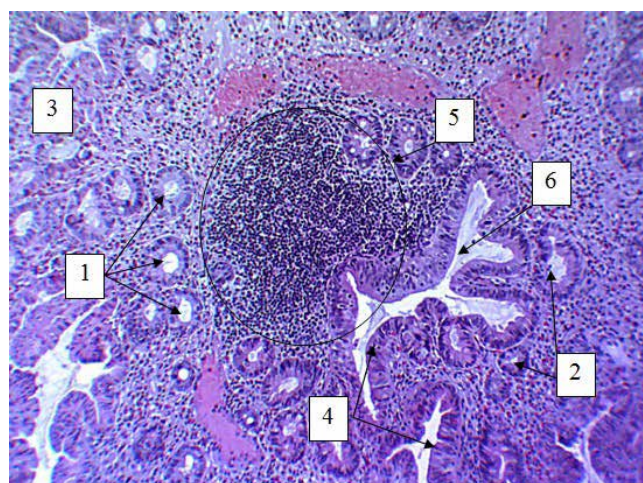
**Notes:** 1 – area of implanted PG-910 surgical thread; 2 – connective tissue of the mucosa; 3 – cross-section of the microvessel; 4 – longitudinal section of the newly formed vessel; 5 – tangential section of microvessels; 6 – crypts of the mucosa of the cecum; 7 – loose fibrous connective tissue of the mucosa; 8 – leukocyte cells.

**Fig. 4.** Angiogenesis of microvessels in the area of the wound defect sutured with PG-910 on day 3 of the experiment. Semi-thin section. Polychrome dye staining. Magnification: Lens ×40, Eyepiece ×10

tively). After seven days of the experimental study, their average number when using PCL-MA was 13.57% lower than when using PG-910 (3.44±0.32 and 3.98±0.32 respectively).

Also, the average number of goblet cells in the crypts of the perivulnar area of the cecum mucosa changed. On day 3 of the experiment, their average number increased sharply, but when PCL-MA was used, this indicator was 17.71% lower compared to PG-910 (72.62±6.12 and





Notes: 1 – lumen of the crypt mouth; 2 – crypts; 3 – hyperhydrated areas; 4 – superficial pit epithelium; 5 – areas of significant leukocyte infiltrate; 6 – lumen of the cecum.

**Fig. 5.** Changes in the histotopography of the depth of the crypt in the perivulnar area of the mucosa of the rabbit cecum on day 3 after suturing the wound defect with PCL-MA. Paraffin section. Hematoxylin and eosin staining. Magnification: Lens  $\times 40$ , Eyepiece  $\times 10$

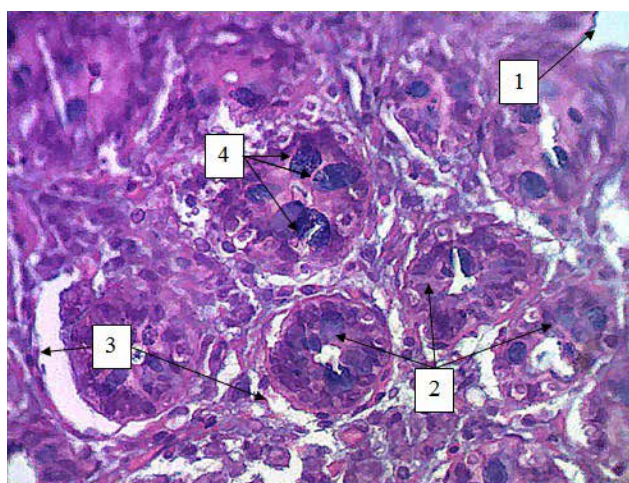
88.24 $\pm$ 6.12 respectively), and after seven days – by 14.48% (86.24 $\pm$ 9.81 and 100.84 $\pm$ 9.81 respectively).

The number of enteroendocrine cells also increased sharply on day 3 of the experimental study, but when using PCL-MA, this indicator was 17.71% lower compared to PG-910 (1.86 $\pm$ 0.18 and 2.43 $\pm$ 0.18 respectively); on day 7 of the experimental research, it also remained 14.48% lower (2.24 $\pm$ 0.26 and 2.85 $\pm$ 0.26, respectively).

After three days of the experimental study, the average number of fibroblasts also decreased sharply, but when using PCL-MA, this indicator was 10.89% lower than when using PG-910 (18.42 $\pm$ 2.12 and 20.67 $\pm$ 2.12, respectively). After seven days, these indicators increased sharply, but when using PCL-MA, this indicator was lower by 7.39% (48.24 $\pm$ 4.32 and 52.09 $\pm$ 4.32, respectively).

In our opinion, the reaction of macrophages of the perivulnar area of the cecum is also important in the process of wound defect healing. It was found that after three days of the experimental study, the average number of macrophages increased sharply, but when using PCL-MA, this indicator was 12.06% lower than when using PG-910 (13.26 $\pm$ 1.12 and 15.08 $\pm$ 1.12 respectively). After seven days of the experimental study, the average number of macrophages was 9.54% lower when PCL-MA was used compared to PG-910 (15.64 $\pm$ 1.81 and 17.29 $\pm$ 1.81 respectively).

The average number of plasmacytes decreased on day 3, but when using PCL-MA, this indicator was 10.76%



Notes: 1 – lumen of the crypt mouth; 2 – crypts; 3 – hyperhydrated areas; 4 – goblet cells.

**Fig. 6.** Changes in the topography of the depth of the crypt in the perivulnar area of the mucosa of the rabbit cecum on day 7 after the closure of the wound defect with PG-910. Paraffin section. Hematoxylin and eosin staining. Magnification: Lens  $\times 40$ , Eyepiece  $\times 10$

lower than when using PG-910 (0.58 $\pm$ 0.02 and 0.65 $\pm$ 0.02, respectively). After seven days, these indicators increased sharply, but when using PCL-MA, this indicator was lower by 6.38% compared to PG-910 (1.32 $\pm$ 0.08 and 1.41 $\pm$ 0.08, respectively).

After three days of the experimental study, the average number of small lymphocytes increased, but when using PCL-MA, this indicator was 9.52% lower than when using PG-910 (18.34 $\pm$ 1.64 and 20.27 $\pm$ 1.64 respectively). After seven days of the experimental study, the average number of lymphocytes was 9,92% lower when PCL-MA was used compared to PG-910 (36.52 $\pm$ 2.82 and 40.54 $\pm$ 2.82 respectively).

Thus, a comparative analysis of the processes of remodelling the structural components of the cecum wall in the perivulnar region using these two sutures has shown an apparent positive effect of PCL-MA on the reparative processes in the area of the wound defect.

## Conclusions

The obtained results of a comprehensive morphological study indicate that PCL-MA is an improved surgical suture material that positively affects the course of morphological and functional processes in the perivulnar area of the mucous membrane of the rabbit cecum for 3–7 days, stimulates tissue regeneration and increases blood flow. Such a modified thread is not conceded by its characteristics to PG-910 and, therefore, can be widely used in surgical practice.

*No conflict of interests was declared by the authors.*

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