UDC 616.345-071-092:612.08

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Morphometric features of the structural organisation of rabbit large intestine crypts after colotomy using polycaprolactone thread modified with L-arginine

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Paediatric Surgery (Ukraine). 2025. 1(86): 33-39. doi: 10.15574/PS.2025.1(86).3339

For citation: Pronina OM, Bilash SM, Ksyonz IV, Kobeniak MM, Oliinichenko YaO, Koptev MM et al. (2025). Morphometric features of the structural organisation of rabbit large intestine crypts after colotomy using polycaprolactone thread modified with L-arginine. Paediatric Surgery (Ukraine). 1(86): 33-39. doi: 10.15574/PS.2025.1(86).3339.

The study of the morphological characteristics of large intestine tissues sutured with various surgical threads under experimental conditions is highly relevant, timely, and holds significant promise for both theoretical and practical medicine, particularly in the field of paediatric abdominal surgery.

The aim is to determine the morphometric parameters of the structural organization of large intestine crypts during experimental colotomy using polycaprolactone thread modified with L-arginine (PCL-MA).

Materials and methods. The study was conducted on 35 sexually mature, non-purebred domestic rabbits aged 9–10 months, in accordance with all bioethical standards. The animals were divided into a control group 5 individuals) and an experimental group (30 individuals). In the control group, all stages of surgical access and sampling of intact caecal wall tissue were carried out. The rabbits in the experimental group underwent colotomy, followed by suturing of the large intestine with PCL-MA. To achieve the aim, histological, semi-thin serial sectioning, morphometric, and statistical methods were employed.

Results. The study showed that when using PCL-MA sutures, due to L-arginine on its surface, the number and functioning of absorptive enterocytes are restored from the 21st day of the experiment. The reaction of goblet cells in the crypts contributes to the formation of a protective mucosal barrier, which is manifested in an increase in their number (from day 3rd to day 14th of the experiment) and the presence of a significant number of cells in the stage of degranulation. Restoration and normalization of the functions of mucosal exocrinocytes of the perivulnar area were observed after the 21st day of the experiment.

Conclusions. Poorly differentiated cells located within the crypts play a key role in the regeneration of the large intestine following surgery, as their proliferation contributes to the restoration of the cellular pool. The application of PCL-MA sutures exerts a positive influence on the processes of differentiation and the formation of undifferentiated cells within the crypts.

No conflict of interests was declared by the authors.

Keywords: intestine, morphometry, L-arginine, crypts, mucosa, intestinal wall, surgical thread.

Морфометричні особливості структурної організації крипт товстої кишки кролів при колотомії з використанням полікапролактонової нитки, модифікованої L-аргініном

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Дослідження морфологічних характеристик тканин товстої кишки при зшиванні різними хірургічними нитками в експериментальних умовах є надзвичайно актуальним, своєчасним і перспективним для теоретичної та практичної медицини, особливо в галузі дитячої абдомінальної хірургії.

Мета – встановити морфометричні показники структурної організації крипт товстої кишки при екпериментальній колотомії з використанням полікапролактонової нитки, модифікованої L-аргініном (PCL-MA).

Матеріали та методи. Дослідження було проведено на 35 статевозрілих безпородних свійських кролях віком 9–10 місяців із дотриманням усіх біоетичних стандартів. Тварин було розподілено на контрольну 5 особин) та експериментальну (30 особин) групи. У контрольній групі було виконано всі етапи оперативного доступу та забір інтактних тканин стінки сліпої кишки. Кролям експериментальних груп проведено колотомію з подальшим ушиванням товстої кишки PCL-MA. Для досягнення мети було застосовано гістологічний, метод напівтонких серійних зрізів, морфометричний та статистичний методи дослідження.

Результати. При використанні хірургічної нитки PCL-MA, завдяки L-аргініну, який знаходиться на її поверхні, відновлюється кількість та функціонування всмоктувальних ентероцитів вже з 21-ї доби експерименту. Реакція келихоподібних клітин у криптах сприяє утворенню захисного слизового бар'єра, що проявляється у збільшенні їхньої чисельності (з 3-ї по 14-ту добу експерименту) та наявності значної кількості клітин у стадії дегрануляції. Відновлення та нормалізація функцій слизових екзокриноцитів перивульнарної ділянки спостерігається після 21-ї доби експерименту.

Висновки. У процесах регенерації товстої кишки після хірургічного втручання важливу роль відіграють малодиференційовані клітини, що містяться у криптах, оскільки їхня проліферація сприяє відновленню клітинного пулу. Використання нитки PCL-MA має позитивний вплив на процеси диференціації та утворення малодиференційованих клітин у криптах.

Автори заявляють про відсутність конфлікту інтересів.

Ключові слова: кишечник, морфометрія, L-аргінін, крипти, слизова оболонка, кишкова стінка, хірургічна нитка.

Introduction

Surgical interventions in general and paediatric surgery require the use of high-quality surgical sutures, which promote rapid wound healing, particularly during large intestine surgery [7,12,18].

Any foreign material, including surgical thread, causes an inflammatory reaction in the body tissues and can lead to postoperative purulent complications [9,16].

One of the directions in the development of metabolitotropic sutures is the creation of threads with bioregulatory effects [8,10,11,14,17], as well as other compounds with antioxidant properties [5]. Thus, absorbable surgical sutures modified with L-arginine, a substrate of NO synthases, have been proposed [15]. Introducing L-arginine into collagen-containing surgical sutures allows for potentiating its antihypoxic and repair properties and activating nitric oxide (NO)-dependent signaling pathways in the cells of sutured structures [1]. L-arginine is a crucial cellular regulator that influences numerous vital functions in the body. It plays a key role in regulating the tone of smooth muscle in the walls of blood vessels, bronchi, and intestines, and is essential in a wide array of physiological and pathophysiological processes. Using sutures modified with L-arginine accelerates the transition to both macrophage and monocyte and fibroblastic stages of wound inflammation [3,12]. The observed proliferation of fibroblasts is limited in time (7–14 days) and corresponds to the period required for surgical wound healing, which prevents the formation of excessive scarring [3]. Our task was to study a new, modern domestic polycaprolactone thread modified with L-arginine (PCL-MA). In our opinion, this will answer whether new suture materials are better than traditional, time-tested old ones.

The aim of the study – to determine the morphometric parameters of the structural organization of large intestine crypts during experimental colotomy using PCL-MA.

Materials and methods of the research

The experiment was conducted at the Department of Anatomy with Clinical Anatomy and Operative Surgery of Poltava State Medical University. The study was carried out on 35 sexually mature outbred domestic rabbits (of both sexes), aged 9–10 months and weighing (2.62±0.21) kg. The animals were housed in a vivarium, in accordance with established sanitary and hygienic standards and regulations.

The animals were divided into two groups. The Control group consisted of 5 animals, where each underwent all stages of surgical access to the large intestine, and samples of intact cecum wall tissue were collected. The Experimental group included 30 animals, where a wound closure was performed using a monofilament absorbable PCL-MA. The thread was used with an atraumatic ne-

edle, metric size 3, for surgical interventions. After the surgical intervention, we studied the condition of the wound and the formed scar on days 3, 7, 14, 21, and 30.

In accordance with the aim, an adequate set of research methods was used experimental, histological, method of semi-thin serial sections, reconstruction, morphometric, and statistical methods. The mean depth of crypts, mean apical width of crypts, mean basal width of crypts, mean diameter of crypts, as well as morphometric changes in the intestinal gland (crypt of Lieberkühn) were measured.

Statistical analysis of the study results was conducted using the InStat software package, designed for processing biomedical and epidemiological data. Differences were considered significant at $p<0.05$.

The study was conducted in accordance with bioethical principles outlined in the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986), Council Directive 2010/63/EU, and Ukraine's Law No. 3447-IV, On the Protection of Animals from Cruelty. According to the decision of the Biomedical Ethics Committee of Poltava State Medical University (Protocol No. 220 of 25.10.2023), no violations in the treatment of laboratory animals were found.

Results of the study

The study revealed that the cecum's surface featured circular folds and crypts.

The crypts were finger-like in shape and protruded into the intestinal lumen. The crypt had its apical and basal parts. The cellular composition of the cecal mucosal crypts in the Control group rabbits included columnar enterocytes with a border, columnar enterocytes without a border, goblet cells, and Paneth cells. Additionally, there were individual endocrinocytes from the diffuse endocrine system associated with the mucosa, as well as intraepithelial lymphocytes.

Morphometric studies showed that the mean number of columnar epithelial cells with a border was 22.67±2.18, the mean number of columnar epithelial cells without a border was 30.17±4.28, the mean number of goblet cells was 31, 02±3.62, the mean number of Paneth cells is 11.67±1.84 and the mean number of endocrinocytes of the diffuse endocrine system associated with the mucosa of the rabbit cecum in the crypt area is 7.82±2.36.

Thus, the ratio of cellular elements in the crypts suggests that the crypt is a dynamic and multifunctional structure that provides several well-known functions of the large intestine.

At the light-optical level, we determined a clear polarity of the crypts and the presence of a lymphatic capillary in its center. The mean height of crypts in the Control group was (179.21±0.54) μm, its mean apical width was (33.97±0.56) μm, the mean basal width of crypts was determined at (39.22+0.55) μm, and the mean diameter of the lumen of the lymphatic capillary of crypts was (12.16±0.81) μm.

The crypts were represented by tubular growth of the epithelial layer of the mucous membrane into its lamina propria. The mean diameter of the crypt was (20.48±1.63) μm, and its mean depth was (79.64±11.01) μm.

When the large intestine was sutured with PCL-MA thread, the processes of restructuring the structural elements of the crypts were observed at different times of the experiment. Thus, in the perivulnar area of the intestine, the crypts statistically significantly (at $p<0.05$) changed their histotopography and location depth; from the 3rd day, they were located 1.07 times deeper.

On the 7th day, compared to the control group, crypts continued to deepen into the mucosa and were 1.09 times deeper, and compared to the previous observation period, crypts were 1.09 times deeper cytotopographically.

On the 14th day of the experiment, the reverse process was observed: the crypts were located more superficially relative to the lumen of the cecum and, compared to the Control group, this indicator changed statistically significantly, at $p<0.05$, by 1.04 times, and compared to the previous observation period, the mean indicator changed by 1.13 times at $p<0.05$.

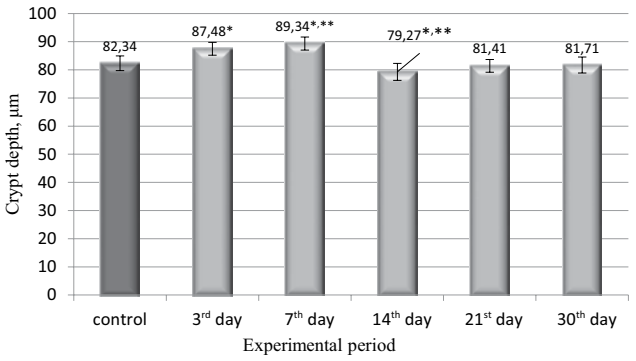
These changes in the crypts' location indicators indicate that these structural and functional units of the cecum's mucosa are actively involved in the inflammatory processes of the perivulnar area.

On the 21st and 30th day of the experiment, the mean depth of the intestinal wall mucosal crypts in the perivulnar area showed no significant difference ($p<0.05$) from the Control group or from the previous observation period, remaining within the statistical margin of error (Fig. 1).

In parallel with the changes in crypt cytotopography, we determined morphometric changes in the mean total width of the crypt in the area of its bottom and mouth.

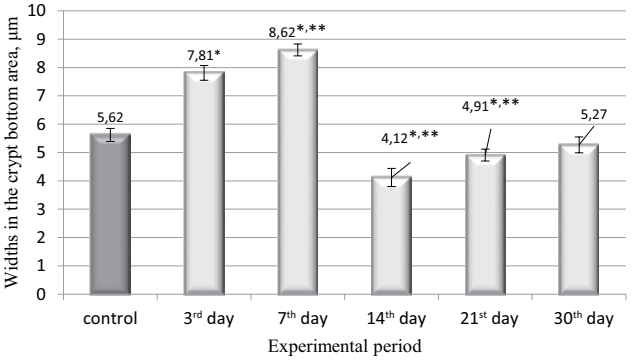
Thus, the mean value of the total width of the crypt bottom changed starting from the 3rd day of the experiment. A statistically significant 1.39-fold expansion of the crypts in the area of their bottom was found compared to the Control group.

After 7 days of the experiment, this indicator continued to increase by 1.54 times compared to the Control group, and compared to the previous period, it was statistically significant, at $p<0.05$, was 1.11 times higher,



Note: * – statistically significant at p<0.05 compared to the Control group; ** – statistically significant at p<0.05 compared to the previous observation period.

Fig. 1. Dynamics of changes in the mean depth of the crypts of the cecum mucosa in the perivulnar area during suturing of the wound defect with PCL-MA suture in the experiment



Note: * – statistically significant at p<0.05 compared to the Control group, ** – statistically significant at p<0.05 compared to the previous observation period.

Fig. 2. Dynamics of changes in the mean width in the area of the bottom of the crypts of the cecum mucosa in the perivulnar area during suturing of the wound defect with PCL-MA suture in the dynamics of the experiment

indicating the continuation of the process of crypt expansion in the bottom area.

After 14 days, the reverse reaction of crypts in the bottom area was observed. Thus, compared to the control values, their mean value was statistically significantly lower by 1.41 times at p<0.05, and, compared to the previous period, it decreased significantly (by 2.09 times) at p<0.05.

After 21 days of the experimental study, compared to the Control group, the above indicator continued to decrease by 1.15 times, and, compared to the previous observation period, this indicator, on the contrary, began to increase (by 1.19 times), indicating the beginning of the restoration of the normal functioning of the crypts of the cecum mucosa.

After 30 days, the mean value of the total thickness of the crypts in the perivulnar area did not differ significantly, at p<0.05, from the corresponding values of the Control group and the values of the previous observation period within the statistical error. Our data on changes in the mean thickness of crypts in the perivulnar zone of the bottom area indicate that even when using PCL-MA suture, no significant effect on the lumen and the main exocrinocytes that make up the crypt is determined after morphometric analysis (Fig. 2).

The inflammatory process caused by a wound defect sutured with PCL-MA sutures also involves structural and cellular elements that were directly involved in implementing focal inflammation and forming a connective tissue scar in the perivulnar area.

Thus, columnar epithelial cells with a border (CECwB) implement in the perivulnar area the processes associated with pericellular and perivascular edema that occur after surgery.

Thus, after 3 days of the experiment, it was morphometrically determined that the mean number of CECwB (MNCECwB) decreased by 4.08 times compared to the Control group.

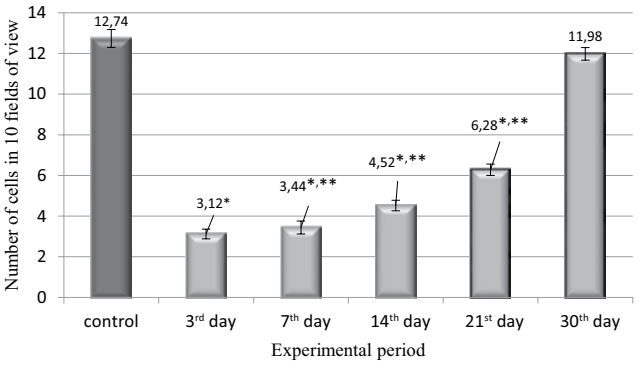
After 7 days, there was a tendency to decrease their mean number compared to the Control group – by 3.71 times, and, on the contrary, an increase by 1.11 times compared to the previous period.

After 14 days of the experimental study, the above indicator was 2.74 times lower compared to the Control group, and, compared to the previous period, the MNCECwB index statistically significantly increased by 1.31 times.

On the 21st day of the experiment, morphometrically, a clear tendency to decrease the mean number of absorptive enterocytes in the perivulnar area by 2.03 times compared to the Control group and increase by 1.39 times compared to the previous observation period was established.

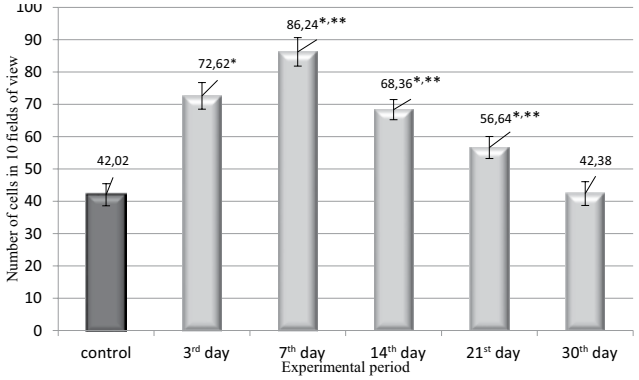
After 30 days, the mean number of absorptive enterocytes in the perivulnar area did not differ from that of the Control group of animals and the previous observation period. It was within the limits of statistical error (Fig. 3).

At the light-optical level, we confirmed the data on the established tendency to decrease the MNCECwB indicator. Thus, after 14 days of the experiment, the crypts of the perivulnar area showed signs of deformation; due to the death of absorptive enterocytes, the population of these cells, compared to the histological sections of the Control group, decreased sharply, given that absorptive enterocytes are the most numerous cell population in the crypts, as evidenced by the detected connective tissue layers around the crypts of the perivulnar area with signs of hyperhydration.



Note: * – statistically significant at p<0.05 compared to the Control group, ** – statistically significant at p<0.05 compared to the previous observation period.

Fig. 3. Dynamics of changes in the MNCECwB indices of the cecum mucosa in the perivulnar area during suturing of the wound defect with PCL-MA sutures in the experiment



Note: * – statistically significant at p<0.05 compared to the Control group, ** – statistically significant at p<0.05 compared to the previous observation period.

Fig. 4. Dynamics of changes in the MNGC indices in the crypts of the cecum mucosa in the perivulnar area during suturing of the wound defect with PCL-MA sutures in the experiment

Thus, we can assert that the absorption of water and electrolytes in the perivulnar area is impaired, resulting in dehydration of faeces formed in the cecum.

In parallel, the reaction of goblet cells (GC) in the perivulnar area of the crypts of the rabbit cecum mucosa was determined. Their mean number (MNGC) changed dynamically during the experiment.

Their number increased by 1.73 times compared to the Control group beginning on the 3rd day of the experiment. By the 7th day, this indicator rose significantly, reaching 2.05 times that of the Control group and 1.19 times that of the previous observation period.

After 14 days, the MNGC index increased by 1.63 times compared to the Control group and decreased by 1.21 times compared to the previous observation period.

After analyzing the changes in the perivulnar area MNGC after 21 days, it was determined that, compared with the control values, it was 1.35 times higher, and, compared with the previous observation period, it decreased by 1.22 times. After 30 days, the MNGC value did not differ from the Control group and the previous observation period and was within the statistical error (Fig. 4).

In the morphometric analysis of the mean number of enteroendocrine cells (MNEC), we found that their mean number changed dynamically, which indicates a significant role of these cell structures in implementing the inflammatory process in the area of the wound defect sutured with PCL-MA suture.

After 3 days of the experimental study, the MNEC value increased by 2.21 times compared to the Control group.

After 7 days of the experiment, the above index increased by 2.67 times compared to the Control group, and compared to the previous observation period, the index of MNEC also increased by 1.21 times, which indicates an increase in the endocrine status in the perivulnar area when using PCL-MA sutures.

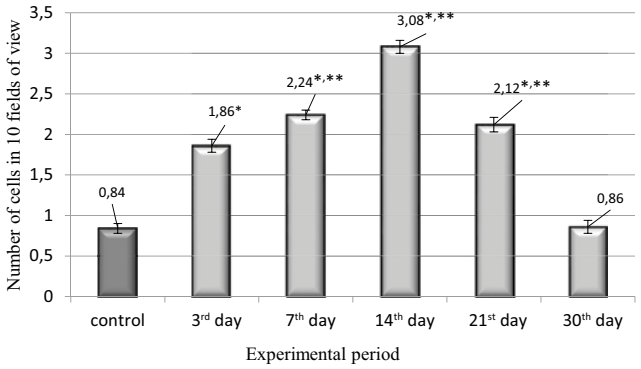
After 14 days of observation, the MNEC index continued to increase: 3.67 times compared with the control values and 1.38 times compared with the previous observation period. This indicates an even greater stress on the endocrine background in the perivulnar area when using PCL-MA sutures.

After 21 days of the experimental study, we found that, compared with the control values, the MNEC was 2.52 times higher, and compared with the previous observation period, it decreased by 1.45 times. Such changes indicate that after 21 days of observation, the tension of the endocrine background decreases due to L-arginine, which modified the polycaprolactone thread.

After 30 days of the experimental study, the mean number of enteroendocrine cells in the crypts of the perivulnar area did not differ from those of the Control group of animals and the previous observation period. It was within the statistical error (Fig. 5).

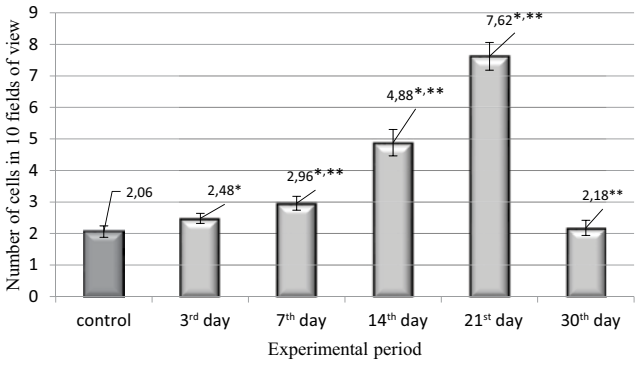
In our opinion, low-differentiated cells in the crypts play a significant role in the regeneration of the perivulnar area after surgery. Their proliferation ensures the restoration of the pool of CECwB, GC, and surface epithelial cells.

Thus, after 3 days of the experimental study, we found that the mean number of low-differentiated cells (MNLDC) increased by 1.24 times. After 7 days, compared to the Control group, the value of the MNLDC increased by 1.24 times and, compared to the previous observation period, by 1.19 times. After 14 days of the experiment, there was a tendency to increase the MNLDC value compared to the Control group and the previous observation period by 2.44 times and 1.65 times, respectively. Morphometric changes in the index of MNLDC after 21 days of the experimental study demonstrate the



Note: * – statistically significant at p<0.05 compared to the Control group, ** – statistically significant at p<0.05 compared to the previous observation period.

Fig. 5. Dynamics of changes in the MNEC indices in the crypts of the cecum mucosa in the perivulnar area during suturing of the wound defect with PCL-MA suture in the experiment.



Note: * – statistically significant at p<0.05 compared to the Control group, ** – statistically significant at p<0.05 compared to the previous observation period.

Fig. 6. Dynamics of changes in the MNLDC indices in the crypts of the cecum mucosa in the perivulnar area during suturing of the wound defect with PCL-MA suture in the experiment

above trend towards an increase in the number of low-differentiated cells in the crypts of the perivulnar area by 3.81 times compared to the Control group, and by 1.56 times compared to the previous period. After 30 days of the experiment, the above indicator did not differ from the Control group and, compared with the previous observation period, it significantly decreased by 3.49 times, indicating the restoration of the MNLDC index to the control level when using PCL-MA suture (Fig. 6).

Our results confirm that the crypts of the caecal mucosa actively participate in post-surgical regeneration. Structural changes are observed not only in the depth and width of the crypt bases but also in their cellular composition. Specifically, both quantitative and qualitative alterations in the MNCECwB contribute to impaired absorption of water and electrolytes. Dynamic fluctuations in the number of GC may indicate disruption of the mucosal barrier function. The increased numbers of low-differentiated and enteroendocrine cells reflect the activation of reparative processes, likely stimulated by the L-arginine component of the PCL-MA suture [2]. Several studies have demonstrated that the caecal mucosa can undergo significant changes under the influence of exogenous factors, particularly during surgical intervention [4]. The choice of suture material plays a critical role in this response [17,18]. Additionally, previous histological studies suggest that monofilament absorbable sutures such as polycaprolactone cause fewer inflammatory changes than multifilament threads [8]. However, our data provide novel morphometric evidence that PCL-MA thread not only minimizes inflammation but also promotes epithelial restoration at the crypt level.

Thus, the use of PCL-MA suture material has a beneficial effect on the differentiation processes and the formation of low-differentiated cells within the crypts of the perivulnar region, thereby accelerating the restoration of the mucosal lining and the intestinal wall as a whole.

Conclusions

1. Using PCL-MA sutures for suturing the wound defect leads to a change in the histotopography of crypts as a structural and functional unit of the cecum mucosa. In the early stages of the experiment, the crypts deepened into the mucosa, reducing the lumen of the intestine in the perivulnar area. The histotopography of the crypts was restored within 21 days of the experiment due to L-arginine, which modified the surgical polycaprolactone thread.

2. It was found that the number of absorptive enterocytes in the crypts at the early stages of the experiment sharply decreased, which is associated with their apoptosis. When using PCL-MA sutures, due to the L-arginine contained on their surface, the number and functioning of absorptive enterocytes were restored from the 21st day of the experimental study.

3. Goblet cells' reaction in the crypts of the perivulnar area in the early stages of the experiment ensures the formation of a protective mucosal barrier, as evidenced by an increase in their number (from the 3rd day to 14th day of the experiment) and the presence of a significant number of cells in the stage of degranulation. When using a PCL-MA suture, the restoration and normalization of the functioning of mucosal exocrinocytes of the perivulnar area occur after 21 days of the experiment.

4. Enteroendocrine cells in the crypts of the rabbit cecum mucosa in the perivulnar area migrated to the periphery of the crypts in the early stages of the experiment, significantly increasing their number after 21 days of observation; local tension of the endocrine background in the perivulnar area decreases due to L-arginine, which modified surgical polycaprolactone thread.

5. Low-differentiated cells in the crypts are crucial for regenerating the perivulnar area post-surgery, as their proliferation restores the pool of absorptive enterocytes, goblet cells, and surface epithelial cells. The use of a PCL-MA suture positively influences the differentiation and formation of these low-differentiated cells within the crypts of the perivulnar area.

No conflict of interests was declared by the authors.

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Стаття надійшла до редакції 15.11.2024 р., прийнята до друку 18.03.2025 р.