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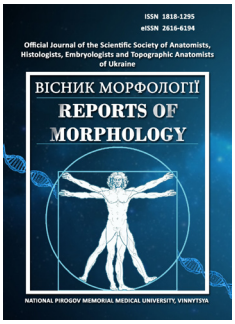

Experimental evaluation of the influence of non-invasive and invasive methods of fixation of prostheses in herniology on the morphology of peripheral nerve structures

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To date, chronic postoperative inguinal pain is one of the most common complications of surgical treatment of inguinal hernias in the late postoperative period. The use of mesh implants is the foundation of almost all inguinal hernia repair techniques. However, the diversity and fundamental difference in their fixation methods necessitate a more targeted comparative evaluation, including regarding their impact on the development of chronic postoperative pain syndrome. The introduction of adhesive fixation techniques into herniology could potentially solve the dilemma between ensuring reliable prosthesis fixation and reducing the risk of chronic inguinal pain in the postoperative period. However, the choice between non-invasive and invasive fixation methods remains a matter of ongoing debate, especially considering their potential implications for peripheral nerve structures. Aim – to conduct a comparative experimental assessment of the influence of invasive and non-invasive methods of mesh implant fixation on the morphology of peripheral nerve tissues and perineural structures. The study included 15 experimental animals (male white Wistar laboratory rats), divided into four groups: group 1 (control) – 3 rats that underwent surgical access to the sciatic nerve only; group 2 (glue) – 4 rats in which tissue fixation in the area of the sciatic nerve was performed using n-butyl-2-cyanoacrylate glue without the use of prosthetic material; group 3 (mesh + glue) – 4 rats in which prosthetic material was fixed near the sciatic nerve using n-butyl-2-cyanoacrylate glue; group 4 (mesh + stapler) – 4 rats in which prosthetic material was fixed near the sciatic nerve using absorbable staples with the AbsorbaTack® stapler. Following the termination of the experiment, perineural tissues were harvested from the sciatic nerve area and subjected to histopathological analysis with a qualitative assessment of tissue changes in all groups. In the glue-induced tissue response, an early macrophage reaction was observed in all samples of the "glue" group and in one sample of the "mesh + glue" group. Histopathological examination demonstrated that the tissue response induced by cyanoacrylate was limited and less pronounced compared to the "mesh + stapler" group. In the "mesh + glue" group, a mixed-cell inflammatory reaction, fibroplasia, and especially neoangiogenesis in the sciatic nerve zone were more pronounced than in the "glue" group, but significantly less intense than in the "mesh + stapler" group. On day 15 of the experiment, a pronounced reduction in cellular inflammatory response, thinning of the fibrotic capsule, and decreased vascularization and neoangiogenesis were observed around cyanoacrylate deposits. In the "mesh + stapler" group, across the entire contact area with the mesh, marked pathological changes were identified, including sclerosis, fibrosis, necrosis, formation of serocellular mass zones, and granulomatous inflammation. This group also showed the most pronounced changes in the sciatic nerve tissue, including axonal cytoplasmic vacuolization, severe intramyelinic edema, endoneural vessel proliferation with marginalization of inflammatory cells, and axonal degeneration. Thus, the obtained morphological data confirm that the use of glue, compared to the stapler technique using AbsorbaTack® in the area of peripheral neural structures, is potentially safer.

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These findings highlight the need for a comparative clinical assessment of the functional outcomes of surgeries, especially in terms of postoperative pain syndrome following different mesh fixation approaches.
Keywords: *inguinal hernia, implants, sciatic nerve, adhesives, foreign-body reaction.*

Introduction

The recent revolutionary changes in herniology in general, and inguinal hernia surgery in particular, are primarily associated with the introduction of synthetic prostheses for abdominal wall reconstruction and the implementation of minimally invasive (laparoscopic) surgical techniques into clinical practice [27, 33].

These advancements have significantly reduced the incidence of inguinal hernia recurrence – from up to 7-15 % in open hernia repairs without mesh, to up to 5 % in open repairs using mesh, and approximately 1.5-2 % in laparoscopic techniques such as transabdominal preperitoneal (TAPP) and total extraperitoneal (TEP) inguinal hernia repair [1, 11, 15]. This progress has led to a shift in the focus of researchers towards evaluating the functional outcomes of surgical interventions [17, 24, 25, 36].

Indeed, the analysis of modern literature convincingly shows that the speed and completeness of physical activity recovery, as well as the comfort and quality of life of patients, are no less important components of surgical success than the "anatomical reliability" of inguinal reconstruction [22]. The main reason for the deterioration of postoperative functional outcomes is chronic postoperative inguinal pain (CPIP) [4, 20]. The mechanism of CPIP development is complex and multifactorial, and the identification of its individual pathogenetic components remains largely conditional [16]. However, most authors undoubtedly recognize the significant role of synthetic prostheses and their fixation methods in affecting peripheral nerve structures during this process [18].

And while the use of mesh implants in modern inguinal hernia repair techniques remains inevitable, the variety and fundamental differences in their fixation methods necessitate a more targeted comparative assessment – particularly regarding their potential impact on the development of CPIP [3].

Aim – to perform a comparative experimental evaluation of the impact of invasive and non-invasive mesh fixation methods on the morphology of peripheral nerve tissues and perineural structures.

Materials and methods

To address this objective, we employed the methodology of an acute experimental model. The study was conducted on laboratory animals by performing surgical access to the sciatic nerve, followed by the application of either invasive (endoscopic hernia stapler AbsorbaTack® 5 mm, Covidien, USA) or non-invasive (n-butyl-2-cyanoacrylate glue LiquiBand® FIX8, Advanced Medical Solutions Group, UK) methods for the fixation of prosthetic material (macroporous monofilament non-absorbable polypropylene mesh Parietene®, Covidien, USA) or surrounding tissues in the affected area.

The study, which involved performing surgical procedures in strict accordance with all requirements of asepsis and antisepsis, was conducted at the National University of Life and Environmental Sciences of Ukraine. The research was carried out in compliance with ethical standards in accordance with the provisions of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (March 18, 1986), as well as the requirements of: the Regulations on the Procedure for Scientific Institutions to Conduct Animal Experiments, approved by the Order of the Ministry of Education and Science, Youth and Sports of Ukraine No. 249 dated March 1, 2012, and the Procedure for Preclinical Study of Medicinal Products and Examination of Preclinical Study Materials, approved by the Order of the Ministry of Health of Ukraine No. 944 dated December 14, 2009. The study protocol was approved by the Ethics Committee for Scientific Research (Meeting Protocol No. 3 dated September 21, 2023).

White male Wistar line laboratory rats (Rattus norvegicus), weighing between 260 and 340 grams (mean weight: 297.3±25.8 g), were used in the study. A total of 15 animals were randomly assigned to four groups: group 1 – Control (n=3): rats that underwent surgical exposure of the sciatic nerve only, without any fixation; group 2 – Glue (n=4): rats that underwent tissue fixation in the sciatic nerve region using n-butyl-2-cyanoacrylate glue, without implantation of prosthetic material; group 3 – Mesh + Glue (n=4): rats that underwent fixation of prosthetic material in the sciatic nerve region using n-butyl-2-cyanoacrylate glue; group 4 – Mesh + Stapler (n=4): rats that underwent fixation of prosthetic material in the sciatic nerve region using a herniostapler with absorbable staples.

The primary objective of the experimental study was to assess the qualitative nature and severity of pathohistological changes in the sciatic nerve and perineural tissues resulting from the use of different types of fixation materials and prosthetic implants.

Throughout all stages of the experiment, the animals were housed individually in cages under controlled room temperature conditions, with a natural light–dark cycle. General anesthesia was induced via intramuscular injection of a combination of tiletamine/zolazepam (Telazol®; 10 mg/kg; 100 mg/mL tiletamine and 100 mg/mL zolazepam) and dexmedetomidine (Dexdor®; 0.25 mg/kg; 0.1 mg/mL).

After induction of anesthesia, preliminary shaving, and surgical site preparation with antiseptic agents, access to the sciatic nerve was performed via gluteal incision. Depending on the experimental group: in the control group, the wound was sutured immediately after nerve exposure without any additional intervention; in the glue group, n-butyl-2-cyanoacrylate glue was applied directly to the nerve area; in the mesh + glue group, a 2×2 cm piece of macroporous polypropylene mesh (Parietene®, Covidien, USA) was fixed

in the sciatic nerve region using cyanoacrylate glue; in the mesh + stapler group, the same mesh was fixed perineurally using an endoscopic herniostapler with absorbable staples. In all groups, the wound was closed with monofilament absorbable suture material (Maxon®, Covidien, USA), size 3-0. On postoperative day 15, the animals were euthanized by intraperitoneal overdose injection of 10 % sodium thiopental solution.

After euthanasia, perineural tissues from the sciatic nerve region were harvested and subjected to pathological examination with qualitative assessment of histomorphological changes across the study groups.

Tissue samples were fixed in 10 % neutral buffered formalin. Following fixation, the specimens were processed and paraffin-embedded according to a standard histological protocol. Paraffin sections with a thickness of 5 μm were stained with hematoxylin and eosin (H&E), which enabled general morphological assessment of the examined material and evaluation of histoarchitectural alterations under experimental conditions. Histological analysis was performed using a Leica DM3000 light microscope.

Results

Throughout the entire observation period, none of the animals in any of the study groups exhibited clinical signs indicative of severe pain or systemic inflammatory response.

On postoperative day 15, histological examination revealed axonal cytoplasmic vacuolization and intramyelinic edema in the sciatic nerve tissue across all samples, with varying degrees of severity. The most pronounced nerve damage was observed in the mesh + stapler group (Fig. 1).

Notably, in the tissue sections, cyanoacrylate glue deposits and mesh components were visualized as optically empty areas with irregular contours, surrounded by inflammatory tissue reactions. In most cases, the mesh itself was not represented in the sectioned plane; however, in one sample (mesh + glue group), mesh elements were partially captured within the histological section. Similar optically clear voids were also observed in the regions corresponding to staple placement, which had been removed during specimen processing prior to paraffin embedding (Fig. 2).

In the control group, sample No. 1 demonstrated perineural edema in the area of surgical access, accompanied by predominantly localized acute inflammatory infiltration composed of lymphocytes, plasma cells, neutrophils, eosinophils, and occasional macrophages. In the remaining two samples (No. 2 and No. 3), the inflammatory response was less pronounced, with a reduction in fibrous thickening and decreased vascularization compared to sample No. 1 (Fig. 3).

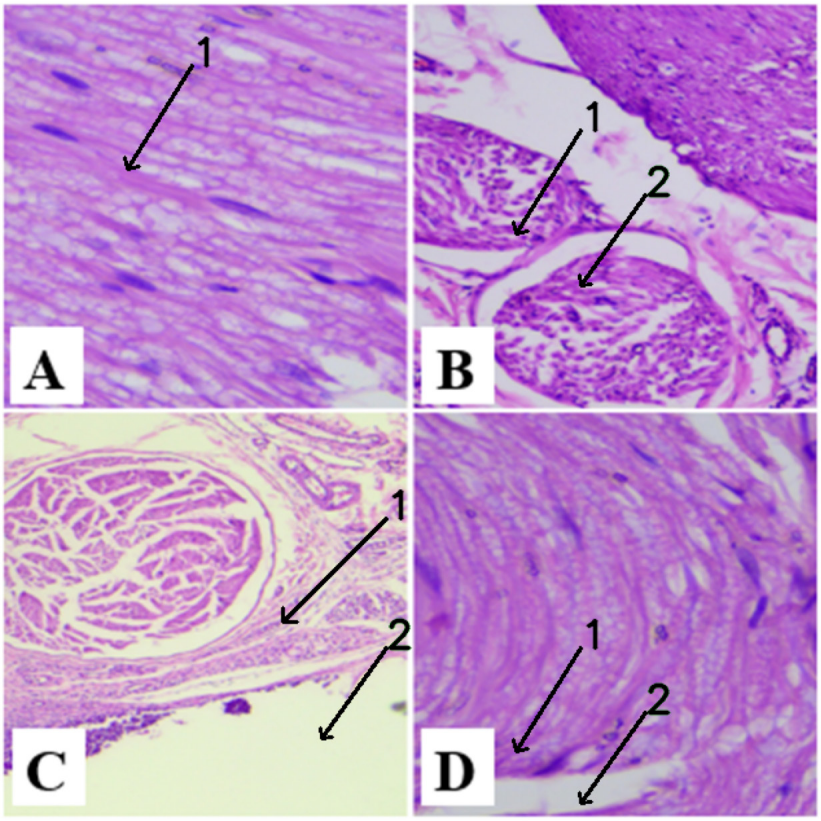

Fig. 1. Section of the femoral segment of the sciatic nerve in a rat. 1 – nerve fibers; 2 – areas of axonal cytoplasmic vacuolization and intramyelinic edema. A – Control group, magnification ×400; B – Glue group, magnification ×100; C – Mesh + Glue group, magnification ×100; D – Mesh + Stapler group, magnification ×100. Staining: hematoxylin and eosin (H&E).

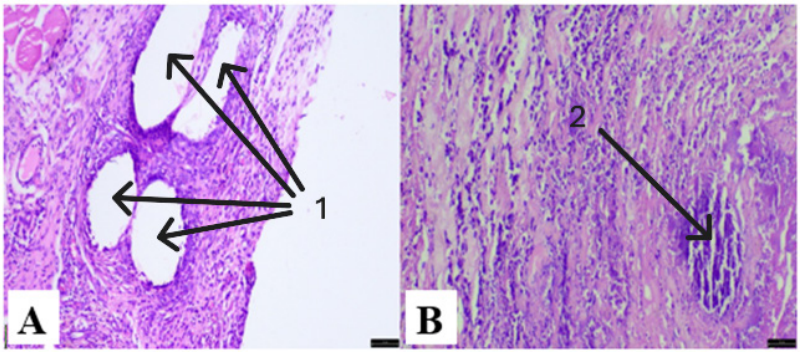

Fig. 2. Section of the femoral segment of the sciatic nerve in a rat. 1 – void areas corresponding to the former location of the mesh; mesh fragments were not preserved in the histological section; 2 – granulomatous inflammatory process. A – Mesh + Stapler group, magnification ×100; B – Mesh + Glue group, visible mesh fragments, magnification ×200. Staining: hematoxylin and eosin (H&E).

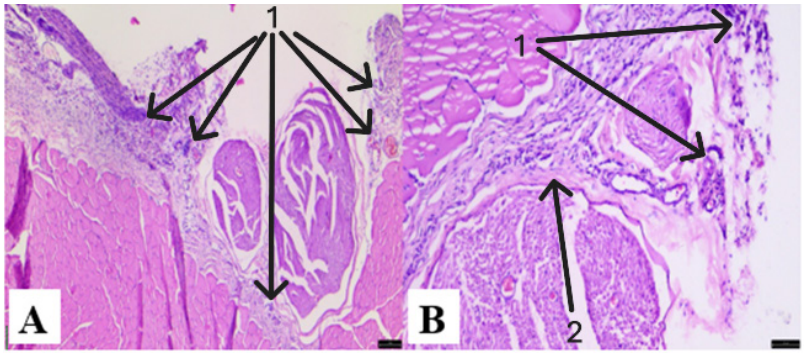

Fig. 3. Section of the femoral segment of the sciatic nerve in a rat (Control group). 1 – infiltration of connective tissue surrounding the sciatic nerve by lymphocytes, and to a lesser extent by eosinophils and neutrophils; 2 – intact muscle tissue. A – sample No. 1, magnification ×40; B – sample No. 2, magnification ×100. Staining: hematoxylin and eosin (H&E).

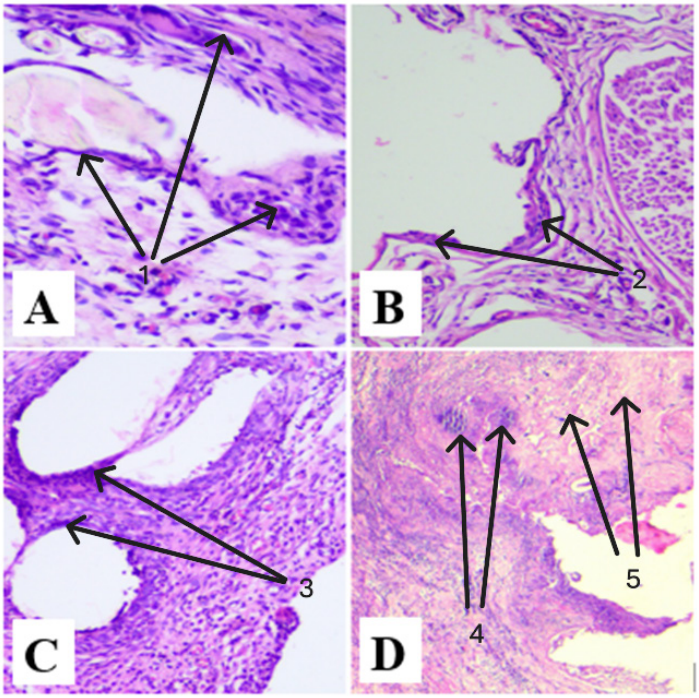

Fig. 4. Section of the femoral segment of the sciatic nerve in a rat. 1 – connective tissue surrounding the sciatic nerve infiltrated predominantly by lymphocytes, with fewer eosinophils and neutrophils; 2 – vascular-fibrous connective tissue capsule around the glue, composed of reactive fibroblasts and infiltrated by lymphocytes and macrophages; 3 – connective tissue capsule around the mesh area, infiltrated mainly by lymphocytes and to a lesser extent by eosinophils and neutrophils; 4 – formation of granuloma-like structures surrounded by a mixed-cell inflammatory ring with the presence of eosinophils and plasma cells; 5 – connective tissue replaced by zones of sclerosis, fibrosis, and areas of necrosis. A – Control group, magnification ×200; B – Glue group, magnification ×100; C – Mesh + Glue group, magnification ×100; D – Mesh + Stapler group, magnification ×40. Staining: hematoxylin and eosin (H&E).

Analysis of the inflammatory infiltrate composition at the periphery of the implantation sites of cyanoacrylate, mesh, and staples in the glue, mesh + glue, and mesh + stapler groups revealed several significant differences.

Tissue changes induced by cyanoacrylate were characterized by an early macrophage response, which was observed in all samples of the glue group and in one sample of the mesh + glue group. Histopathological examination demonstrated that the tissue reaction to cyanoacrylate was limited and less pronounced compared to the mesh + glue and mesh + stapler groups. On day 15 postoperatively, the cyanoacrylate deposits were surrounded by markedly reduced cellular inflammatory response, thinning of the fibrotic capsule, and decreased vascularization and neoangiogenesis.

In the mesh + glue and mesh + stapler groups, the inflammatory response in the sciatic nerve region surrounding the mesh was more intense than in the glue group. In the mesh + stapler group, three of the samples (№ 8, № 10, № 11) exhibited extensive pathological changes along the entire mesh-tissue interface, including sclerosis, fibrosis, necrosis, formation of serocellular masses, and granulomatous inflammation. The mixed-cell inflammatory response was also most pronounced in this group. Moreover, the sciatic nerve tissue in the mesh + stapler group exhibited the most severe alterations: axonal cytoplasmic vacuolization (in all samples), pronounced intramyelinic edema (in all samples), proliferation of endoneurial vessels with marginalization of inflammatory cells (in all samples), and axonal degeneration (in one sample).

In the mesh + glue group, a mixed-cell inflammatory response, fibroplasia, and especially neoangiogenesis in the sciatic nerve region were also more pronounced than in the glue group, but considerably less intense than in the mesh + stapler group (Fig. 4).

Discussion

CPIP is one of the most common complications following surgical treatment of inguinal hernias in the long-term postoperative period. Pain plays a critical role and is often the most indicative factor of a patient's functional outcomes and quality of life. Its intensity can lead patients to perceive the surgery as unsuccessful, even in cases where the procedure was technically correct and the hernia was "anatomically" resolved.

The most frequent cause of CPIP is mechanical injury to the branches of the genitofemoral, ilioinguinal, and lateral femoral cutaneous nerves, often resulting from entrapment of nerve fibers by suture material or staples – this type of pain is referred to as neuropathic. It is essential to distinguish this form of pain from nociceptive pain, which is caused by the release of neurotransmitters as a reactive response to tissue trauma and local inflammation in the area of surgical intervention and mesh implantation [2]. Nociceptive pain typically subsides over time as the inflammatory process resolves and generally responds well to conservative management with non-steroidal anti-inflammatory drugs (NSAIDs) and paracetamol. In contrast, neuropathic CPIP is persistent and continuous, responds poorly to NSAIDs, and may even necessitate repeat surgical intervention, including neurectomy [5, 14]. A pain duration of at least three months postoperatively is one of the primary diagnostic criteria for CPIP [19].

Despite the potential risks and disadvantages associated with invasive prosthetic fixation methods, the routine use of non-fixation techniques aimed at reducing the incidence of chronic pain – particularly in laparoscopic TAPP repair– remains controversial and likely unjustified due to an increased risk of recurrence. This is especially true for large medial hernia defects (Nyhus type IIIa) and recurrent hernias (Nyhus type IV) [12].

In a randomized controlled trial conducted by Zwaans W. A. R. et al. [36], long-term outcomes over a 3-year follow-up were evaluated in patients with primary inguinal hernias treated using the traditional open technique versus those treated with ProGrip® self-gripping mesh. The authors reported no significant difference in the incidence of chronic inguinal pain between the groups. However, the recurrence rate in the ProGrip group was notably higher compared to the standard treatment group – 11.5 % vs. 5.0 %, respectively ($p=0.05$).

Thus, the topic of prosthetic fixation in hernia repair remains relevant and is likely to remain a focal point of surgical debate in the near future.

A thorough understanding of both classical and laparoscopic inguinal anatomy, along with the concept of the "pain triangle" – which includes branches of the genitofemoral and lateral femoral cutaneous nerves – is critically important for surgeons. These anatomical considerations must be taken into account when planning mesh fixation. However, even a comprehensive understanding of this so-called "forbidden triangle" does not fully eliminate the risk of iatrogenic injury to small nerve branches that may lie outside its borders. According to some reports, the risk of intraoperative nerve injury reaches up to 80 % for the lateral femoral cutaneous nerve and approximately 30 % for the femoral branch of the genitofemoral nerve [6, 34].

The ongoing concern of nerve injury during mesh fixation has prompted the development of a wide range of fixation devices aimed at optimizing the balance between fixation reliability and functional outcomes. Initial research focused on the evolution of stapling materials – from non-absorbable titanium tacks (ProTack®) to absorbable variants such as AbsorbaTack®, Permasorb®, and SorbaFix®. Further investigations explored the impact of tack insertion angle and the introduction of rotating staplers (e.g., ReliaTack®) in hernia repair [8]. Although some studies have reported slight improvements in fixation reliability with angled or rotating staplers, the incidence of CPIP has largely remained unchanged [23, 29, 30, 35].

Attempts to reduce the number of fixation points likewise failed to significantly improve postoperative pain outcomes [26].

These limitations led to the development of fundamentally new fixation strategies, guided by the following criteria: reliability (to prevent mesh dislocation and shrinkage), low recurrence rates, non-invasiveness (minimizing direct mechanical nerve injury), biological inertness (reducing long-term indirect pro-inflammatory effects that may contribute to CPIP), and cost-effectiveness.

These considerations laid the foundation for the introduction of fibrin and synthetic adhesives as alternative fixation tools in herniology. Subsequent studies have shown no statistically significant difference in recurrence or reoperation rates between glue fixation and traditional stapling methods [9, 21, 28].

Importantly, long-term CPIP incidence in the adhesive fixation group was only 2.4 %, which was significantly lower than the 4.7 % observed in the stapler fixation group [32]. These findings have been corroborated by multiple large-scale randomized trials [7, 31].

In a 2021 meta-analysis by Habib Bedwani N. A. R. et al. [10], cyanoacrylate glue use was associated with a significant reduction in CPIP incidence compared to stapling – from 7.5 % to 3.0 % (p=0.002).

Nevertheless, a number of researchers continue to emphasize the need for further studies on the safety, effectiveness, and long-term outcomes of adhesive-based fixation in hernia surgery [13].

Conclusions

1. The use of widely accepted herniological techniques for prosthetic material fixation and tissue approximation in the sciatic nerve region of laboratory animals resulted in the development of a mixed-cell inflammatory response and post-inflammatory perineural changes. These included sclerosis, fibrosis, necrosis, the formation of serocellular masses, and granulomatous inflammation, as well as inflammatory alterations in the sciatic nerve itself in the form of axonal cytoplasmic vacuolization, intramyelinic edema, endoneurial vessel proliferation with marginalization of inflammatory cells, and axonal degeneration.

2. The minimal severity of the inflammatory response in the group with isolated use of glue, its moderate intensity in the mesh + glue group, and maximal manifestations in the mesh + stapler group suggest a combined effect of the prosthetic material and the chosen fixation method on the development of inflammation.

3. The obtained morphological data indicate that the use of cyanoacrylate glue, compared to the stapling technique with AbsorbaTack®, is potentially safer for peripheral nerve structures, and underscore the need for comparative clinical evaluation of functional surgical outcomes, particularly with regard to postoperative pain syndrome.

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ЕКСПЕРИМЕНТАЛЬНА ОЦІНКА ВПЛИВУ НЕІНВАЗИВНИХ ТА ІНВАЗИВНИХ МЕТОДІВ ФІКСАЦІЇ ПРОТЕЗІВ У ГЕРНІОЛОГІЇ НА МОРФОЛОГІЮ ПЕРИФЕРІЙНИХ НЕРВОВИХ СТРУКТУР

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На сьогоднішній день хронічний післяопераційний пахвинний біль є одним із найпоширеніших ускладнень хірургічного лікування пахвинних кил у віддаленому післяопераційному періоді. Використання сітчастих імплантатів є основою в практично усіх методиках пластики пахвинних гриж, однак різноманітність та принципова відмінність методів їх фіксації зумовлює потребу їхньої більш прицільної порівняльної оцінки, в тому числі – стосовно впливу на розвиток хронічного післяопераційного больового синдрому. Впровадження клейових методик фіксації для застосування в герніології потенційно могло б вирішити проблему вибору між надійністю фіксації протезного матеріалу та ризиками розвитку хронічного пахвинного болю в післяопераційному періоді. Проте вибір між неінвазивними та інвазивними методами фіксації все ще залишається предметом дискусії з потенційними наслідками для периферичних нервових структур. Мета роботи – провести порівняльну експериментальну оцінку впливу інвазивних та неінвазивних методів фіксації сітчастих імплантатів на морфологію тканин периферичних нервів та перинервальних структур. До дослідження увійшло 15 експериментальних тварин (білі лабораторні щури-самці лінії Wistar), розділені на 4 групи: група 1 (контроль) – 3 щури, котрим виконували лише хірургічний доступ до сідничного нерву; група 2 (клей) – 4 щура, котрим в зоні сідничного нерву фіксували тканини n-бутил-2-ціаноакрилатним клеєм без застосування протезного матеріалу; група 3 (сітка+клей) – 4 щури, котрим фіксували протезний матеріал в зоні сідничного нерву n-бутил-2-ціаноакрилатним клеєм; група 4 (сітка+степлер) – 4 щури, котрим протезний матеріал в зоні сідничного нерву фіксували герніостеплером AbsorbaTack®. Після виведення тварин з експерименту проводили забір перинервальних тканин із ділянкою сідничного нерва з подальшим патогістологічним дослідженням та якісною оцінкою змін в досліджуваних групах. Зміни в тканині індуковані ціанокрилатом характеризувалися ранньою макрофагальною відповіддю, яка спостерігалася в усіх зразках групи «клей», та в одному зразку групи «сітка+клей». Гістопатологічні дослідження продемонстрували, що реакція, викликана ціанокрилатом, була обмеженою та менше вираженою в тканинах порівняно з групою «сітка+степлер». В групі «сітка+клей» змішаноклітинна запальна реакція, фіброплазія та особливо неоангіогенез зони сідничного нерву були також більш виражені ніж у групі «клей», однак значно меншою мірою ніж у групі «сітка+степлер». На 15 добу дослідження навколо відкладень клею спостерігалися виражене зниження клітинної запальної реакції, потоншення фібротичної капсули, знижена васкуляризація і неоангіогенез. В групі «сітка+степлер» на всій площі контакту із сіткою, розвинулися явища склерозу, фіброзу, некрозу, формування зон сероцелюлярної маси та гранульоматозу. Також в цій групі відмічалися найбільш виражені зміни в тканині сідничного нерву, що проявлялися розвитком аксональної цитоплазматичної вакуолізації, вираженим інтрамієліновим набряком, проліферацією ендоневральних судин із маргінізацією клітин запалення та аксональною дегенерацією. Таким чином, одержані морфологічні дані дозволяють стверджувати, що використання клею порівняно зі степлерною методикою з використанням AbsorbaTack® в ділянці периферійних нервових структур є потенційно більш безпечним та доводять необхідність порівняльної клінічної оцінки функціональних результатів операцій.

Ключові слова: *пахвинна грижа, імплантати, сідничний нерв, клеї, реакція на чужорідне тіло.*

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Trots A. V. – work concept and design, data collection and analysis, responsibility for statistical analysis, writing the article.
Perekhrestenko O. V. – critical review, final approval of the article.