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Prevention of Post-Traumatic Adhesive Process Around the Achilles Tendons of Rabbits

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The problem of treatment of tendon injuries remains unresolved despite on the variety of ways for its restoration. Unsatisfactory outcomes after surgical treatment according to different authors range from 15 to 62 %. The most important problem of tendon surgery is restoration of its function of sliding.

***Objective**: To study the structural organization of the injured tendon by implementation of medications around tendon suture.*

***Methods**: The experiments were performed on 12 mongrel rabbits aged 12-18 months, weight 1850-2000 g. Partial Achilles tendon injury was modeled after cutting it at 1/2 diameter. In the control group (1st series) stitches were not treated. In experimental animals we injected around the tendon suture in the 2nd series — 1 ml 1 % hyaluronic acid (Syn- gial™), 3rd series — 1 ml of 4.5 % three-dimensional polyacrylamide polymer (Noltrex™), 4 ,h series — 1 ml 64IU hyaluronidase/ ml (Lydasa-Biolek™). The study was conducted by means of light and polarization microscopy.*

***Results**: after 60 days in the area of traumatic injury tendon-like tissue that contained diverse bundles of collagen fibers made of collagen types I and III formed. Maximum accumulation of collagen type III was detected in regenerates of the animals in 1 and 4 series. Destructive tendon disorders in the areas located above or below of the injury zone found in all series of experiments: the most pronounced in the 4 (II stage), less pronounced — in 2 and 3 (I stage). The growth of connective tissue that violates sliding of bundles of collagen fibers was the most pronounced in the 1st and 4th series of experiments.*

***Conclusion**: For restoration of sliding function of the tendon andfor prevention of tendon adhesions can be recommended medications Syngial™ and Noltrex™.*

Проблема лікування хворих з ушкодженнями сухожиль залишається невирішеною, незважаючи на різноманітність способів його відновлення. Незадовільні результати після хірургічного лікування, за інформацією різних авторів, становлять від 15 до 62 %. Найважливішою проблемою хірургії сухожиль є відновлення їх функції ковзання. Мета: вивчити структурну організацію травмованого сухожилля шляхом введення навколо сухожильного шва медикаментозних препаратів. Методи: експерименти виконані на 12 безпородних кролях віком 1218 міс., масою 1850-2000 г. Часткове ушкодження ахіллового сухожилля моделювали, перетинаючи його на 1/2 діаметру. У контрольній групі (1-а серія) шви не обробляли. У дослідних тварин навколо сухожильного шва вводили: 2-а серія — 1 мл 1 % гіалуронової кислоти (Сингіал™), 3-я серія — 1 мл 4,5 % тривимірного поліакриламідного полімеру (Нолтрекс™), 4-а серія — 1 мл 64 ОД гіалуронідази/мл (Лідаза-Біолік™). Дослідження проведено за допомогою світлової та поляризаційної мікроскопії. Результати: через 60 діб в ділянці травматичного ушкодження формувалася сухожилкоподібна тканина, яка містила різноспрямовані пучки колагенових волокон, виконаних колагеном I і IIIтипів. Максимум накопичення колагену IIIтипу виявлений у регенератах тварин 1 та 4-ї серій. Деструктивні порушення в ділянках сухожилля, розташованих вище або нижче зони травми, встановлені в усіх серіях експерименту: найбільш виражені в 4-й (II стадія), менш виражені — у 2 і 3-й (I стадія). Розростання сполучної тканини, що порушує ковзання пучків колагенових волокон, виявилося найбільш вираженим у 1 і 4-й серіях експерименту. Висновок: для відновлення ковзної функції сухожилля і профілактики спайок можна рекомендувати препарати Сингіал™ і Нолтрекс™. Ключові слова: ахіллове сухожилля, експеримент, травматичне ушкодження, відновлення, медикаментозні препарати, спайки.

Key Words: Achilles tendon, experiment, traumatic injury, rehabilitation, medications, adhesions

Introduction

The problem of treatment of tendon injuries remains unresolved despite on the variety of ways for its rehabilitation [1, 2]. Unsatisfactory outcomes after surgical treatment according to different authors range from 15 to 62 % [3, 4]. The functional outcome after surgical repair of damaged tendons is difficult to predict

due to the high risk of cicatrical block formation, which prevents their free sliding [5, 6]. In this regard, the most important problem of tendon surgery is the restoration of its sliding function [7].

The appearance of adhesions of a damaged tendon is a natural process during the regeneration. Connecting the tendon ends with the surrounding tissues, adhesions with a well-developed vascular network contribute to the restoration of the blood supply in the damaged tendon and the union of its ends. But, having played a positive role at the beginning of the regeneration, adhesions become an obstacle and disrupt the sliding function of the tendon later [4].

In the modern literature, there is not enough data reflecting experimental and morphological studies on reparative regeneration of tendons under conditions of using biomaterials that prevent the formation of cicatricial adhesive process.

Purpose of the study: to study the structural organization of the injured tendon by administration of medications around the tendon suture.

Material and Methods

The experiments were performed on 12 mongrel rabbits aged 12-18 months, weight 1850-2000 g. 12Achilles tendons were operated on.

A model was created for the partial injury of the Achilles tendon by transecting it by 1/2 of the diameter. Then the injured tendon was sutured (modified locking Kessler suture and twisted suture). The monofilament poliamide 3/0, 6/0 suture material was used.

After the traumatic injury, the animals were divided into four groups: one control and three experimental, three rabbits in each:

1st series — the control group. After the tendon suture, the wounds were sutured;

2nd series — the experimental group. 1 mL of 1% hyaluronic acid (Synhyal™) was administered around the tendon suture to animals.

3rd series — the experimental group. Animals received 1 mL of 4.5% three-dimensional polyacrylamide polymer (Noltrex™) around the tendon suture.

4th series — the experimental group. Animals were injected 1 ml of 64 U of hyaluronidase/ml (Lydasa-Biolek™) around the tendon suture.

After the injection of medications, the wounds were sutured. The animals were removed from the experiment 60 days after the surgery. The work with animals was carried out in accordance with the requirements of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes", as well as the Ukrainian legislation [8].

The study was carried out using the methods of light and polarizing microscopy.

Tendons were fixed in a 10% solution of neutral formalin, dehydrated in alcohols of increasing concentration, and embedded in celloidin. Longitudinal histological sections were prepared, which were stained with hematoxylin and eosin, as well as van Gieson's picro-fuchsin [9] and analyzed using an Axiostar Plus microscope (Carl Zeiss). To assess gl**y**cosaminoglycans, sections were stained with toluidine blue at pH 2.5; and for the assessment of collagen — picrosirius red [10]. Collagen types were analyzed in polarized light (Polmy-A microscope). The preparations were photographed using a Canon EOS-300D digital camera.

The effect of medications (when compared with control animals) on the following indicators of the injured tendon was studied: post-traumatic regeneration; the condition of the tendon in the areas located above and below the area of traumatic injury; the sliding function of the bundles of collagen fibers in the tendon and the relation of the tendon to the surrounding tissues.

To assess the structural organization of the tendon outside the injured area, longitudinal sections of the tendon sections located above the zone of the experimentally reproduced injury were analyzed (2 fields of view of the microscope, magn. 80), using the semi-quantitative rating scale Movin and Bonar [11] in our modification (table). The indicators were evaluated in points from 1 to 4, analyzed in each series of 9 sections.

The results of the semi-quantitative assessment are presented on a scale within the following limits: from 1 to 8 points — the norm, from 9 to 16 — mild disorders (stage I), from 17 to 24 — moderate disorders (stage II), expressed from 25 to 32 points (III stage).

Results and Discussion

The postoperative period in animals was uneventful, the wounds healed by primary intention. The average healing time of postoperative wound in the control and experimental groups did not differ and was 7 days.

Tendon regeneration under conditions of modeling traumatic injury, control group

Macroscopic examination. The tendon in the area of traumatic injury and adjacent areas is fused with the surrounding tissue.

Microscopic examination. In the area of traumatic injury, a tendon-like tissue was formed, represented by dense bundles of collagen fibers filled with type I collagen (**Fig. 1**). The fiber bundles were located both perpendicular to the tendon axis and at an angle (**Fig. 2**). The refraction of type I collagen (red glow) in the regenerate was high, which is typical for tendon-like tissue. In the regenerate, among the type I collagen fibers, foci of loose, chaotically located thin collagen fibers with type III collagen (green glow) were found.

The distribution of tenocytes among collagen fibers was disrupted due to the appearance of areas with increased cell proliferation (cluster-like structures), as well as cell-free areas. Tenocytes mainly had large oval nuclei and slightly elongated cytoplasm.

There were few blood vessels in the regenerate.

In the areas of the tendon located above and below the area of traumatic injury, the bundles of collagen fibers had a convoluted appearance, were disfigured and separated from each other by endotendineum with different density of fibroblasts in the areas (**Fig. 3**).

Table

Histological assessment of tendon arrangement in sites located above the area of the traumatic injury

Study object	The severity of disorders, grade			
	0	I	II	III
	mark (points)			
	1	2	3	4
Tenocytes	Long narrow nucleus with poorly contoured cytoplasm	Change in the shape of nuclei: increased roundness. The cytoplasm is weakly colored	Change in the shape of nuclei: increased number of cells with rounded nuclei. The cell cytoplasm in the form of a narrow rim	Cell nuclei are large, rounded. The cytoplasm is abundant. The appearance of lacunae with chondrocytes
Cell density	Relatively even distribution of tenocytes	Focal cell proliferation, small cell-free areas	Cell proliferation fields among large cell-free areas	Cell clusters, large cell-free territories
Collagen fibers with type I collagen	Tightly packed bundles. Uniform coloring	Separation of bundles into individual fibers with bundle demarcation. Color change within the fiber	Separation of fibers with loss of demarcation, discoloration of fiber bundles	Loosening of fiber bundles with a complete loss of architectonics, impaired coloration of collagen fibers over a large area
Refraction of type I collagen in collagen fibers	Uniform	Reduced in small areas	Loss of refraction (up to 50% of the microscope field of view)	Loss of refraction (over 50% of the microscope field of view)
Refraction of type III collagen in collagen fibers	In single fibers of peritendinium and epitenonium	In single fiber bundles located between collagen fibers with type I collagen	In fiber bundles located between fibers with type I collagen, in small areas of the tendon	Refractive fields of type III collagen (about 30% of the microscope field of view)
Glycosamino-glycans (total)	Mainly in epitenonium	In epitenonium, endotenonium and peritenonium	Between collagen fiber bundles	Extensive fields in the tendon structure
Endotendinium	Narrow cell-free spaces separating collagen fiber bundles	Single fibroblasts present	The density of fibroblasts is increased, there are single collagen fiber bundles	The density of fibroblasts is high, the collagen fiber bundles are fused
Epitenonium, peritendinium	The connective tissue membrane of a characteristic structure	Loosened of collagen fiber bundles, increased density of fibroblasts	Loosened collagen fiber bundles, ruptures, increased density of fibroblasts, expansion of fields and refraction of type III collagen	Ruptures of collagen fiber bundles, their dislocation, uneven density of fibroblasts, formation of clusters. Extensive fields of collagen fibers with type III collagen

Collagen fibers were colored in an uneven manner. The peritendinium differed in width; the growth of loose connective tissue with an increased density of fibroblasts and single narrow blood vessels was recorded. Collagen fibers with type I collagen had weak refraction, uneven along the fibers, interspersed with

single thin fibers with type III collagen. Cell proliferates found in these areas were represented by tenocytes of varying degrees of differentiation including tenocytes with round and oval nuclei, mature cells with long narrow nuclei.

In accordance with the Movin and Bonar semi-quantitative scale for assessing the condition of the tendon [11] in our modification (**table**), the severity of disorders in the tendon sections located above the area of traumatic injury was estimated at 18.6 points, which corresponds to moderate disorders (stage II).

In the area of the formed regenerate, an increased density of myoblasts and fibroblasts was identified. The blood vessels of the capillary type were found. Due to adhesions of the tendon with the surrounding connective tissue, a clear boundary between the peritendinium and the epithendinium was not fixed.

Thus, the tendon regeneration 60 days after the surgery in animals ends with the formation of tendon-like tissue in the area of the traumatic injury. In the sections of the tendon located above the site of traumatic injury, abnormalities of the intercellular substance of the tendon and cells are observed. The formation of connective tissue adhesions of the tendon with the surrounding tissues was recorded.

Tendon regeneration using Singial™

Macroscopic examination. In the area of the traumatic injury of the tendon and in the adjacent areas, no adhesions with the surrounding tissue were found.

Microscopic examination. In the area of traumatic injury, a tendon-like tissue was formed with dense bundles of collagen fibers (**Fig. 4**) with type I collagen (**Fig. 5**), which were mainly longitudinal and not typical for the tendon only in areas. The coloration and refraction of type I collagen were uniform along the fiber length, which indicates its orientational ordering in the structure of the newly formed collagen fibers of the regenerate.

Collagen fibers with type III collagen were identified as small short bundles among type I collagen fibers (**Fig. 5**).

In the injured area, an epithendinium-like structure was formed, consisting of collagen fibers, represented by collagen types I and III, with an uneven density of fibroblasts. However, the union of the tendon with the surrounding tissues was not revealed.

In the areas of the tendon located above and below the area of traumatic injury, the peritendinium had a characteristic structure. No adhesions with the surrounding tissues were found (**Fig. 6**).

Tenocytes were relatively evenly distributed among collagen fibers, their density was low. Only small ribbon-like proliferates of cells (3-4) in the areas were found. The tenocytes generally had a narrow, long nucleus and an elongated cytoplasm, which is characteristic of mature cells.

In the areas of the tendon located above and below the area of traumatic injury, the bundles of collagen fibers retained a characteristic orientation along the axis of the tendon. Most of the fibers contained type I collagen, and a few thin ones contained type III collagen.

In contrast to the control series, cell proliferates forming cluster-like structures were not found in these areas. The blood vessels were located in the endothendinium, and their density was low.

Thus, the introduction of hyaluronic acid into the area of the tendon suture led to the separation of the wound surfaces of the tendon and the surrounding tissue, which serves as a prevention of the development of cicatricial adhesions. The reparative process in the area of traumatic injury on the 60th day ended with the formation of tendon-like tissue with a high density of type I collagen fibers, which are characteristic for tendon tissue, and partial restoration of the structure of the peritendinium. The restoration of the sliding function of the tendon contributed to a decrease in the manifestations of post-traumatic injury in the areas located above and below the area of injury. When using a semi-quantitative scale for assessing the structural organization of the tendon located above the area of traumatic injury, violations were assessed as mild, at 14.8 points (stage I).

Tendon regeneration using Noltrex™

Macroscopic examination. The tendon is not adherent to the surrounding tissue.

Microscopic examination. Collagen fibers in the area of traumatic tendon injury formed dense longitudinally arranged bundles alternating with bundles with a disturbed axial organization. Structural organization characteristic of the tendon was not found (**Fig. 7**). A polarization-optical study revealed that the composition of the bundles of collagen fibers of the regenerate includes type I collagen (**Fig. 8**). Collagen type III was detected only in fine fibers. The collagen refraction was relatively uniform, bright and was visible along the entire length of the collagen fiber bundles. Only small areas of collagen fibers were colored unevenly. A high density of tenocytes was between the collagen fibers. Most of the cells had long and narrow nuclei which are characteristic for cells of a normal tendon, but single cells with rounded nuclei were also identified.

The tissue in the area of the regenerate is characterized as tendon-like.

When examining the areas of the tendon located above and below the area of traumatic injury, no distinctive features from the previous series of experiments were revealed. Collagen fibers containing type I collagen formed dense bundles oriented along the tendon axis (**Fig. 9**). Collagen type III was found in small areas of the tendon.

Tenocytes were relatively evenly located between collagen fibers and had a characteristic structure for mature tendon tissue. Small cell proliferations were found only near the lesion in the area of the expanded endothendinium (**Fig. 10**).

Single blood vessels with narrow lumens in the tendon were located along the collagen fibers.

Complete recovery of the peritendinium in the area of traumatic injury was not revealed. Collagen fibers in the epithendinium were oriented in different directions, the density of fibroblasts between them was increased, but the development of cicatricial adhesive process was not found. In the histological assessment of tendon tissue by the method of Movin and Bonar [11], changes in the superior areas of the tendon were estimated at 15.4 points, which corresponds to mild disorders (stage I).

A distinctive feature of the tissue response of peri tendon tissues to the medication was an increased macrophage response aimed at utilization of the synthetic polymer Noltrex™, the main component of which is polyacrylamide with silver ions.

Thus, the use of Noltrex™ prevents the formation of adhesions between the tissue of the injured tendon and the surrounding tissue. The medication does not disrupt the course of the reparative process in the area of traumatic injury and reduces the development of destructive disorders in the areas of the tendon located above and below the area of injury.

Tendon regeneration using Lydasa-Biolek™

Macroscopic examination. Small foci of connection of the regenerate with the surrounding tissue were found, and in the higher and lower areas of adhesions with the surrounding tissue were not found.

Microscopic examination. Collagen fibers filling the area of traumatic tendon injury formed multidirectional bundles (**Fig. 11**). When examining in polarized light, wide beams of collagen fibers containing collagen I and III types were found among the newly formed beams oriented characteristic of tendon tissue (**Fig. 12**). The refraction of collagen fibers was uneven in areas and along the length. Clusters of fibroblastic diferon cells were noted between the collagen fibers.

In areas located above and below the area of the traumatic injury, extensive foci of destruction of bundles of collagen fibers, which did not have clear demarcation lines, remained. In such areas, single tenocytes were noted, most of which had large rounded or oval nuclei surrounded by abundant cytoplasm.

Fibers with type III collagen were determined not only in the area of the regenerate, but also in the higher and lower areas. The endotendinium in the areas had irregular gaps. In the area of its expansion, lysed cells and edematous fluid were found (**Fig. 13**). Also, single blood vessels were localized in the endothendinium, sometimes they formed aggregations.

In the histological assessment of tendon tissue (**table**), changes in the higher areas of the tendon were estimated at 18.7 points, which corresponds to medium disorders (stage II).

In the area of the formed regenerate and in small areas of the tendon located above and below from the traumatic injury, adhesions were formed, which lead to a violation of its sliding function.

So, the use of Lydasa-Biolek™ does not interfere with the tendon regeneration process. Tendon-like tissue forms in the injured area. When examining the areas located above and below the injury zone, an increase in areas with destructive disturbances was found in comparison with other experimental series (Noltrex™ and hyaluronic acid). The sliding function of the collagen fibers within the tendon and the sliding function of the tendon in relation to the surrounding tissues is impaired.

Thus, in an experimental study conducted on 12 rabbits, the effect of the preparations Singial™, Noltrex™ and Lydasa-Biolek™ (in comparison with control animals) on the following parameters of the injured tendon: post-traumatic regeneration, the state of the tendon in the areas adjacent to the damage, the sliding function of the collagen fiber bundles in the tendon, and the relationship of the tendon to the surrounding tissue.

The direction of the reparative process was practically the same in all series of the experiment and at the end of the study (day 60), in the area of traumatic injury, the regenerate was represented by tendon-like tissue. Multidirectional bundles of collagen fibers with collagen types I and III were found in the regenerate. Fibers formed by type I collagen are the main component that provides the strength properties of the intercellular substance when the tendon is stretched. In this regard, collagen fibrils with collagen type I are the main constituent of the dense connective tissue of the tendon. In all the series of experiments carried out at the end of the study, the regenerate was made with tendon-like tissue containing collagen fibers with type I collagen. However, in the process of tendon regeneration, collagen fibers with type III collagen are also formed. The study of this type of collagen makes it possible to assess the features of tendon regeneration and indirectly characterize its strength qualities, since it is known that type III collagen has a lower tensile strength and, thus, its high content in the tendon is a predisposing factor in the violation of strength qualities [12]. In a normal tendon, type III collagen is found mainly in the epithendinium. In addition, being located in a small amount between bundles of collagen fibers, it takes part in limiting the growth of bundles in width. Collagens of types I and III are synthesized by fibroblasts during tendon regeneration. As a result of the study, it was revealed that type III collagen is present in tendon regenerates in all series with the maximum accumulation in regenerates of the control series, as well as in tendons treated with Lydasa™.

When examining the areas of the tendon located above or below the area of injury, destructive disorders were revealed in animals of all experimental series. In the tendons of the control series and the tendons treated with Lydasa™, moderate impairments (stage II) were recorded (according to the Movin and Bonar scale in our modification). Less noticeable disorders in the tendon structure were revealed in a series of experiments with Singial™ and Noltrex™ (mild disorders, stage I).

Analysis of the sliding function of collagen fiber bundles in the tendon structure was carried out in the assessment of the peritendinium. It was found that the proliferation of connective tissue, which disrupts the sliding of collagen fiber bundles, is most pronounced in

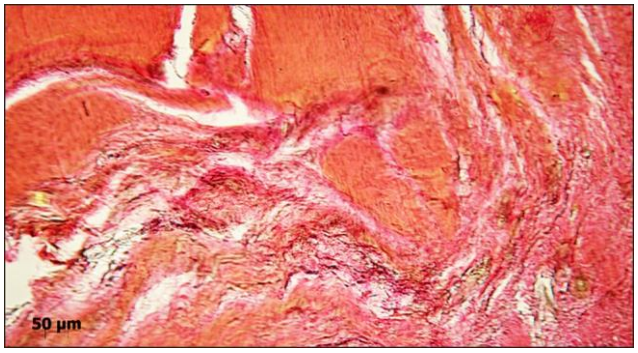

Fig. 1. Area of traumatic injury. Collagen fiber bundles multidirectional with respect to the tendon axis. Control series. Van Gieson's picro-fuchsin. Magn. 100

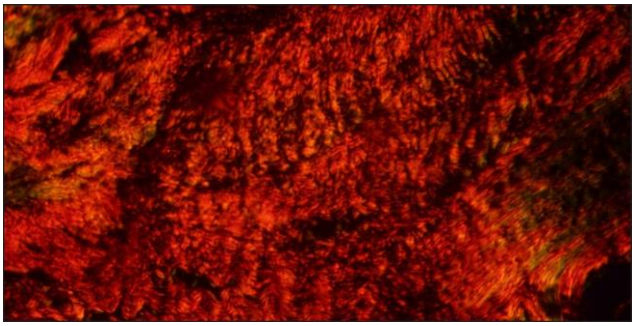

Fig. 5. Area of traumatic injury. Tendon-like tissue. Bundles of collagen fibers with type I collagen predominate. Singial™. Polarized light. Red picrosirius. Magn. 200

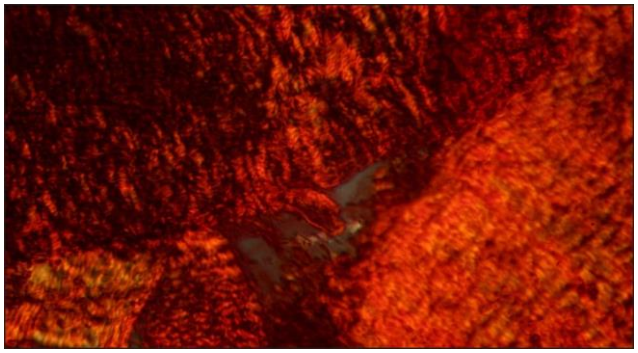

Fig. 2. Area of traumatic injury. Collagen fiber bundles in tendon-like tissue. Control series. Polarized light. Red picrosirius. Magn. 80

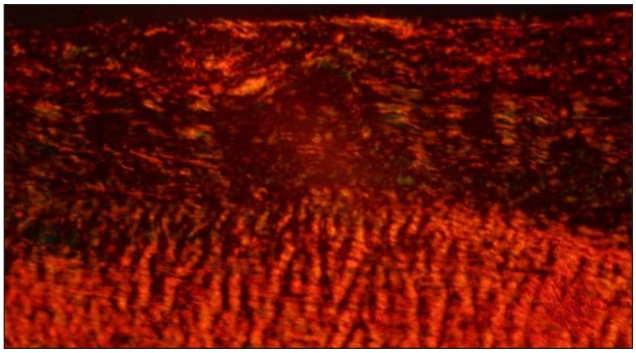

Fig. 6. The perithendinium is thickened and has no characteristic structure. There is no adhesion with the surrounding tissues. Singial™. Polarized light. Red picrosirius. Magn. 200

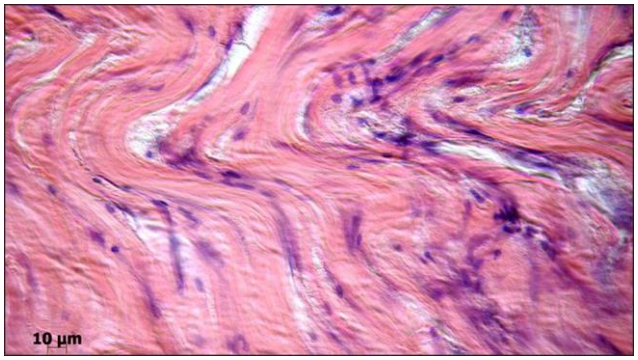

Fig. 3. The area of the tendon located above the area of traumatic injury. Bundles of collagen fibers are wavy, disfigured and dissociated. Control series. Hematoxylin and eosin. Magn. 100

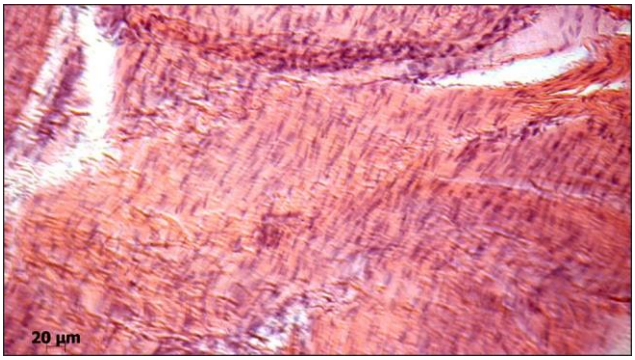

Fig. 7. Dense, mostly longitudinally arranged bundles of collagen fibers in the area of traumatic tendon injury. Increased tenocyte density. Noltrex™. Hematoxylin and eosin. Magn. 200

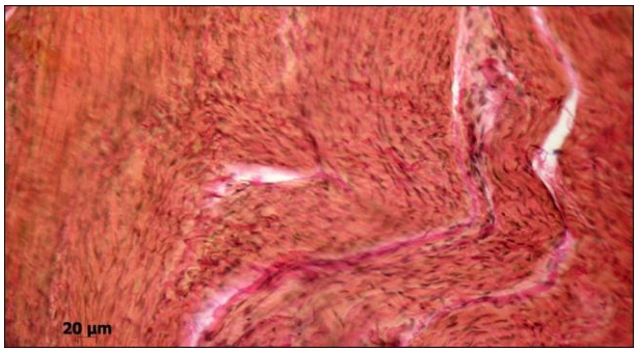

Fig. 4. Area of traumatic injury. Newly formed bundles of collagen fibers. Singial™. Van Gieson's picro-fuchsin. Magn. 200

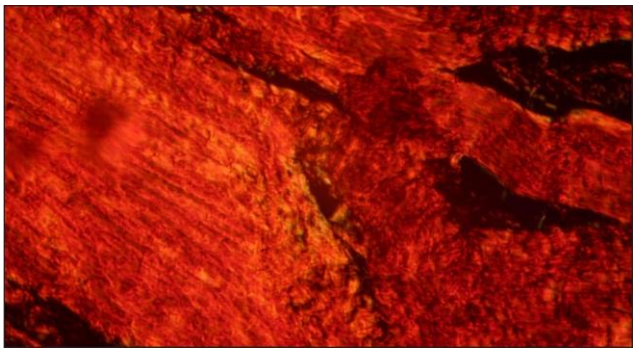

Fig. 8. Tendon-like tissue in the area of traumatic injury is represented by type I collagen. Noltrex™. Polarized light. Red picrosirius. Magn. 200

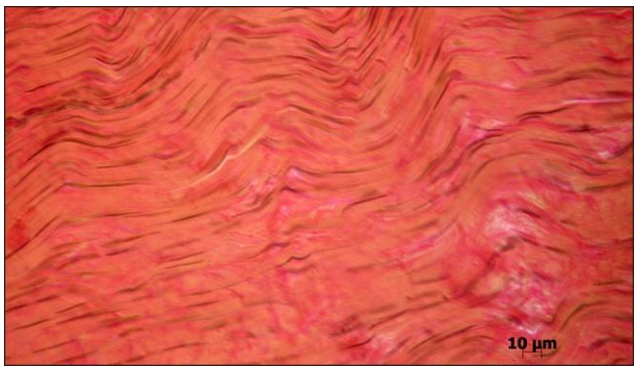

Fig. 9. Dense bundles of collagen fibers, parallel to the tendon axis, intertwine in areas with thin fibers. Noltrex™. Van Gieson's picro-fuchsin. Magn. 400

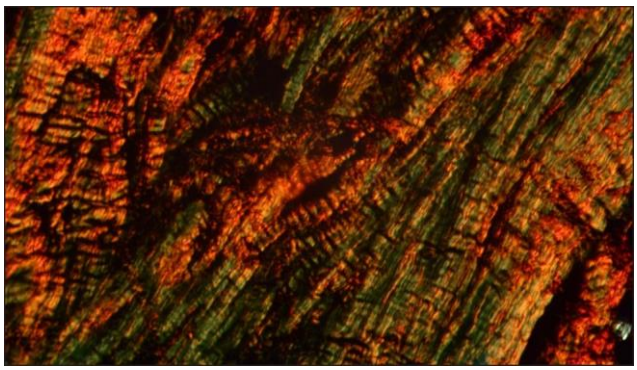

Fig. 12. Fragment of the regeneration area. High density of collagen fiber bundles with collagen type III. Lydasa-Biolek™. Polarized light. Red picrosirius. Magn. 200

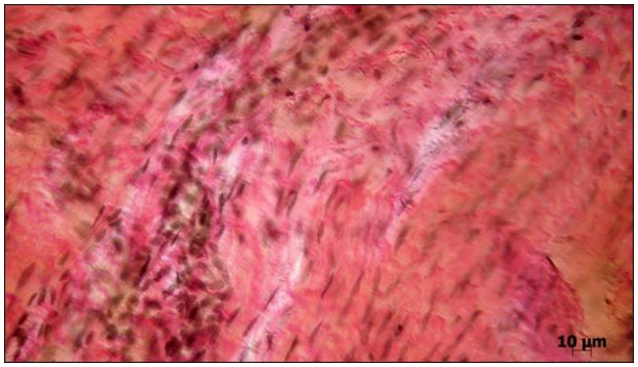

Fig. 10. Proliferation of fibroblastic differon cells in the endothendinium. Noltrex™. Van Gieson's picro-fuchsin. Magn. 400

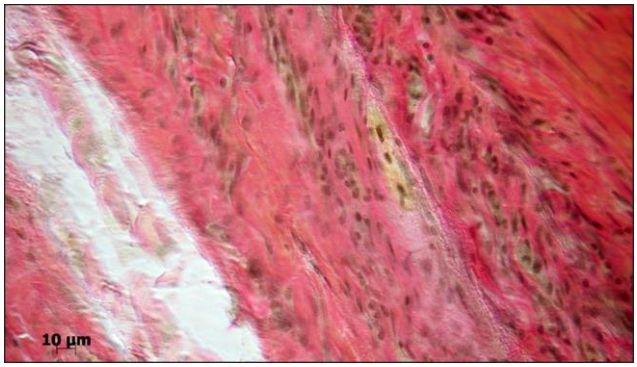

Fig 13. Violation of the structural organization of the tendon. The endothendinium is dilated, filled with edematous fluid and lysed cells. Lydasa-Biolek™. Hematoxylin and eosin. Magn. 100

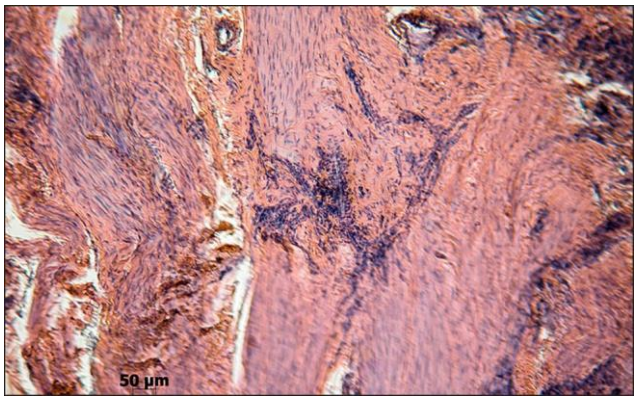

Fig. 11. Collagen fibers filling the area of traumatic tendon injury. The orientation of the bundles is different from the normal tendon. The density of tenocytes is high. Accumulations of cells between bundles of collagen fibers. Lydasa-Biolek™. Hematoxylin and eosin. Magn. 100

the control series of the experiment and in the series using the Lydasa™ medication. It was revealed that the use of the Singial™ and Noltrex™ medications contributed to the separation of the wound surfaces of the tendon and the surrounding tissue, and this was the prevention of the development of cicatricial adhesions.

As a result of the experimental study, it was revealed that for the restoration of the sliding function of the tendon and the prevention of adhesions, the Singial™ and Noltrex™ medications can be recommended.

Conclusions

In the process of post-traumatic restoration of the tendon under conditions of adhesion prophylaxis, destructive disorders were found in all series of the experiment with the maximum manifestation in the tendons of the control series and those treated with hyaluronidase. The use of hyaluronic acid and a three-dimensional water-containing polyacrylamide polymer with silver ions led to the separation of the wound surfaces of the tendon and the surrounding tissue, which served as the prevention of post-traumatic cicatricial adhesions. When all medications are used, tendon-like tissue is formed in the injured area.

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PREVENTION OF POSTTRAUMATIC ADHESIONS AROUND THE ACHILLES TENDONS IN RABBITS

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