

Modeling the tendon suture with its isolation to demonstrate primary regeneration of the junction area

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Abstract

Introduction Functional recovery of the finger flexor tendon after surgical treatment is a problem due to formation of unavoidable blocking adhesions. For prevention of the adhesions, the mechanical separation of the sliding surfaces of the tendon and the wall of the fibro-osseous canal with a polymer tube was proposed. However, despite good clinical results, it is not possible to morphologically confirm the healing of the suture zone after such an intervention. **Objective** To present an experimental model and technique of surgical intervention on the calcaneal tendon of laboratory animals for isolation and blocking with a polymeric tube, longitudinally cut, for obtaining and studying the regenerate in the suture site. **Materials and methods** In an experiment under inhalation anesthesia with Isoflurane on both hind paws of 10 laboratory Wistar rats, the tendon suture was simulated with blocking and isolation of the tendon suture ($n = 14$) and the control operation without isolation ($n = 6$). The tendons were excised three weeks post-surgery, and the appearance and histological picture of the regenerates in the suture zone were examined. **Results** The surgery model with isolation of the suture site on the calcaneal tendons of rats reproduces well and adequately simulates the operation on the human hand tendons. The morphology of the obtained regenerates demonstrates the restoration of continuity with the formation of tendon-like tissue at the junction. In the experiment, subtotal isolation did not lead to tendon necrosis, but slowed down the healing of the suture zone. The tube prevented the formation of adhesions and maintained tendon nutrition through the fissure space. **Conclusion** Modeling of the operation of tendon suture on the rat calcaneal tendon with its isolation provides material for demonstration the primary tendon regeneration in vivo. Regeneration of the suture site during subtotal isolation slows down and occurs due to the cells of the tendon itself.

Keywords: tendon, tenotomy, tendon suturing, suture site isolation, tendon regenerate, laboratory animals

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INTRODUCTION

Restoration of the fine anatomy and normal functioning of human hand tendons after traumatic injuries has been a problem in hand surgery for decades [1]. The greatest difficulty is the reconstruction of the flexor apparatus in the area of osseo-fibrous canals. However, unsatisfactory outcomes of the operations such as tenogenic contractures occur mainly due to the impossibility of ideal restoration of sliding structures and the formation of blocking adhesions between the tendon and the canal wall. Ingrown vessels and neovascularization of the suture zone can be considered the only positive consequence of the formation of adhesions around the tendon [2].

To prevent cicatricial and tenogenic contractures, surgeons should comply with the requirements for an “ideal suture” [3]. For the same purpose, tendons are mobilized after surgery by their daily movement in the canal [1, 4]. However, a mechanically weak suture or uncontrolled rehabilitation can lead to a rupture of the suture zone and a repeated operation which is more traumatic.

Since the proposed numerous methods for the prevention of adhesions by anti-inflammatory drugs [5–7], irrigation

of cytostatic drugs [8] and hyaluronic acid preparations into the cavity of the canals [9, 10], separation with barriers of biological origin [11–14] and membranes from biodegradable synthetic polymers [15] are not widely used, the main means of preventing adhesions after surgery is tendon mobilization from the first days after surgery [1, 16].

In one of the works [17], a longitudinally cut polymer tube temporarily fixed to the tendon was used to protect the suture zone from scar ingrowth and formation of the canal wall. The new technology presents an alternative to a two-stage tendoplasty in cases where the osseofibrous canal is scarred, and the ends of the deep flexor tendon can be brought together and sutured with little tension. However, despite good clinical and functional results of using the new technology, it is not possible to study the process of tendon healing in the patients. A previous experiment on tenotomy and suture on the calcaneal tendons of small laboratory animals [18] demonstrated the possibility of creating a relatively simple experimental model for reproducing operations performed on the tendons of the human hand and for obtaining tissue of the repaired tendon for morphological studies.

Purpose To present an experimental model and technique of surgical intervention on the calcaneal tendon of laboratory animals for isolation and

blocking with a polymeric tube, longitudinally cut, for obtaining and studying the regenerate in the suture site.

MATERIALS AND METHODS

Experimental animals, their tendons and the ethics of the experiment Ten Wistar rats of both sexes weighing 250–300 grams were used to practice the intervention technique. The calcaneal (Achilles) tendon was well contoured and palpated in passive dorsiflexion of the foot.

Modeling of the tendon suture with its isolation was performed in 4 animals on both hind legs. It was also modeled in 6 more animals on the right hind leg ($n = 14$, experiment) but tenotomy and tendon suture without isolation was performed on their left hind leg ($n = 6$, control).

The experimental methodology and work with laboratory animals were approved by the local committee on biomedical ethics of the educational institution Gomel State Medical University. The experimental model presented in the article was developed and tested in accordance with current ethical and regulatory documents [19–21]. All operations on the animals were performed with sterile instruments under aseptic conditions in the operating room of the research laboratory of the Medical University.

Anesthesia Animals were anesthetized by inhalation anesthesia with Isoflurane. After being removed from the cage, the animal was placed in a desiccator, on the bottom of which a cloth impregnated with an anesthetic was placed. In the absence of a dispenser, the relaxing

concentration of Isoflurane in a 1-liter desiccator was created by evaporation of the drug applied to the tissue, not exceeding a dose of 2 ml. Upon a decrease in the motor activity of the animal, it was removed, laid down and fixed on the operating table with its back up. To maintain anesthesia, a plastic tube filled with anesthetic-moistened cotton was placed near the nose and mouth of the animal. A sufficient and safe level of anesthesia during the operation was judged by the spontaneous regular breathing of the animal and the absence of reactions to pain stimulation.

Histological study of the regenerate Immediately after the operation, the blocking sutures were removed from the tube, and a soft tissue cord was removed from the lumen of the tube. Threads from the suture zone of all tendons were carefully removed. The tendons were placed in a neutral formalin solution. Histological tissue processing was performed on an automatic apparatus with accessories STP 120 Thermo Scientific (Thermo Fisher Scientific Inc., USA). Longitudinal sections with a thickness of 5 μm were obtained on a sledge microtome HM 450 Thermo Scientific. The preparations were stained with hematoxylin and eosin and according to Van Gieson. The study of tendon morphology and photodocumentation were performed using a Levenhuk MED 10T optical microscope (Levenhuk LLC, USA) with a TouPCam 10.0 MP digital video eyepiece.

RESULTS

The experiment required preliminary preparation: 1) acquisition of a set of surgical instruments for operations on small structures; 2) preparation of conditions, means and equipment for anesthesia; 3) planning the method of tendon suture, tenotomy technique and determining their sequence; 4) selection of an atraumatic suture material and a tube suitable for isolation of the calcaneal tendon of the rat.

The most suitable instruments for the experimental operation were the instruments from the set for hand surgery: forceps, a needle holder, a ‘mosquito’ clamp, scissors, a pointed scalpel No. 11.

Inhalation anesthesia is accepted as the most effective and safe method for performing surgical interventions in rodents [22]. In our study, Isoflurane became the anesthetic of choice, since it has the lowest dissolution rate in the blood and, therefore, minimal toxicity. The drug ensured the fastest entry of the animal into anesthesia, as well as a fairly rapid reversion. Anesthesia and monitoring of motor reactions during the operation was carried out by a trained assistant. The entry of rats into anesthesia was accompanied by a

successive disappearance of reflexes. The rolling reflex was the first to disappear. The loss of the pedal reflex indicated a sufficient level of sedation and the moment when the animal could be fixed on the operating table. The onset of the surgical stage of anesthesia was noted by the absence of palpebral and lingual reflexes. With a negative ‘wrist squeeze’ reflex, the disappearance of pain sensitivity was noted and the operation was started. In disorders of the breathing rhythm or the appearance of muscle tone during anesthesia, the experiment was limited to the isolation of the sutured tendon only on the right hind leg. Thus, a suture with isolation was performed on 14 calcaneal tendons (in 6 rats on the right hind paw, in 4 rats on both paws). Six stitched non-isolated tendons on the left legs served as control material.

Anesthetized animals were shaved off the hair around the perimeter of the shins of both hind legs, after which the shins and feet were treated with the Septocid Synergy solution (ZAO BelAseptika, Belarus).

Technique of the intervention with tendon isolation A skin incision 1.5–2 cm long was made in the projection

of the tendon along the posterior surface of the lower leg. By dissecting the mesentery, the Achilles tendon was mobilized from the calcaneal tuber to the tendon-muscle junction. For the convenience of subsequent connection of the ends before tenotomy, the tendon shaft was sutured with two threads (Fig. 1). Long-term absorbable polyglycolide threads of 3/0 on an atraumatic stabbing needle were used as suture material. The level of tenotomy was visualized by retracting the threads and stretching the tendon. It was performed with a sharp scalpel perpendicular to the course of the fibers so as not to cross the threads. The ends of the tendon were then adapted end-to-end and the threads were tied.

To isolate the tendons, fragments of elastic transparent infusion guide made of non-toxic polyvinyl chloride (UE FreBor, Belarus) were used that were 1 cm long with an inner diameter of 1.5 mm and a wall thickness of 0.3 mm. The tube was cut lengthwise, placed over the sutured tendon, and stitched to it with U-shaped sutures above and below the junction zone. To keep it from moving during stitching, the tube was temporarily fixed to the tendon with thin injection needles. After applying the tube, a fissure-like gap of 0.3 mm was left for the ingrowth of the mesentery and the supply to the suture zone. The skin of the paws of the animals was sutured with a continuous twist suture and treated with brilliant green.

The length of the calcaneal tendon of rats was 1.5 cm, and the thickness reached 1.8–2 mm. It was sufficient to perform an experimental operation without using a microscope. Figure 1 shows that the calcaneal tendon of the rat was first stitched with two threads (a), and then a transverse tenotomy was performed (b). This sequence technically simplified and accelerated the surgical intervention. In the case of the traditional sequence, tenotomy and subsequent suture of the tendon, there are difficulties with equally accurate suturing of the trunk and matching the ends of the thin tendon. Such manipulations make the intervention significantly longer and lead to deformation of the suture zone.

Since the suture area was subsequently isolated and blocked in the tube, the tendon suture in this experiment was not subjected to such a requirement as strength. The ends were held only by two interrupted sutures along the lateral surfaces of the tendon. An atraumatic polyglycolide suture material was used on a 3/0 needle. The suture resorption period of 60 days was significantly longer than the duration of the experiment.

The following requirements were followed for choosing a tube for isolating a sufficiently thin rat tendon. The tube had to be elastic, pyrogen-free, sterile, and transparent. The inner diameter of the implant had to be such that the tube that was cut longitudinally and laid on the tendon covered at least 2/3 of the tendon diameter and formed a fissure-like gap for the ingrowth of the connective tissue and blood vessels. The wall of the tube also had to be thin enough so that it could reversibly deform as the animal moved and did not create tension on the surrounding soft tissues. The tube we chose optimally met the given requirements, with the exception of elasticity. The intact infusion guide was soft and easily deformed when pinched. However, its 1-cm fragments were not sufficiently elastic, making it difficult to hold the cut tube on the rat tendon during blocking stitching. To fix the tube during stitching, it was pinned to the tendon with a thin injection needle. The appearance of the tendon blocked in the tube is shown in Figure 1 (d).

The surgical technique used for the control tendons was similar to the described above with exception of tendon isolation with a polymeric tube.

Postoperative period After the operation, the animals were placed in a cage, where they became active after 1–3 minutes. The animals were kept and observed in the usual conditions of the vivarium. Due to the complexity of creating external immobilization of tiny paws of the rats and presence of internal fixation of the tendons with polymer tubes, the animals were followed in the early mobilization regime.

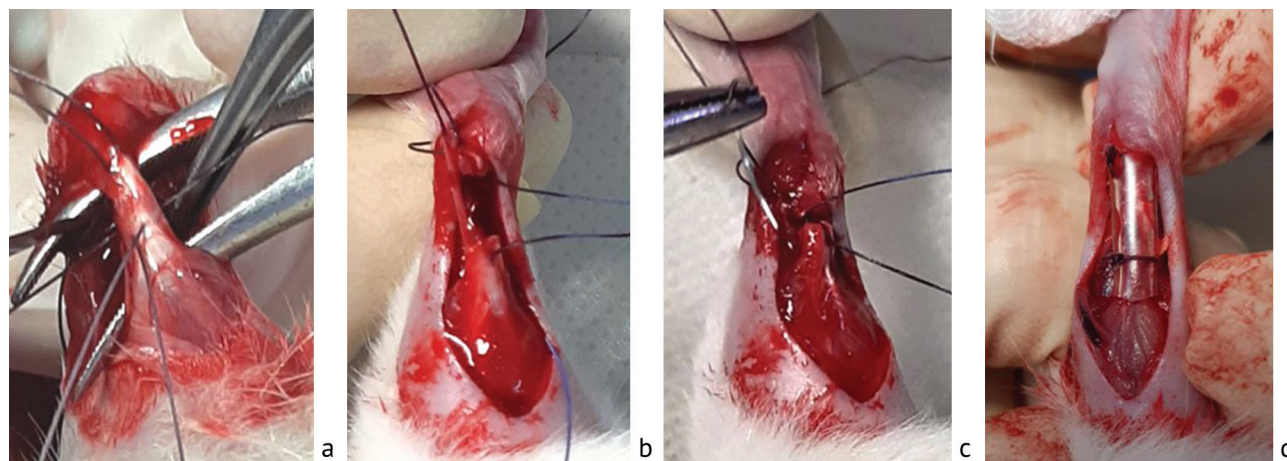


Fig. 1 Stages of the operation on the calcaneal tendon of a rat: suturing (a), tenotomy (b), joining the ends of the tendon (c) and covering the tendon with a cut tube (d)

Tendon dissection The second operation was performed three weeks after the first one under inhalation anesthesia. The approach ran along the postoperative scar.

After dissection of the skin, the tubes with the tendons enclosed in them were easily detected and separated from the surrounding tissues; the tendon suture looked consistent (Fig. 2). The tubes were surrounded by a pale pink tissue with a smooth sliding surface. Loose bleeding connective tissue grew into the tendon through the fissure-like space. The calcaneal tendon immobilized in the tube was cut along the edges of the tube and removed from the wound as a single block. The skin of the paw of the animal was sutured with a continuous twisting suture and treated with an antiseptic solution. The rat was returned to the cage.



Fig. 2 View of the tube and surrounding tissues three weeks after tenotomy and stitching with tendon isolation

The tendons extracted from the tubes looked like connective tissue bands with a smooth surface. Their entire diameter was 1.3–1.5 mm, which is smaller than the transverse size of the narrowest part of a healthy rat calcaneal tendon (1.8–2 mm). Thus, the polymer tube lying on the tendon shaped and formed the sliding surface of the tendon and its sheath. Obviously, the use of a more elastic tube would preserve the natural anatomical configuration of the tendon-muscle junction. The integrity of the suture zone under slight stretching of the samples after removal of the suture material indicated the healing of all tendons.

The collection of tendons stitched without isolation on the left paws of animals caused difficulties due to tissue changes such as loss of the tendon sliding sheath, its fusion with surrounding tissues and deformation. To obtain the material, the tendon was isolated from the scars with its excision. In the middle part of the tendon, a fusiform thickening with a diameter of 2.5–3 mm was seen a suture material enclosed in it.

Observation of the animals

After the completion of the experiment, the animals were observed for 5–7 days until their condition returned to normal, as evidenced by their active motion in the cage and the appearance of repulsion with their hind legs from a hard surface.

Regenerate morphology in isolation of the tendon with a cut polymer tube In the microphotographs of the longitudinal sections of the experimental tendons, the sutured ends are connected by a loose immature regenerate along the entire contact zone (Fig. 3a). Its structure is a well vascularized tissue (Fig. 3b). It characterizes the peak of the repair phase.

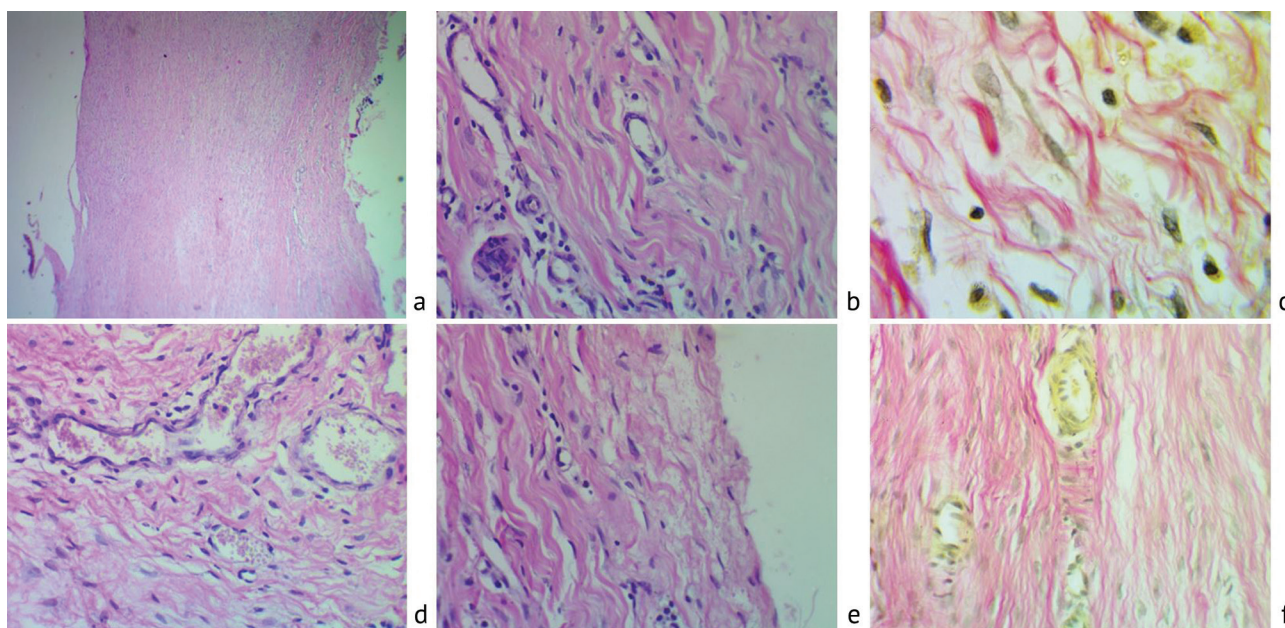


Fig. 3 Microphotos of the junction area in a suture simulation experiment with tendon isolation on a rat calcaneal tendon. The ends of the tendon are united by a connective tissue regenerate (a) with an abundance of immature blood capillaries (b), large fibroblasts and lymphocytic-macrophage infiltration (c). Along the tendon fibers and bundles that are in contact in the junction zone, there is a new formation of capillaries (d). The surface of the tendon is covered by a smooth layer of loose connective tissue (e). In the thickness of the regenerate and near its surface, the accumulation of collagen in the form of bright orange fibers (e) is seen. Paraffin sections, stained with hematoxylin and eosin – a, b, d, e; Van Gieson staining – c, f. Mag. 40× (a); 400× (b, d, e, f); 1000× with immersion (c)

The metabolic activity of the emerging regenerate is evidenced by the proliferation of fibroblasts, both young and more mature types, by histiocytes, macrophages, plasma and lymphoid cells (Fig. 3c), and new young immature capillaries near tendon fibers and tendon bundles that are in contact in the junction plane (Fig. 3d). These cells and microvessels form an immature granulation tissue that fills the traumatic microdefect and subsequently rebuilds into a tendon-like tissue. The surface of the tendon adjacent to the tube is represented by a smooth layer of delicate soft fibrous connective tissue growing on the regenerate (Fig. 3e). The sections stained according to Van Gieson showed signs of collagen synthesis and its deposition in the form of multidirectional fibers and bright orange bundles along the vessels evenly throughout the entire thickness of the tendon regenerate (Fig. 3f). The morphology of a three-week-old calcaneal tendon regenerate of an animal demonstrates the presence and implementation of a cell type of regeneration inherent in connective tissue. The macroscopical restoration of the integrity of the tendon and the microscopically visible filling of the traumatic defect with tissue identical to the lost one indicate the

course of tendon restitution in the absence of external sources of regeneration.

Morphology of the tendon regenerate in control animals In the area of healing, thickening of the tendon is determined (Fig. 4 a). In paraffin sections, it is a mature structurally organized regenerate of dense fibrous connective tissue. The cells of the regenerate are represented mainly by spindle-shaped tenocytes with rounded, less often with elongated nuclei without visible cytoplasm, single fibroblasts and rare macrophages and leukocytes (Fig. 4b). In the central part of the regenerate, short bundles of collagen fibers of the 1st and 2nd orders tightly adjacent to each other are structured, located at an angle or perpendicular to the main axis of the tendon. Closer to the surface of the tendon and in the transition zones, the fibers are elongated and oriented longitudinally in the direction of pulling forces. The blood supply of the regenerate is poor, observed between the bundles in the layers of loose connective tissue of 1–2 capillaries per field of vision at high magnification (Fig. 4b). The surface of the tendon is covered with connective tissue adhesions damaged at the extraction (Fig. 4c). The histological picture demonstrates the completion of the tendon repair phase.

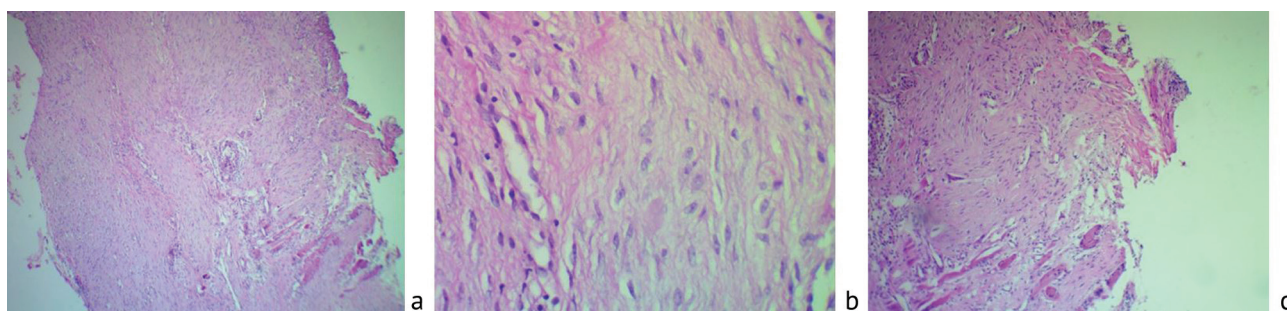


Fig. 4 Microphotos of the regenerate formed after tenotomy and suture without tendon isolation. The mature regenerate is thickened (a), is formed by bundles of differently directed mature collagen fibers with single capillaries penetrating inbetween (b). The surface of the tendon is covered with connective tissue adhesions (c). Paraffin sections stained with hematoxylin and eosin. Mag. 40× (a); 100× (c); 400× (b)

DISCUSSION

The choice of Wistar rats as experimental animals was due to their availability, ease of maintenance and observation. These animals feature high-quality and rapid tissue regeneration.

The three-week duration of the experiment was based on the achievement of the calcaneal tendon healing in the rats after tenotomy and suture without isolation within this period [18], as well as by the results of the experiment in which the primary regeneration of the rabbit tendon in the avascular medium occurred 3 weeks after the suture [23, 24]. In humans, the repair phase (fibroplastic) occurs from the 2nd to the 6th week. It is a period when the strength of the suture zone progressively increases so much that it is possible to proceed to a more active rehabilitation of the patient [1, 16, 25].

Under normal conditions, the suture zone in laboratory animals revascularizes approximately

17 days after stitching. The newly formed vessels grow through adhesions from the surrounding tissues along the surface of the tendon and penetrate into the suture zone through normally avascular areas [26]. In the experimental model presented by us, the healing of the isolated tendon slowed down by half a phase. It was possible only due to the synthetic activity of tenocytes and tenoblasts of the tendon itself. The peak of the repair phase with activation of collagen and angiogenesis occurred at the end of the third week.

For three weeks, the tube was a mechanical barrier to the ingrowth of scar tissue and excluded the influence of external sources of regeneration. Vessels grew into the suture zone only from the capillaries in the surrounding tissues in a limited area from the side of the fissure-like space in the cut tube. At the same time, stimulation of reparative regeneration and tissue differentiation occurred due to the preservation of the dominant

regulatory mechanism of tendon regeneration, the functional mechanism [27, 28]. A positive effect of tube implantation was the formation of sliding surfaces of the

tendon and its sheath, which are necessary in clinical practice for free excursion of the tendon and restoration of normal finger ROM.

CONCLUSIONS

The study presents an experimental model of toμία, suture and isolation of the calcaneal tendon in laboratory rats. It simulates a reconstruction operation with temporary isolation of the suture zone with a longitudinally cut polymer tube in patients with chronic injuries of the deep flexor tendons of the fingers. The operation on the calcaneal tendon of rats is well reproduced. It can be used to study the process of tendon healing in an in vivo experiment, as well as to study the influence of various factors on the formation of the regenerate.

Subtotal isolation with polymer tubes in the

experiment did not cause necrosis of the tendon and the suture zone. The histological study confirmed the viability of the tendon and its primary reparative regeneration due to the cells of the tendon itself. The slowdown in the regeneration under the condition of tendon isolation should be considered in post-surgery management and for prescribing active rehabilitation.

The advantage and humane aspect of the experimental model is that there is no need to withdraw the animals from the experiment. They remain alive and can be used in other studies.

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