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FEATURES OF A LOCAL BIOINTERFACE FORMED UPON INTEGRATION OF A KNITTED NICKEL-TITANIUM MESH INTO STRIATED MUSCLE TISSUE

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Introduction. Injuries to skeletal muscles at various sites remain a pressing clinical problem, necessitating the development of biocompatible implants that enable full regeneration and functional restoration of muscle tissue.
Objective. Assessment of the safety and efficacy of a knitted nickel–titanium fabric following its implantation into striated muscle tissue in an *in vivo* model of mechanical loading.
Materials and methods. The study was performed on 30 Wistar rats weighing 400–500 g aged 70–90 days. An implantable material made from a low-modulus superelastic TiNi wire with a diameter of 40 μm, grade TN-10, was tested. An experimental specimen was prepared in the form of a warp-knitted tape. The animals were divided into the following groups. In group "A" (*n* = 10), the material under test was implanted into the area of a fenestrated defect of the anterior abdominal wall muscle. In group "B" (*n* = 10), the material was implanted into the area of the dissected parietal peritoneum under the left dome of the diaphragm. In group "C" (*n* = 10), the material was implanted under the muscles of the anterior neck surface. The laboratory animals were withdrawn from the experiment following 14, 30, 60, and 90 days after implantation of the metal-knit fabric. The biointerface between the metal-knit fabric and skeletal muscle tissue was assessed based on histological examination and scanning electron microscopy in the setting of a long-term experiment on laboratory animals. The research was conducted using an Axio Lab.A1 microscope, an AxioCam ERc 5s camera, and the AxioVision Rel. 4.8 software.
Results. Across all test groups, on day 14 post-implantation, a thin layer of newly formed connective tissue in the implantation area was observed. On days 30, 60, and 90 of the experiment, a trend toward denser ingrowth of collagen fibers was noted, with the formation of bundles around the NiTi wire. The formation of muscle buds and fibers was recorded on day 60. By day 90, a mature tissue regenerate in the implantation zone had been identified. This regenerate consisted of muscle fibers and the metal-knit fabric, between which fibrocytes, fibroblasts, bundles of collagen fibers, and blood vessels were located.
Conclusions. The NiTi wire-based metal-knit fabric is a safe, biocompatible material, which promotes the reparative regeneration of skeletal muscle tissue under various biomechanical conditions. This material holds promise for the surgical treatment of skeletal muscle tissue defects, thus deserving further clinical studies.

Keywords: reparative regeneration; skeletal muscle tissue; nickel-titanium alloy; metal-knit fabric; implant

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ОСОБЕННОСТИ ЛОКАЛЬНОГО БИОИНТЕРФЕЙСА ПРИ ИНТЕГРАЦИИ ТРИКОТАЖНОЙ НИКЕЛИД-ТИТАНОВОЙ СЕТКИ В ПОПЕРЕЧНОПОЛОСАТУЮ МЫШЕЧНУЮ ТКАНЬ

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Введение. Повреждения скелетных мышц различной локализации остаются актуальной клинической проблемой, требующей разработки биосовместимых имплантов, обеспечивающих полноценную регенерацию и восстановление функции мышечной ткани.
Цель исследования. Оценка безопасности и эффективности применения трикотажного никелида титана после имплантации в поперечнополосатую мышечную ткань на *in vivo* модели механического воздействия.
Материал и методы. Исследование выполнено на 30 крысах Wistar массой 400–500 г возрастом 70–90 сут. Апробировали опытный образец имплантационного материала в виде трикотажно-вязаной ленты, выполненной из сверхэластичной TiNi проволоки марки ТН-10. Животных распределяли по группам: группа «А» (*n* = 10) — металлотрикотаж в виде вязаной ленты из низкомодульной сверхэластичной никелид-титановой (TiNi) проволоки диаметром 40 мкм имплантировали в зону окончатого дефекта мышцы передней брюшной стенки; группа «Б» (*n* = 10) — имплантация образца в зону рассеченной париетальной брюшины под левым куполом диафрагмы; группа «В» (*n* = 10) — имплантация образца под мышцы передней поверхности шеи. Лабораторных животных

выводили из эксперимента на 14, 30, 60 и 90 сут после имплантации металлотрикотажа. Биоинтерфейс металлотрикотажа и скелетной мышечной ткани оценивали на основе гистологического исследования и сканирующей электронной микроскопии в условиях хронического эксперимента на лабораторных животных. Исследования проведены с использованием микроскопа Axio Lab.A1, видеокамеры AxioCamERc 5s и программного обеспечения AxioVision Rel. 4.8.

Результаты. На 14 сут в зоне имплантации во всех группах выявлено появление тонкого слоя вновь образованной соединительной ткани. На 30, 60 и 90 сут эксперимента в исследуемых группах наблюдали тенденцию к более плотному врастанию коллагеновых волокон с формированием пучков вокруг TiNi проволоки. На 60 сут зафиксировано формирование мышечных почек и волокон. На 90 сут в зоне имплантации определялся зрелый тканевой регенерат, включающий мышечные волокна и металлотрикотаж, между которыми были расположены фиброциты, фибробласты, пучки коллагеновых волокон и кровеносные сосуды.

Выводы. Металлотрикотаж из TiNi проволоки безопасен, биосовместим и способствует репаративной регенерации скелетной мышечной ткани в различных биомеханических условиях. Материал перспективен для хирургического лечения дефектов скелетной мышечной ткани и требует дальнейших клинических исследований.

Ключевые слова: репаративная регенерация; скелетная мышечная ткань; никелид титана; металлотрикотаж; имплант

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Соответствие принципам этики: исследование выполнено с соблюдением правил биоэтики, утвержденных Европейской конвенцией о защите позвоночных животных, используемых для экспериментальных и других целей. Проведение исследований одобрено на заседании биоэтического комитета ФГБОУ ВО «Сибирский государственный медицинский университет» Минздрава России (протокол № 732 от 06.10.2020).

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INTRODUCTION

Mechanical impacts or excessive physical exertion experienced during military conflicts, emergency situations, sports events, and everyday life can lead to impaired integrity of chest or limb muscles in humans. The severity of injury determines the selection of treatment approaches and the duration of rehabilitation. A muscle can be injured at its base, tendon, muscle belly, or musculotendinous junction, leading to fiber rupture, inflammation, and impaired contractile function [1–4]. In surgical practice, iatrogenic injuries to striated muscle tissue occurring when performing surgical access are highly common. Such injuries result from the dissection of soft tissues, which inevitably compromises the integrity of the perimysium and separates the muscle fiber bundles [5, 6].

Red, intermediate, and white muscle fibers are present in all human skeletal muscles. The predominance of their certain type determines the speed of response and contraction. Red muscle fibers are characterized by a slow contraction speed and are fatigue-resistant. Conversely, white muscle fibers respond and contract rapidly, although fatigue rather rapidly. Rupturing of a small number of muscle fibers leads to the formation of muscle buds at their ends; these subsequently fuse, thereby filling the damaged area. In cases of extensive muscle defects, another mechanism of reparative regeneration after injury is involved, characterized by pronounced proliferation of myosatellite cells, their migration into the defect area, and the formation of new myotubes. This requires specific conditions, such as the absence of fibroblasts in the regeneration area and preserved motor innervation of the muscle fibers. Otherwise, a dense connective tissue scar forms [7–9]. In order to prevent fibrosis and scarring in the area of surgical trauma, which lead to impaired muscle function, biological and synthetic implants can be used. The development and clinical implementation of such implants are actively pursued in scientific centers worldwide, including Russia. There are reports on the use of porous and mesh materials made from metal alloys, hyaluronic acid-based hydrogel, sponge biomaterial, polypropylene, polytetrafluoroethylene (Gore-Tex), prolene, Parietex Composite, etc. [10–12].

Nickel–titanium-based alloys (NiTi) are increasingly attracting interest in the fields of traumatology and orthopedics, reconstructive thoracoabdominal surgery, and regenerative medicine. This interest is primarily driven by the biocompatibility of NiTi-based implants. Previous studies have shown that implants made of NiTi wire-based metal-knit fabric possess not only sufficient elasticity to conform to the contours of biological tissues, but also high resistance to long-term cyclic loading, while also facilitating the formation of a strong, unified tissue regenerate in the damaged area [13–15]. Morphological changes associated with the use of NiTi implants for integration into skeletal muscle tissue at various locations with differing physical loads have not been previously described.

The aim of this study was to assess the safety and efficacy of a knitted nickel-titanium alloy following its implantation into striated muscle tissue under an *in vivo* model of mechanical loading.

MATERIALS AND METHODS

The study was conducted at the Central Research Laboratory of the Siberian State Medical University using 30 certified male Wistar rats weighing 400–500 g and aged 70–90 days. The animals were obtained from the Department of Experimental Biological Models of the Goldberg Research Institute of Pharmacology and Regenerative Medicine (Tomsk National Research Medical Center). The animals were housed under standard vivarium conditions.[1] Prior to the experiment, the animals underwent a two-week quarantine in the vivarium in compliance with established housing requirements. A 12-h light-dark cycle was maintained, and food and water were provided *ad libitum*. No more than two animals were housed per cage. Body weight was recorded for all animals in each group before the surgical procedure and again at the end of the experiment prior to euthanasia.

During the experiment, a prototype implantable material in the form of a warp-knitted tape made of superelastic NiTi wire grade TN-10 was tested (Fig. 1).

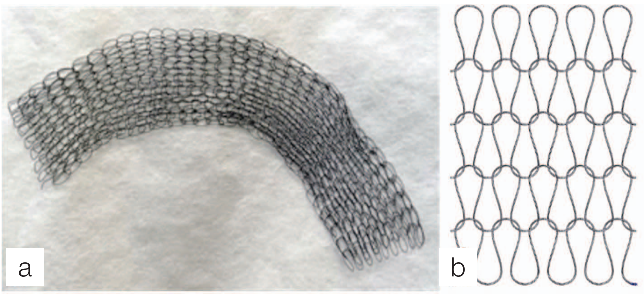

Photo taken by the authors

Fig. 1. Nickel-titanium alloy wire metal-knit fabric: a — general view of the two-layer tape; b — geometric model of a single-layer specimen

The implant was manufactured at the Laboratory of Superelastic Biointerfaces, National Research Tomsk State University.

The metal-knit tape is a mesh structure made of biologically inert, corrosion-resistant NiTi wire with a diameter of 40 µm and mesh sizes ranging 0.3–3.5 mm (Fig. 2). The NiTi-based wire is a composite material characterized by the presence of an outer rough layer with a thickness of up to 1 µm.

A warp-knitted structure of the metal-knit fabric ensured non-unraveling and non-fraying edges, while the specific intertwining imparted to the fabric not only dimensional stability but also limited, uniform stretchability in all directions. To form a knitted sleeve and tape, thin NiTi wire was knitted on a Rishikesh Wire circular knitting machine (Rishikesh Electromatic Pvt. Ltd., India). The circular knitting created a closed tubular structure with a regular loop pattern, which facilitated subsequent technological transformation and ensured isotropic mechanical properties in the plane of the fabric. After knitting, the preform underwent a stabilizing anneal. To shape the metal-knit fabric into a tape, the metal-knit sleeve was subjected to hot rolling at a temperature of 500°C, forming a symmetrical two-layer structure with a predetermined porosity and cell topology, which played a key role in its functional properties.

The perioperative management of the animals in the groups did not differ, except for the area of surgical intervention.

The animals were divided into three groups:

- In group “A” (*n* = 10), the NiTi metal-knit fabric was implanted into a thoracoabdominal defect adjacent to the muscles of the anterior abdominal wall;
- In group “B” (*n* = 10), the NiTi metal-knit fabric was implanted adjacent to the dissected parietal peritoneum under the left dome of the diaphragm;

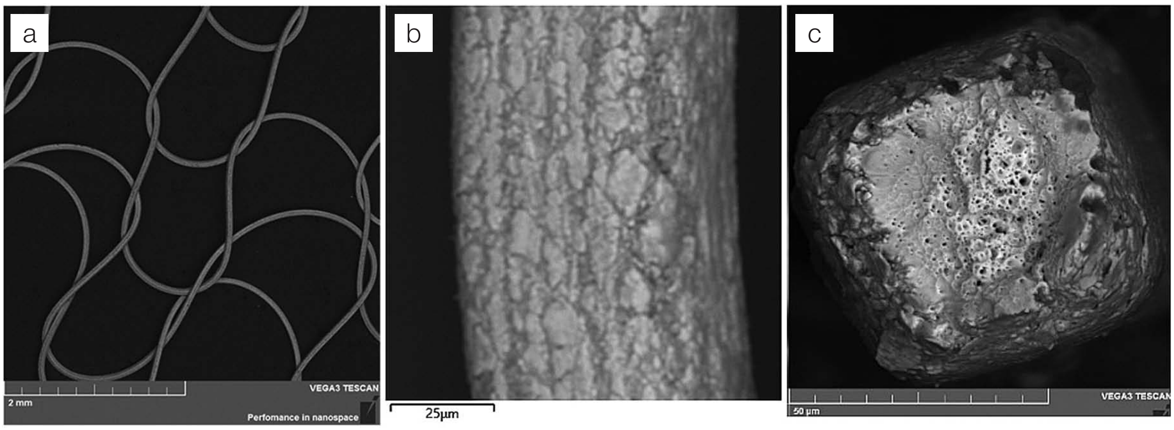

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Fig. 2. Spatial and morphological structure of a nickel-titanium alloy wire metal-knit fabric: a — warp-knitted structure of a single-layer specimen under scanning electron microscopy (magn. ×50); b — rough surface (magn. ×2500); c — cross-section of the NiTi wire under scanning electron microscopy (magn. ×2500)

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[1] GOST 33215-2014. Guidelines for the Housing and Care of Laboratory Animals. Rules for Facilities and Procedural Organization. Moscow: Standartinform; 2019.

- In group “C” (n = 10), the NiTi metal-knit fabric was implanted in the pretracheal space, beneath the muscles of the anterior neck surface.

The implants were placed in skeletal muscles differing in their level of natural physiological load and the nature of contractile activity, without applying any additional external loads on the animals. This choice of implantation sites was dictated by the objective of modeling different physiological biomechanical conditions for the implant within a single experimental series.

Laboratory animals, under zolazepam-xylazine anesthesia, were secured to an operating table; the surgical area was shaved and treated with an antiseptic agent.

In group “A”, a midline laparotomy was performed, and the xiphoid process along with a musculofascial portion of the anterior abdominal wall were resected in an extrapleural manner. The post-resection thoracoabdominal defect was replaced with a metal-knit fabric tape, which was fixed along its perimeter with a continuous suture using a 4/0 polypropylene atraumatic suture material. In group “B”, following laparotomy and incision of the parietal peritoneum under the left dome of the diaphragm, the tape was placed without additional fixation. In group “C”, after an incision was made along the anterior surface of the neck and the muscles were retracted, the metal-knit fabric was placed pretracheally, beneath the deep muscles. The surgical wound was closed completely with a 4/0 Vicryl® atraumatic suture material.

After the surgical intervention, all animals were housed in individual cages (VELAZ, Czech Republic). To assess the general condition of the animals, daily clinical examination data were recorded throughout the entire observation period. The parameters evaluated included the nature of excretion, the state of the fur, skin and mucous membranes, appetite, respiration, mobility, neuromuscular excitability, gait, aggression, miosis/mydriasis/exophthalmos, etc. (SOP: CPS-SV-008). During the postoperative period, for five days, each animal received preventive antimicrobial therapy with Enrofloxacin (API SAN LLC, Russia) *per os* via drinking water at a dose of 15 mg/kg bw; the drug was pre-dissolved in 200 mL of drinking water. For analgesia during the first two days after implantation, the animals received Ketoprofen (VIK — Animal Health, Belarus) subcutaneously at a dosage of 5 mg/kg bw once daily.

Laboratory animals were euthanized following 14, 30, 60, and 90 days after implantation of the metal-knit fabric via carbon dioxide inhalation in accordance with the Standard Operating Procedure (SOP: CPS-PDI-023). The biological specimen was excised with surrounding tissues for macroscopic analysis. For histological examination, fragments of striated muscle tissue and surrounding connective tissue were collected directly from the implantation site, as well as from the visually unaltered muscle tissue at a distance of no less than 10 mm from the implant edge, which served as an internal control. The biological samples, comprising the tissue regenerate (striated muscle tissue with adjacent connective tissue) from the implantation site, were fixed in 10% neutral buffered formalin. Upon completion of fixation, the regenerate was precisely separated along its boundary with the mesh metal-knit implant, since metal wire is not amenable to standard histological processing and sectioning. The remaining tissue was dehydrated, embedded in paraffin, sectioned at 5 μm thickness, and stained with hematoxylin and eosin and Van Gieson’s stain (for connective tissue).

For morphological examination, an Axio Lab.A1 microscope (Carl Zeiss Microscopy GmbH, Germany) was used in conjunction with an AxioCam ERc 5s video camera (Carl Zeiss Microscopy GmbH, Germany) and AxioVision Rel. 4.8 software (Carl Zeiss Microscopy GmbH, Germany). The analysis of tissue integration features with the mesh structure of the metal-knit fabric was performed using a Tescan Mira analytical scanning electron microscope (Brno, Czech Republic). After preliminary fixation in a 10% solution of neutral buffered formalin, tissue fragments containing the implanted material samples were isolated. Following freeze-drying, the samples were mounted on conductive carbon tape in the chamber of the scanning electron microscope.

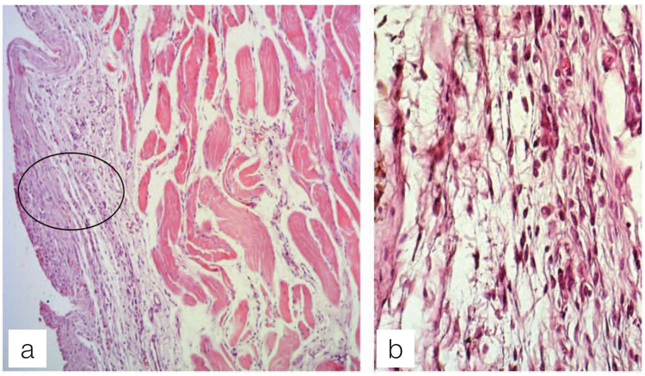
RESULTS

In all laboratory animals, the postoperative wounds healed by primary intention. At day 14 and subsequent time points, no visual deformation of the anatomical structures adjacent to the implant was recorded in groups “A” and “C”. During macroscopic examination at autopsy, a trend toward denser adhesion of the implant to the adjacent muscle tissues was observed over the experimental period from the moment of implantation in groups “A” and “B”.

On day 14, in all groups, a layer of delicate connective tissue between the implant and the muscle structures was observed. In group “C”, on days 14 and 30, the metal-knit fabric and the neck muscles could be easily separated from each other due to the absence of dense adhesion between them. At all time points in the study groups, migration of the mesh implant was not observed. At later time points in group “B”, the tissue regenerate in the implant area had limited mobility due to denser adhesion; however, this did not generally restrict diaphragmatic motility. In group “C”, on days 60 and 90, the metal-knit fabric was more densely adherent to the neck muscles compared to earlier time points in the study.

Histological examination of biological samples from groups “A” and “B” on day 14 post-implantation revealed the formation of a layer of young connective tissue with small newly formed blood vessels and collagen fibers between the metal-knit fabric and the striated muscle fibers (Fig. 3). At the same time point, all groups predominantly exhibited lympho-macrophagic inflammatory infiltration, along with minor edema and isolated neutrophils in the implant area. Lymphocytes, macrophages, and fibroblasts predominated among the cellular elements. However, in group "C" on day 14, the formation of loose, immature connective tissue on the surface of the implant was noted.

On day 30, in the implantation site in all groups, more mature connective tissue was observed, characterized by an increased number of collagen fibers and the presence of isolated, newly formed bundles composed therefrom. Histological examination of samples at day 60 post-implantation of the NiTi metal-knit fabric revealed that the collagen fibers were more structured and formed bundles (Fig. 4), with fibrocytes predominating in the cellular composition, fibroblasts being less common, and isolated neutrophils being present. In all study groups, at day 60 post-implantation, muscle buds — a structural sign of striated muscle tissue regeneration — were detected at the sites of muscle fiber transection (Fig. 5).



Photos taken by the authors

Fig. 3. Tissue regenerate on day 14 post-implantation: a — a layer of granulation tissue is visualized between the metal-knit fabric (element removed during slide preparation) and the striated muscle fibers of the abdominal wall (outlined) (magn. ×150); b — a fragment of the same granulation tissue area at higher magnification: fine collagen fibers, cellular elements, and newly formed blood vessels (magn. ×400)

By day 90 post-implantation, histological examination in groups "A" and "B" revealed the formation of a tissue regenerate consisting of muscle fibers and mature connective tissue components. The regenerate structure contained cellular elements represented by fibrocytes and fibroblasts, exhibiting an organized, predominantly unidirectional orientation of collagen fibers. Furthermore, pronounced vascularization was observed, indicating the presence of a developing network of blood vessels that provide the regenerating tissue with the necessary metabolic conditions for further restoration and functional integration.

Examination using scanning electron microscopy on day 14 post-implantation in all groups recorded the initial formation of connective tissue on the implant surface and within the interstices of the NiTi metal-knit fabric wire (Fig. 6a). By day 30, collagen fibers and fibroblasts had been completely filled the pores of the two-layer implant in isolated areas, promoting material integration with the surrounding tissue and ensuring mechanical stability (Fig. 6b). At later time points, on days 60 and 90, dense encasement of the wire by collagen fibers was observed in the groups, with these fibers forming bundles oriented in mutually perpendicular planes. This led to the creation of a distinctive structural mesh, providing optimal conditions for implant fixation and biocompatibility (Fig. 6c). The formation of collagen fiber bundles creating various types of interweaving between the muscles of the anterior abdominal wall was noted, accompanied by a pronounced cellular ridge along the surface and at the intersections of the NiTi wire. The density of these structures progressively increased toward the later observation time points.

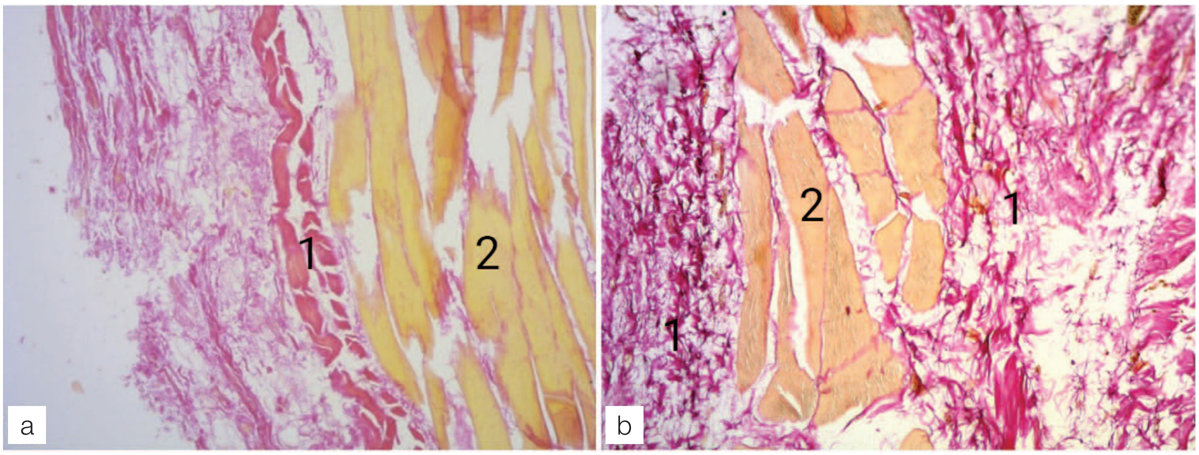


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Fig. 4. Proliferation of collagen fibers (1) between the muscle fibers (2) of the abdominal wall at various time points following NiTi metal-knit fabric implantation: a — observation period 30 days; b — observation period 60 days; staining with picrofuchsin according to Van Gieson (magn. ×150)

DISCUSSION

During the process of reparative regeneration of skeletal muscle tissue following traumatic injury, the formation of a connective tissue scar is inevitable. This scar partially or completely replaces the defect, leading to motor dysfunction of the muscle and clinically manifesting impairment of mobility in the corresponding anatomical region. The mechanisms of reparative regeneration of muscle tissue are still under investigation and remain a relevant area of scientific research [4, 16–18]. Despite significant progress in elucidating the molecular and cellular processes regulating the restoration of striated muscle tissue, many aspects, including the role of the extracellular matrix and the interaction of various cell populations, require further detailed study [19–21].

A wide range of biomaterials and tissue-engineered constructs have been proposed for reconstructing extensive post-resection soft tissue defects, reflecting the ongoing search for an optimal solution that accounts for individual anatomical and functional requirements. Key criteria for the effective application of such materials include biocompatibility to ensure adequate integration with the surrounding stroma without immunogenic reactions and mechanical strength under cyclic multiaxial loads to prevent deformation and postoperative complications. These characteristics are essential for anatomical and physiological restoration and long-term functional rehabilitation of the damaged area [22, 23].

The present study established that the fundamental biological processes of regeneration, including granulation tissue formation, neoangiogenesis, fibroblast proliferation, and subsequent organization of the collagen matrix, exhibited similar morphological features regardless of the location of the tissue regenerate. This indicates the versatility of restoration mechanisms under the conditions of metal-knit scaffold implantation [24–26]. Previous studies, including comparisons with polypropylene implants, have shown that the formation of a vascularized collagen regenerate around the implant is a key biological phenomenon determining successful material integration and restoration of the supporting function of soft tissues [9, 13, 27–30].

When using a NiTi metal-knit fabric as an implant in muscle tissues, the absence of a pronounced inflammatory reaction and a reliable, stable fixation of the implanted element without migration were noted. Long-term implantation of the metal-knit fabric promoted improved integration of the artificial material with biological tissues, further confirming its high level of biocompatibility. The NiTi metal-knit implant served as a scaffold for the formation and ingrowth of a connective tissue regenerate, creating a complete anatomical structure. The interaction between the mesh implant made of NiTi wire and the collagen fibers within the regenerate area ensured the restoration of anatomical and physiological integrity,

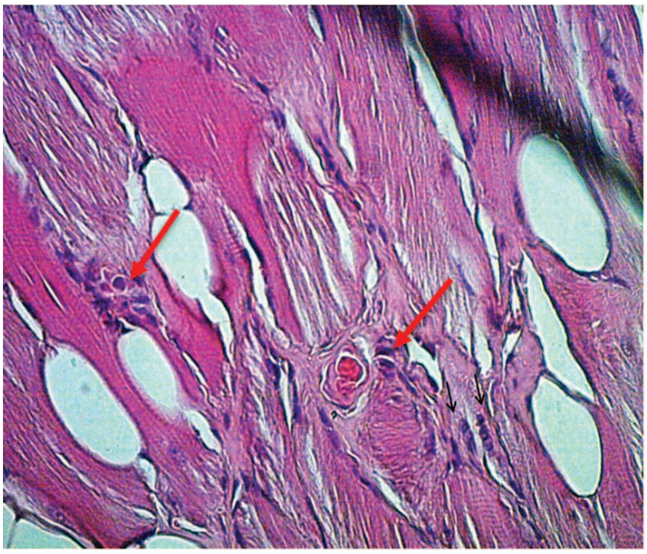

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Fig. 5. Regeneration of skeletal muscle fibers in group “A” at 60 days post-implantation of NiTi metal-knit fabric: muscle buds are indicated by red arrows; hematoxylin and eosin staining (magn. ×600)

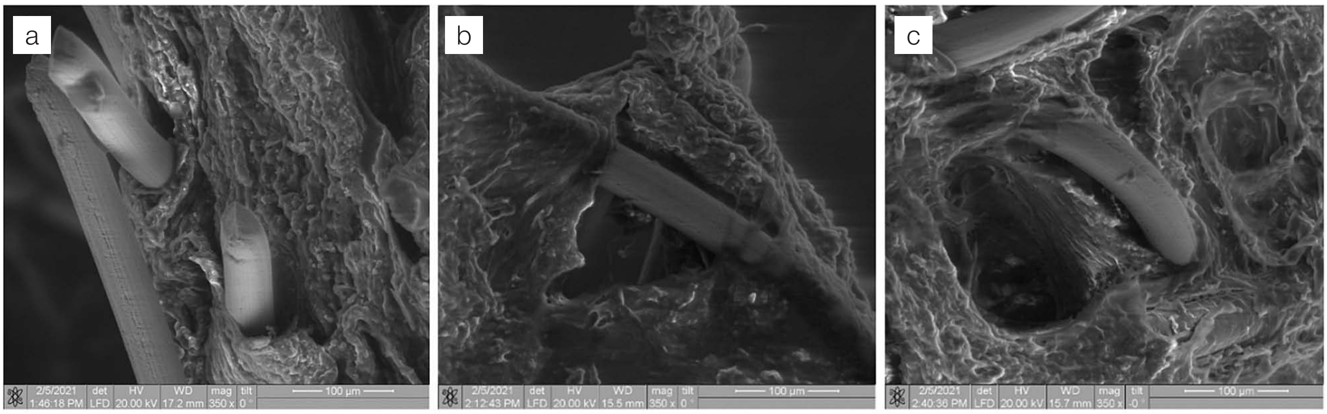

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Fig. 6. Tissue regenerate in group “A” at various time points post-implantation: a — day 14; b — day 30; c — day 60 (magn. ×350)

which was a key factor in the regeneration and functional integrity of the muscle tissue.

The data obtained indicate that the integration of the implant into muscles with different baseline functional loads was accompanied by the formation of a unified tissue regenerate without clinically significant restriction of mobility or signs of rigidity in the corresponding muscle groups. This allows us to consider the formed scaffold to be biomechanically and functionally adequate to the recipient tissues under different physiological muscle operating conditions. The mesh NiTi material implant, after replacing the soft tissue defect, functioned as a mobile reinforcing structure, functionally analogous to the tendinous part of a muscle. The use of a metal-knit fabric with a filament diameter of 40 μm optimized the combination of mechanical strength and biocompatibility, minimizing the risk of postoperative complications and the formation of dense connective tissue scars. The presumed physiological load on the muscle group of a specific anatomical area was successfully transferred through the NiTi-based material, confirming its long-term ability to support the motor function of the muscle structure. It was additionally noted that such integration and interaction of the implant with the tissue contributed to the preservation of microcirculation and prevented the development of coarse fibrosis, which is an essential condition for sustainable functional restoration.

CONCLUSION

The investigated metal-knit fabric based on thin NiTi wire was shown to be promising as a biomaterial for reconstructive surgery in addressing extensive skeletal muscle defects. The combination of biocompatibility, mechanical stability under dynamic loads, and pronounced capacity for tissue integration provides reliable functional support to the muscular framework, promotes anatomical-physiological restoration, and allows for the minimization of excessive fibrosis. Overall, this forms a basis for an optimal clinical outcome.

Further research should be focused on a comprehensive assessment of the long-term biomechanical characteristics and clinical efficacy of metal-knit fabric implants in various traumatic and surgical scenarios. This must include a mandatory quantitative comparative analysis of the cellular and matrix components of the regenerate. Such an approach will allow for the formation of a reliable evidence base to facilitate their broad adoption and targeted refinement in clinical practice.

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