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Innervation of Facial Muscles Using an Allogeneic Biomaterial in an Experiment

Lyalya A. Musina[1], Olga R. Shangina[1], Anna I. Lebedeva[1], Alexey V. Prusakov[2], Anatoliy V. Yashin[2], Vladimir S. Ponamarev[2]✉, Vladimir D. Radnatarov[3], Alexandr M. Lunegov[2]

[1] Bashkir State Medical University, 47, Zaki Validi str., Ufa, Republic of Bashkortostan, 450077, Russia
[2] Saint Petersburg State University of Veterinary Medicine, 5, Chernigovskaya str., Saint Petersburg, 196084, Russia
[3] Federal State Budgetary Educational Institution of Higher Professional Education "Buryat State Agriculture Academy by V.R.Philippov"; FSBEI HPE Buryat SAA, 8, Pushkina str., Ulan-Ude, 670024, Russia

✉ **Corresponding author:** Vladimir S. Ponamarev. **E-mail:** psevdopyos@mail.ru

ORCID: Lyalya A. Musina – https://orcid.org/0000-0003-1237-9284; Olga R. Shangina – https://orcid.org/0000-0003-1686-1254; Anna I. Lebedeva – https://orcid.org/0000-0002-9170-2600; Alexey V. Prusakov – https://orcid.org/0000-0001-5582-5155; Anatoliy V. Yashin – https://orcid.org/0000-0002-3614-4730; Vladimir S. Ponamarev – https://orcid.org/0000-0002-6852-3110; Vladimir D. Radnatarov – https://orcid.org/0009-0006-5115-7501; Alexandr M. Lunegov – https://orcid.org/0000-0003-4480-9488.

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Abstract

Introduction. It is known that the problem of regeneration of damaged peripheral nerves is one of the leading problems in traumatology and neurosurgery.

Aim. Aim of the study is to determine the features of the innervation of facial muscles when using allogeneic biomaterials.

Materials and methods. The experiment was performed on Chinchilla rabbits ($n = 36$). The facial nerve was cut in the animals. In the control group ($n = 9$), the wound was sewn up, in the 1st experimental group ($n = 12$), a fragment of their masticatory muscle was sewn together with a neurovascular bundle to the denervated buccal muscle. In the 2nd experimental group ($n = 15$), dispersed allogeneic biomaterials "Regeneration stimulator" and "Vasculogenesis stimulator" were injected into the muscle junction in the operation zone. Rabbits were withdrawn from the experiment for 10, 30, 60 and 180 days. Tissue pieces from the surgery area were examined by transmission electron microscopy.

Results and discussion. In the control and 1st experimental groups, the experiment ended with scarring of the operating area and contracture of facial muscles. In the 2nd experimental group, signs of tissue revascularization and axon germination to the buccal muscle with the restoration of individual neuromuscular synapses were revealed.

Conclusion. The use of allogeneic biomaterials in operations to restore damaged peripheral nerves accompanying muscles creates conditions not only for the restoration of muscle fibers, but also the vascular bed, as well as nerve elements with neuromuscular connections.

Keywords: muscle reinnervation, allogeneic biomaterials, axon growth

Conflict of interest. The authors declare that they have no obvious and potential conflicts of interest related to the publication of this article.

Contribution of the authors. Lyalya A. Musina, Olga R. Shangina and Anna I. Lebedeva – provision of allogeneic biomaterial, development of research methodology, literature review. Alexey V. Prusakov, Anatoliy V. Yashin, Vladimir S. Ponamarev and Alexandr M. Lunegov – conducting an experiment, sampling, designing an article, communicating with the editors. Vladimir D. Radnatarov – writing an article, literature review. All authors participated in the analysis of the obtained results.

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Реиннервация мимических мышц с использованием аллогенного биоматериала в эксперименте

Л. А. Мусина[1], О. Р. Шангина[1], А. И. Лебедева[1], А. В. Прусаков[2], А. В. Яшин[2], В.С. Понамарёв[2]✉, В. Д. Раднатаров[3], А. М. Лунегов[2]

[1] Федеральное государственное бюджетное образовательное учреждение высшего образования «Башкирский государственный медицинский университет» Министерства здравоохранения Российской Федерации (БГМУ), 450077, Россия, Республика Башкортостан, г. Уфа, ул. Заки Валиди, д. 47
[2] Федеральное государственное бюджетное образовательное учреждение высшего образования «Санкт-Петербургский государственный университет ветеринарной медицины», 196084, Россия, г. Санкт-Петербург, ул. Черниговская, д. 5
[3] Федеральное государственное бюджетное образовательное учреждение высшего профессионального образования «Бурятская государственная сельскохозяйственная академия имени В. Р. Филиппова», 670024, Россия, г. Улан-Удэ, ул. Пушкина, д. 8

✉ **Контактное лицо:** Понамарёв Владимир Сергеевич. **E-mail:** psevdopyos@mail.ru

ORCID: Л. А. Мусина – https://orcid.org/0000-0003-1237-9284; О. Р. Шангина – https://orcid.org/0000-0003-1686-1254; А. И. Лебедева – https://orcid.org/0000-0002-9170-2600; А. В. Прусаков – https://orcid.org/0000-0001-5582-5155; А. В. Яшин – https://orcid.org/0000-0002-3614-4730; В. С. Понамарёв – https://orcid.org/0000-0002-6852-3110; В. Д. Раднатаров – https://orcid.org/0009-0006-5115-7501; А. М. Лунегов – https://orcid.org/0000-0003-4480-9488.

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Резюме
Введение. Известно, что проблема регенерации поврежденных периферических нервов является одной из ведущих в травматологии и нейрохирургии.
Цель. Цель исследования – определить особенности иннервации мимических мышц при использовании аллогенных биоматериалов.
Материалы и методы. Опыт выполняли на кроликах породы Шиншилла (n = 36). У животных была произведена перерезка лицевого нерва. В контрольной группе (n = 9) зашивали рану, в 1 опытной группе (n = 12) к денервированной щечной мышце подшивали фрагмент своей жевательной мышцы вместе с нервно-сосудистым пучком. Во 2 опытной группе (n = 15) в место соединения мышц в зоне операции вводили диспергированные аллогенные биоматериалы «Стимулятор регенерации» и «Стимулятор васкулогенеза». Кроликов выводили из опыта на 10, 30, 60 и 180 сутки. Кусочки тканей из зоны операции исследовали методом трансмиссионной электронной микроскопии.
Результаты и обсуждение. В контрольной и 1 опытной группе эксперимент закончился рубцеванием операционной зоны и контрактурой мимических мышц. Во 2 опытной группе были выявлены признаки реваскуляризации тканей и прорастания аксонов к щечной мышце с восстановлением отдельных нервно-мышечных синапсов.
Заключение. Использование аллогенных биоматериалов в операциях по восстановлению поврежденных периферических нервов, сопровождающих мышцы, создает условия не только для восстановления мышечных волокон, но и сосудистого русла, а также нервных элементов с нервно-мышечными соединениями.

Ключевые слова: реиннервация мышц, аллогенные биоматериалы, рост аксонов

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Вклад авторов. Л. А. Мусина, О. Р. Шанигина и А. И. Лебедева – предоставление аллогеного биоматериала, разработка методики исследования, обзор литературы. А. В. Прусаков, А. В. Яшин, В. С. Понамарёв и А. М. Лунегов – проведение эксперимента, отбор проб, оформление статьи, общение с редакцией. В. Д. Раднатаров – написание статьи, обзор литературы. Все авторы участвовали в анализе поученных результатов.

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INTRODUCTION

It is known that the problem of damaged peripheral nerves regeneration is one of the leading in traumatology and neurosurgery [1, 2]. On this basis studying of nervous control recovery patterns in injured muscles under different condition has significant theoretical and practical value [3–7]. This knowledge allows improving scientific understanding of neuroplasticity and recognizing factors necessary for successful management of damaged peripheral nerves recovery processes. The choice of reconstructive intervention to restore the nerve trunk should be based not only on the size of the nerve defect, but also take into account a combination of other various factors, one of which is frequent scarring of the surgical area [8–12]. The aim of the study is to determine the features of the innervation of facial muscles when using allogeneic biomaterials.

MATERIALS AND METHODS

The experiment was carried out on 36 Chinchilla rabbits (weight 2500–3500 g). When working with animals, the international principles of the Helsinki Declaration on the Humane Treatment of Animals (2000) and the Federal Law of the Russian Federation "On the Protection of Animals from Cruelty" dated 01.01.1997 were observed.

Dispersed allogeneic biomaterials Alloplant® ("Regeneration stimulator" and "Vasculogenesis stimulator", manufactured according to specifications 9398-001-04537642-2011 at the All-Russian Center for Eye and Plastic Surgery) were used in the experiment. In all experimental animals, the right facial nerve, which innervates the buccal muscle, was transected. The separated facial nerve was exposed in the area of exit from the temporal bone. In order to exclude the possibility of germination of the fibers of the facial nerve, its trunk was ligated with a compression silk ligature. The incision of the main nerve trunk was performed along the outer edge of the ligature.

In the control group (9 rabbits), reinnervation was not performed, allogeneic biomaterials were not used, soft tissues were sutured in layers. In the 1st experimental group (12 rabbits), after nerve transection, a myoplasty operation was performed with a muscle flap (from the chewing muscle) with a neurovascular bundle. The formed muscle autograft retained the vascular and nervous structures associated with the main part

of the belly of the masticatory muscle. It was sutured to the denervated layer of mimic muscles. In the 2nd experimental group (15 rabbits), an identical myoplastic operation was performed, but a layer of dispersed Alloplant® biomaterials ("Regeneration stimulator" and "Vasculogenesis stimulator") was formed between the muscle autograft of the masticatory muscle and the mimic muscles, manufactured according to TU 9398-001-04537642-2011). Animals were taken out of the experiment on the 10th, 30th, 60th and 180th day after the operation by an overdose of anesthesia. Pieces of tissue from the operation area 1–2 mm in size were studied by electron microscopic examination. The material was fixed and poured into epon-812 using standard methods [8]. Tissue pieces were fixed in 2.5 % glutaraldehyde in cacodylate buffer (pH 7.2–7.4) for 2 hours, followed by additional fixation in 1 % osmium tetroxide (OsO4) (1 hour in a refrigerator at +4 °C). The material was dehydrated in a battery of alcohols of increasing concentration (ethanol) and absolute acetone, poured into epon-812 resin with polymerization at a temperature of 37 and 60 °C in a thermostat. Ultrathin sections were prepared on an LKB-III 8800 ultramicrotome (Sweden). Sections were counterstained with 2 % aqueous solution of uranyl acetate and lead citrate, studied and photographed using a Jem-1011 electron microscope (Japan, JEOL) at magnifications of 2500–20000.

RESULTS AND DISCUSSION

Atrophic processes in muscle cells developed right after deep dystrophic changes in the control group. Muscle fibers gradually homogenized, subjected to decay (Figure 1). Thinning of the fibers, hypertrophy of the nuclei were noticed, the pattern of transverse striation was smoothed out. Synapses with nerve processes were not detected on the collapsing muscle cells. In this zone, the number of active fibroblast cells increased, which in the cytoplasm contained more dilated channels of the granular endoplasmic reticulum (GER), indicating an increase in their collagen-synthetic function. Instead of destroyed muscle fibers, bundles of collagen fibers grew. There were more of them by the end of the experiment, the processes occurring in the muscle tissue led to fibrosis or scarring of the muscle tissue left without nutrition.

Connective tissue regenerate began to form in the border zone in the 1st experimental group a month later. Single thin nerve fibers were detected, probably growing from the branches of the trigeminal nerve, in the autograft of the masticatory muscle. Despite this, the fibers of the dissected facial nerve gradually underwent Waller's degeneration, and the fibers of the buccal muscle – to destructive processes (Figure 2). The majority of the buccal muscle fibers were destroyed and atrophied, without additional innervation and nutrition six

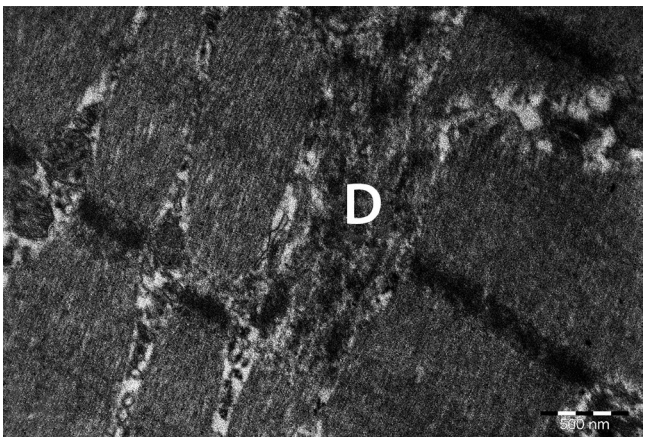

Figure 1. Ultrastructure of the denervated buccal muscle of the rabbit of the control group: destruction (D) of the muscle fiber on the 30th day after resection of the facial nerve. Magnification x20000

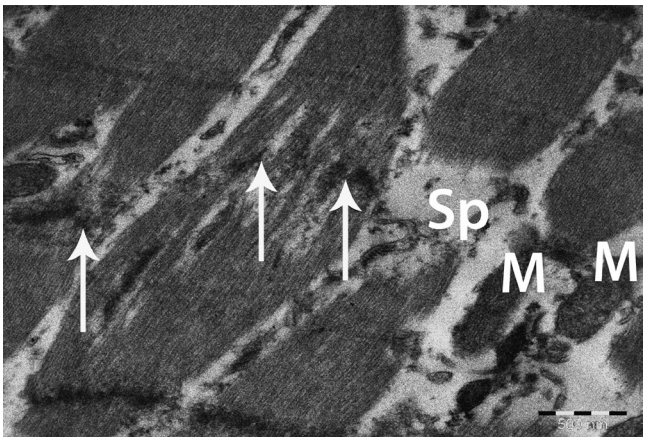

Figure 2. Ultrastructure of the denervated buccal muscle of the rabbit of the 1st experimental group after surgery without the use of allogeneic biomaterials. Destruction of buccal muscle fibers on day 60; destruction of mitochondria (M); absence of glycogen granules in the sarcoplasm (Sp), lysis of individual sarcomeres (↑). Magnification x20000

month later. In the border zone, a dense connective tissue scar was determined, and partially preserved muscle fibers near it were subjected to pronounced contraction (Figure 3).

In the 2nd experimental group, a month after the operation, the structure of the buccal muscles differed from that of the denervated muscles of the animals of the 1st experimental group in that a zone of loose connective tissue was formed in the border zone between the transected buccal muscle and the autologous flap of the supplied masticatory muscle. It contributed to the rapid germination of blood vessels and axons from the bundles of the trigeminal nerve to the denervated buccal muscle of rabbits. Two months later and in further periods of the experiment, blood capillaries and separate bundles of unmyelinated axons of neurons were detected between the regenerating muscle fibers in the connective tissue (Figure 4). In such areas on the

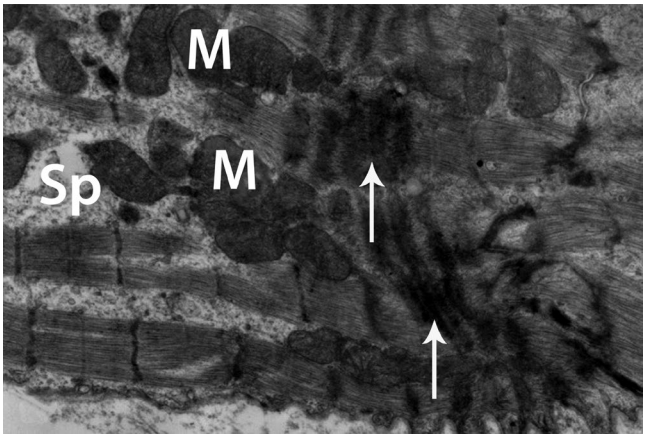

Figure 3. Ultrastructure of the denervated buccal muscle of the rabbit of the 1st experimental group after surgery without the use of allogeneic biomaterials. Contraction (↑) of the fibers of the buccal muscle near the connective tissue scar on the 180th day after the operation; Sp, sarcoplasm; M – mitochondria. Magnification x15000

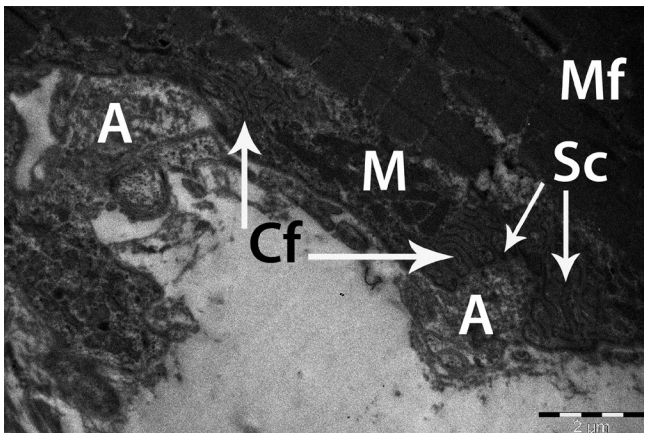

Figure 5. Neuromuscular synapses on the fiber surface of the denervated buccal muscle in a rabbit of the 2nd experimental group on day 180 after surgery using allogeneic biomaterials; Mf – striated muscle fiber; A – the end of the axon of the motor nerve; M – mitochondria: Cf (↑) – contact folds; Sc (↑) – synaptic clefts. Magnification x 8000

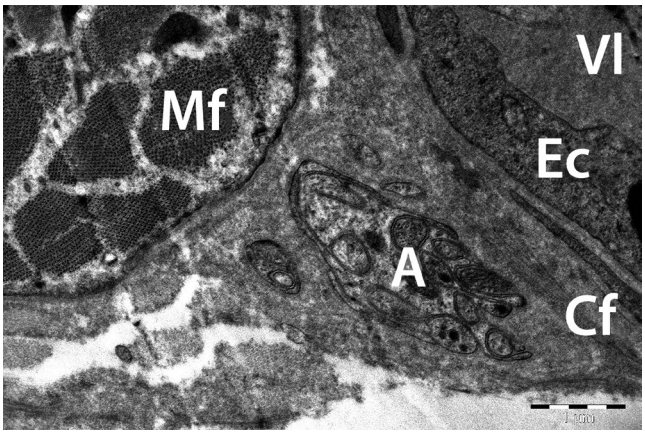

Figure 4. Surgical area in a rabbit of the 2nd experimental group using allogeneic biomaterials after 30 days. Loose connective tissue with a blood vessel and a bundle of axons along the muscle fiber. Mf – striated muscle fiber; Cf – collagen fibers; A – axons; Vl – lumen of a blood vessel; Ec – endothelial cell in the wall of a blood vessel. Magnification x 10000

surface of muscle fibers, motor end plates (neuromuscular junctions) were detected. Synapses were characterized by numerous folds and with numerous synaptic vesicles in them (Figure 5).

Around and between the synapses, clusters of optically dark oblong mitochondria were detected, since the processes occurring in the neuromuscular junctions are energy intensive. Muscle cells contained round or oval nuclei with coarse clumps of heterochromatin on the inner karyolemma. In the cytoplasm, a moderate amount of rounded mitochondria with thin cristae, short channels of the granular endoplasmic reticulum, numerous accumulations of ribosomes and polyribosomes, and star-shaped small clumps of glycogen were determined. In the muscle fibers, transverse striation was clearly visible. By day 180, the structure of the buccal muscle was identical to the normal one, and the striated striation of the tissue was clearly visible. Between the bundles of muscle fibers, the profiles of blood vessels were determined, as well as individual myelinated and unmyelinated axons lying along the muscle cells.

It is a well-known fact that after damage to the facial nerve as a result of a subsequent violation of structural and functional relationships with the buccal muscles, muscle paralysis, atrophy of muscle tissue, scarring and contracture of facial muscles most often develop, which was determined in the control group [2]. In the group where autotransplantation of a part of the masticatory muscle was performed, the processes of tissue destruction of the buccal muscle slowed down significantly. Probably, the sutured "leg" of the masticatory muscle had a certain trophic effect, but this was not enough to stimulate tissue regeneration. Destructive processes in the damaged muscle intensified, and compensatory-restorative processes also ended in fibrosis and scarring of the border zone [2]. The scar tissue prevented the germination of blood vessels and axons.

In the experimental group, where allogeneic biomaterials were used, a zone of loose connective tissue was formed between the denervated muscle and the autograft, into which blood vessels and axons from the trigeminal nerve bundles grew. Vessel profiles were determined in the interfascicular spaces, and characteristic neuromuscular synapses appeared on the regenerating muscle fibers. The results of many years of scientific research conducted at the All-Russian Center for Eye and Plastic Surgery (Ufa) showed that, when implanted into the tissues of a living organism, allogeneic biomaterials have the ability to inhibit scarring of various tissues, including muscle [13–15]. Used allogeneic biomaterials are gradually resorbed by macrophages and replaced by a newly formed fully differentiated tissue [15–20]. Non-

specific stimulation of macrophages with allogeneic biomaterial contributes to the restoration of cellular mechanisms for controlling regeneration in tissues that were disturbed during pathology, which leads to reparative processes proceeding on the basis of the laws of physiological regeneration. Therefore, the use of allogeneic biomaterials in surgical practice makes it possible to achieve full reparation and avoid scarring of tissues, including muscle tissue. Thus, the data presented by us on the reinnervation of muscle tissue are used in reconstructive operations on the auxiliary apparatus of the eye (eyelids, extraocular muscles). In particular, in the work of A. B. Nuraeva [20], using the factor of reinnervation of mimic muscles during allotransplantation, the possibility of not only anatomical, but also functional restoration of the eyelids was proved.

CONCLUSION

The use of allogeneic biomaterials in operations to restore damaged peripheral nerves accompanying muscles creates conditions not only for the restoration of muscle fibers, but also for the restoration of nerve elements with neuromuscular junctions, as well as the vascular bed of the nerve bundle.

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