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ARTIFICIAL NERVE CONDUIT FOR GUIDING PERIPHERAL NERVE GROWTH (CADAVERIC STUDY)

A.G. Fedyakov[1, 2], E.A. Nemets[3], O.N. Dreval[2], A.V. Gorozhanin[1, 2], L.A. Sidneva[2], Z.H. Plieva[2, 4], M.A. Razin[2], N.V. Perova[5], V.I. Sevastianov[3, 5]

[1] Botkin Hospital, Moscow, Russian Federation
[2] Russian Medical Academy of Continuous Professional Education, Moscow, Russian Federation
[3] Shumakov National Medical Research Center of Transplantology and Artificial Organs, Moscow, Russian Federation
[4] Orthospine Clinic, Moscow, Russian Federation
[5] Institute of Biomedical Research and Technology, Moscow, Russian Federation

At present, the search for effective ways of restoring peripheral nerves with anatomical damage continues. Autoplasty still remains the gold standard, which, however, is not without its drawbacks. The use of nerve implants for promoting directional axon growth is essential and promising. **Objective:** to study the biomechanical properties of laboratory samples of an artificial nerve conduit (NGC) made of hybrid biomaterials and to, on cadaveric material, assess the technical feasibility of using them in surgical practice to repair extended peripheral nerve defects. **Material and methods.** The objects of the study were three electrospun NGC samples: from synthetic material (polycaprolactone, PCL) and hybrid biomaterials (PCL + gelatin or PCL + collagen). The work compared the physical and mechanical properties of NGC: stiffness, plasticity, elasticity, brittleness, resistance to chemical attack, their ability to be impregnated with liquid media, permeability, possibility of making an anastomosis between the implant and the nerve during surgical procedure. Cadaveric material was the object of the study: we used a dissected superficial sensory branch of the human right radial nerve, 2 mm in diameter, isolated on the forearm, about 12 cm in length, because it most corresponded to the diameter of the NGC samples tested. After surgery, the echogenic features of the implants and their anastomoses with the nerve were assessed by ultrasound imaging. **Results.** It was found that hybrid NGC samples, based on their biomechanical properties, are fundamentally suitable for use in surgical practice, to ensure growth and replacement of a peripheral nerve defect. However, the best composition of a nerve guide can be established after comparative preclinical study of the biocompatible and functional properties of hybrid material samples. **Conclusion.** The physical and mechanical properties of the investigated NGC samples made of hybrid biomaterials meet the technical requirements for implantable nerve conduits for surgical application.

Keywords: peripheral nerve damage, artificial nerve guide, nerve conduit, polycaprolactone, collagen, gelatin, regeneration.

The frequency of peripheral nerve injuries from limb injuries ranges from 1.5% to 13% and ranks first in terms of degree of disability of the injured. Disability in all nerve injuries reaches 60% [1], and about 45% of cases of nerve injuries in the Russian Federation occur among able-bodied citizens aged 21 to 35 years [2].

Every year in Russia, 4,000 to 7,000 people require surgical treatment for this condition [3]. Among the patients who underwent surgical treatment, only half of them have complete functional recovery of the nerve, 3% of patients have reduced sensitivity, while the motor function of the nerve is restored in less than 25% of patients [4, 5]. Such a low percentage of rehabilitation of patients with peripheral nerve injury is mainly due to the incomplete regenerative potential of the injured axon, as well as to the lack of necessary conditions for directed axonal growth from the proximal end to the distal end [6]. The high frequency of peripheral nerve injuries, accompanied by loss of ability to work, up to disability, makes finding new effective surgical approaches for surgical repair of damaged nerves an urgent task.

To restore the anatomical integrity of the damaged peripheral nerve, neurorrhaphy (surgical restoration of nerve trunk integrity by mobilizing and suturing its ends) is traditionally used in clinical practice, and if it is impossible, autoplasty with the patient's own nerve is used. Nerve autotransplantation is currently the gold standard treatment for peripheral nerve injuries accompanied by diastasis >3 cm. However, the neurological deficit arising in the area of innervation of the nerve used for autoplasty,

Corresponding author: Larisa Sidneva. Address: 2/1, bldg. 1, Barrikadnaya str., Moscow, 123242, Russian Federation. Phone: (988) 991-08-26. E-mail: larisa.sidneva@inbox.ru

the mismatch between the diameters of the donor and recipient nerves, and the significant duration of surgical intervention limit the use of this technique. An alternative approach to restoring the anatomical integrity of damaged nerve is to create and use peripheral nerve implants made of synthetic and/or natural polymeric materials called nerve guidance conduits (NGC) (also referred to as an artificial nerve conduit or artificial nerve graft), which are designed to promote directed axonal growth and provide good conditions for regeneration of damaged nerve.

Various variants of nerve conduits were proposed back in the 19th century, but the feasibility of their use was questioned until the second half of the 20th century, since simpler surgical techniques for nerve fiber mobilization and tensioning were used in parallel. Later, these surgical methods were abandoned because it became clear that nerve tension significantly reduces their regenerative potential [7, 8]. Nerve conduits began to be registered as medical devices since the mid-1980s, and they are becoming commercially available in clinical practice [9].

Meanwhile, there is still no universal artificial nerve conduit with perfect biocompatibility. Literature asserts the importance of the following basic requirements for fabrication of peripheral NGCs [5, 10–14]:

a) Biocompatibility;
b) Biodegradation/bioresorption rate should not exceed the nerve regeneration time;
c) Permeability and wall thickness (the ideal scaffold should be semi-permeable; conduit permeability increases with pore size: nerve channels with larger pores better support axonal growth, the optimal pore size range is 10–20 μm, which facilitates nutrient inflow and at the same time prevents fibroblast penetration and growth);
d) Acceptable mechanical properties (strength, elasticity, tension, flexibility, resistance to destruction and stretching, stitching capability);
e) Ability to create optimal conditions for accelerated and directed axonal growth followed by full functional and structural restoration of denervated tissue;
f) Implant production technology should facilitate the production of a linear range of products of different diameters and lengths;
g) Affordable price.

By structure, NGCs can be divided into hollow (in the form of closed tubes or longitudinally dissected cylinder) and filled with various materials.

NGCs can be made from biodegradable/bioabsorbable synthetic (e.g., polyvinyl alcohol, polyglycolic acid, polycaprolactone) and natural polymers (usually collagen/gelatin, chitosan, polyoxybutyrate). Each of these groups has its own advantages and disadvantages [15, 16]. Highly biocompatible products made of natural polymers are rapidly resorbed, have poor mechanical properties, and are expensive to produce. The qualities of medical products made of synthetic polymers are more reproducible, have good mechanical properties, but are inferior to natural polymers in terms of biocompatibility and are not bioactive.

In our opinion, a promising approach to the creation of artificial nerve grafts is to use hybrid biomaterials comprising both synthetic and natural polymers. Synthetic polymers allow NGCs to provide the required mechanical characteristics, while natural polymers ensure high biocompatibility and provide bioactive properties in terms of stimulating the regenerative processes of the damaged nerve. In our work on creation of laboratory NGC samples from hybrid biomaterial, we chose a synthetic polymer, polycaprolactone (with a low degradation rate), and a natural biopolymer, collagen (or gelatin, its denatured form), the main extracellular matrix protein.

The aim of this work was to study the technical feasibility of surgical application of NGC samples made of hybrid biomaterials based on polycaprolactone and collagen/gelatin on a nerve isolated from cadaveric material.

MATERIAL AND METHODS

Tubular NGCs with an internal diameter of 2 mm (Fig. 1) were made by electrospinning method, previously developed to create tissue-engineered constructs of small-diameter blood vessels [17], from a 10% (w/w) polycaprolactone solution (PCL, MM 80000, Sigma-Aldrich, USA, sample #1), PCL with added gelatin (Sigma-Aldrich, USA (sample #2) and PCL with collagen (Collost, Russia, sample #3) in hexafluoroisopropanol (NPO PIM-INVEST, Russia) using electrospinning

Fig. 1. NGC samples

device NANON-01A (MECC CO, Japan) at 25 kV voltage between electrodes, solution feed rate 4 ml/h, distance to collector 100 mm, spindle speed 1000 rpm, using 18 G needle. After the end of the solution application process, the obtained samples were dried in a thermostat at 37 °C for 2 hours, followed by evacuation to remove traces of solvent at a residual pressure of 10–20 mm Hg and temperature 37 °C for 24 hours.

NGC samples were subjected to mechanical testing on a Shimadzu EZ Test EZ-SX test frame (Shimadzu Corporation, Japan) at a tensile speed of 5 mm/minute. The following mechanical characteristics of the specimens were recorded: elongation at break, tensile strength at break, and Young's modulus, which characterizes the degree of elasticity of the NGC. Young's modulus was calculated in Trapezium X software, version 1.2.6.

Experimental work was carried out in a room with a temperature of +18 °C. During the study, the following physical, mechanical and technical properties of the samples were evaluated: stiffness, plasticity, elasticity, brittleness, chemical resistance, ability to be impregnated with liquid media, porosity, and possibility to make anastomosis between the implant and nerve (stitching with surgical needle, conducting a ligature). We used lever scales with loads of different weights, a caliper, liquid media (centrifuged human blood plasma, 0.5% novocaine solution, native human blood, 3% H_2O_2 solution), edible indigo carmine for staining transparent liquids, suture surgical material with non-absorbable monofilament thread and atraumatic cutting needle (Prolene 6-0, 45 cm).

Cadaveric material was the object of the study: we used a dissected superficial sensory branch of the human right radial nerve, 2 mm in diameter, isolated on the forearm, about 12 cm in length, because it most corresponded to the diameter of the presented samples (Fig. 2).

We simulated restoration of the injured nerve integrity by tandem suturing of the NGC with distal and proximal fragments of the crossed nerve. The surgery was microsurgical: a 3.5× headband magnifier and microsurgical instruments were used.

After layer-by-layer suturing of the wound, the echogenic characteristics of the implants and their anastomoses with the nerve were assessed through ultrasound-guided percutaneous needle biopsy in the tissues with a 7.5 MHz linear ultrasound probe.

RESULTS

Three NGC variants with the following dimensions were investigated: length and diameter of samples #1 (PCL), #2 (PCL + gelatin) and #3 (PCL + collagen) were 97.9 mm and 2.3 mm, 60.4 mm and 2.5 mm, and 49.1 mm and 3.2 mm, respectively.

The stiffness (based on tactile sensation) of the dry samples decreased in the following order: #3 > #2 > ##1. When dissecting the samples with a scalpel, it was noted that they were electrified in the dry state, sticking to the tool, the first and third samples slightly disentangled.

The physical and mechanical characteristics of NGC samples are summarized in Table. As can be seen from Table, the presence of gelatin in the nerve conduit (sample #2) does not affect its elongation at break, accom-

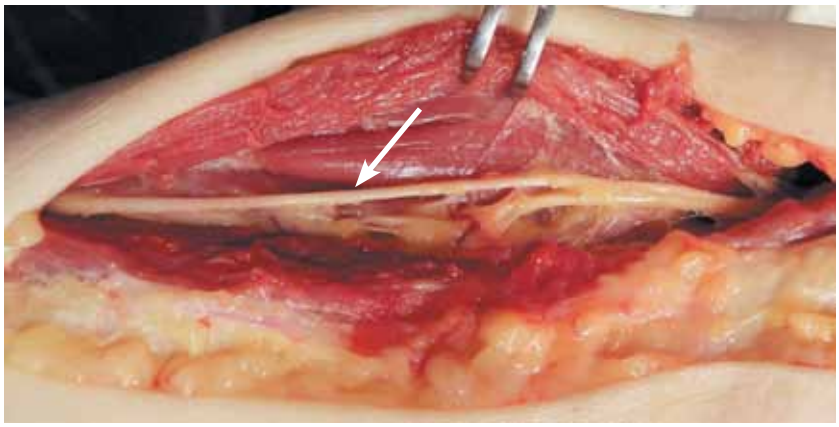

Fig. 2. Superficial sensory branch of the right radial nerve (indicated by arrow)

Table

Physical and mechanical characteristics of NGC

NGC	Young's modulus (MPa)	Tensile strength at break (N)	Elongation at break (%)
#1 (PCL)	5.5 ± 1.1	10.9 ± 1.6	477 ± 38
#2 (PCL + gelatin)	7.8 ± 2.6	24.3 ± 7.6	452 ± 32
#3 (PCL + collagen	10.5 ± 3.1	33.2 ± 6.9	357 ± 47

panied by a 30% increase in Young's modulus and a 2.5-fold increase in tensile strength at break compared to specimen #1 made of PCL. With collagen in the hybrid material (sample #3), the Young's modulus increases twice as much as that of NGC made of PCL, tensile strength at break increases 3-fold, and elongation at break decreases by 25%. Thus, introduction of gelatin and collagen increases the strength of NGC with a simultaneous slight decrease in its elasticity, especially noticeable when using a hybrid material with collagen (sample #3).

The obtained results were confirmed when assessing the resistance (rigidity) of the specimens to deformation changes. We evaluated the deformation of sample fragments, 5 mm long, in dry state under the influence of loads of different weights. Sample #1 began to deform under a load of 5 g or more. Deformation of the other two samples occurred under a 20 g weight (Fig. 3). The samples were not brittle under physical stress and did not crumble when cut with a scalpel.

To determine possible changes in the properties of the samples when interacting with various biological media and chemical compounds encountered during the operation, they were wetted: 1) in 0.5% novocaine solution; 2) 3% H_2O_2 solution; 3) in native human blood; 4) human blood plasma. Changes in the samples after 0.5, 1.0, 1.5, and 2.0 hours were assessed. All samples were gradually impregnated with solutions, becoming more elastic (based on tactile sensations). Sample #3, which is more hydrophilic in comparison with the others, was the best and fastest to be impregnated in the media. Sample #1 was the most hydrophobic. Dissolution, change of shape, significant loss of elasticity (deformation under own weight) was not noted in any of the examined samples.

Fig. 3. Assessment of stiffness and elasticity (deformation of sample #1 under a 5 g load)

We used 20-mm-long sample fragments to create anastomoses between the NGC and the nerve. Each specimen was tandemly sutured using an atraumatic needle (Prolene 6-0) to the pre-transected superficial sensory branch of the radial nerve (Fig. 4). Epineural sutures were placed on the nerve, and the NGC was sutured through its entire thickness. There were no difficulties with stitching in any of the specimens; however, sample #1 was the easiest to stitch. When creating an anastomosis, it is more convenient to inject from the epineurium side and then stitch the implant to its full thickness, with the knot better left on the outside of the nerve conduit to avoid formation of scar changes in the nerve tissue area. After wetting with novocaine solution, the specimens were easier to suture to the nerve because they became more elastic.

Permeability of the samples was assessed by filling their lumen from the neural side of the anastomosis with an aqueous solution of edible indigo carmine (Fig. 5). After dye injection, the sutures were noted to be tight, permeability of the NGC depended on the volume of the injected solution. All specimens passed the dye after some time, the most permeable being specimen #1 made of PCL.

Ultrasound imaging of the created anastomoses after layer-by-layer suturing of the wound was performed (Fig. 6). The following data were obtained during the study:– Anastomoses between the nerve and NGC spe-

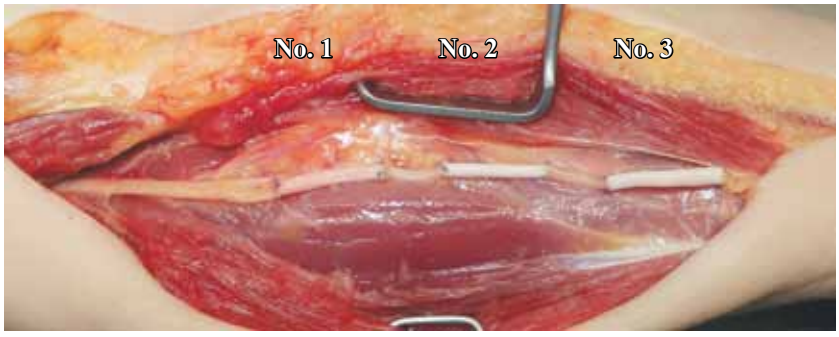

Fig. 4. Tandem anastomoses between the NGC and the superficial sensory branch of the radial nerve

cimens after soft tissue suturing was preserved in all cases;

– Echogenicity of all specimens relative to the nerve and surrounding soft tissues increased;
– Cross-sectional scanning of the implant revealed a hyperechogenic circumference, which differed from the nerve in ultrasound image;
– The NGC samples had different resistance to mechanical pressure on the surrounding tissues. Sample #1 had the lowest stiffness and elasticity – it shrank under the pressure of the ultrasound probe, flattened and did not fully recover its original rounded shape. Specimens #2 and #3 had high stiffness and elasticity, and did not change their configuration under pressure. Sample #3 was the most rigid.

DISCUSSION

NGC samples made of hybrid biomaterials (#2, PCL + gelatin; #3, PCL + collagen) are surgically suitable for creating artificial nerve conduits to repair extended peripheral nerve defects.

When hydrated, NGCs are lightweight, flexible, elastic, resilient, not brittle, porous tubes convenient for suturing to peripheral nerves. Hybrid nerve conduits are moderately hydrophilic, which *in vivo* is important to ensure cell adhesion as the initial stage of the regenerative process in the damaged nerve. Also, these samples have acceptable surgical porosity and are more preferable due to the ability to retain their shape, including when exposed to various liquid media and pressure of the surrounding tissues [16].

Surgical manipulations on the cadaveric material showed that all the presented NGC samples are convenient for surgical application (easily sutured, form tight anastomoses with the nerve, are not deformed and do not dissolve when exposed to various fluids used during surgery). The best nerve conduit composition will be selected during comparative preclinical studies of biocompatible and functional properties of hybrid material samples.

Ultrasound is the main method of monitoring the surgical restoration of the anatomical integrity of peripheral nerves: direct neurorrhaphy, autotransplantation, and

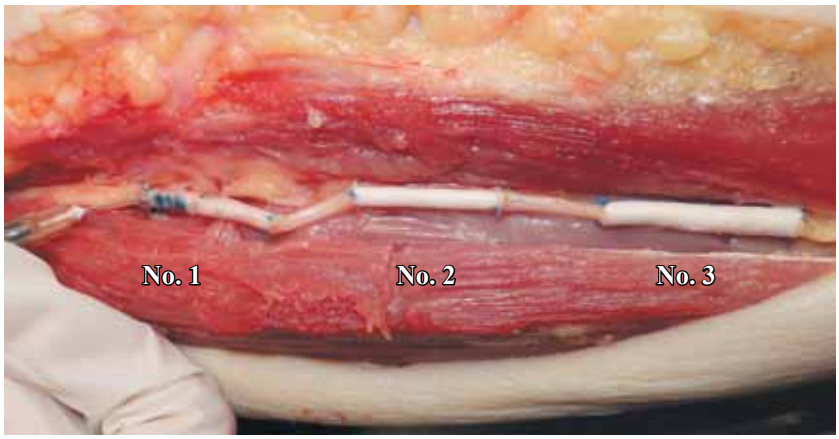

Fig. 5. Dye injection (aqueous solution of edible indigo carmine) into the NGC lumen

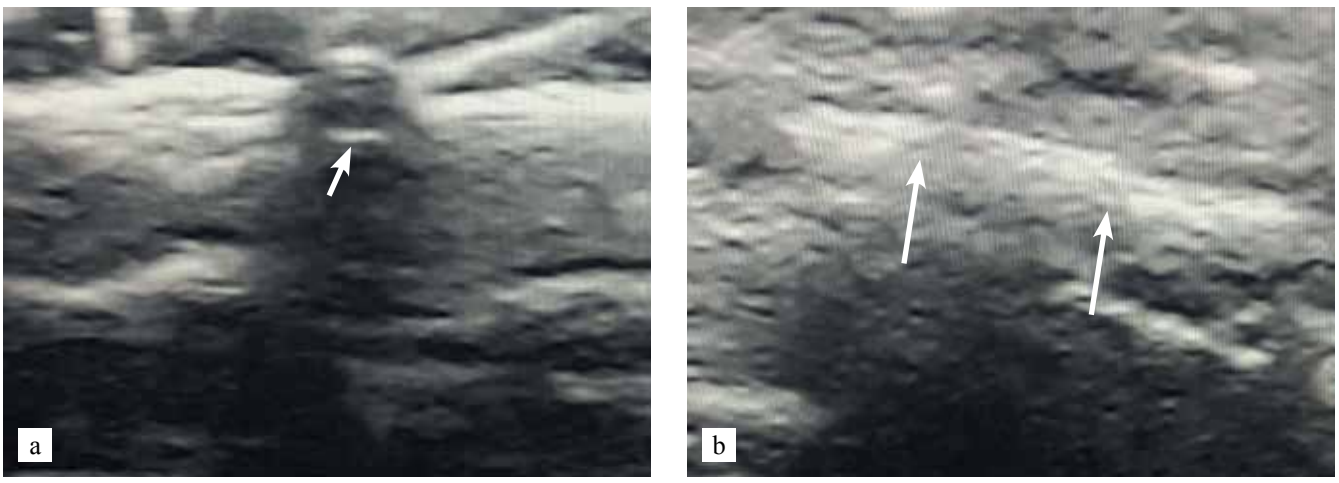

Fig. 6. Ultrasound picture of the NGC (indicated by arrows). a, cross section; b, longitudinal section

plasty using NGCs. This is due to the fact that morphological changes occurring in the postoperative period are ahead of the functional nerve, which can be assessed clinically or by electroneuromyography. To determine the adequacy of such interventions and their probable effectiveness, it is necessary to assess the formation of an end neuroma, regeneration of nerve fibers, severity of the cicatricial adhesive process in the surgical area, and anastomosis viability. In the case of NGCs, the carrying capacity of their membranes for ultrasound signal, which determines the possibility to visualize the regenerating nerve tissue in the graft lumen, is important. Creating an optimal material for NGC in terms of performing perfect ultrasound imaging is extremely difficult. By focusing only on this criterion, one will have to sacrifice more important (chemical, physical and biological) properties of NGC. Even the polyhydroxybutyrate-based membrane, which is several microns thick and used in neurosurgical practice for the prevention of cicatricial adhesions, reduces the capabilities of ultrasound imaging in the postoperative period [18].

During the reparative processes in the area of surgical intervention, the ultrasound image of prosthetic biotransformation will change over time. This is due to many factors: development of cicatricial adhesion process, neoangiogenesis, nerve fiber regeneration, and biotransformation of the NGC itself. The most important early (first 1–2 months after intervention) criterion of successful regeneration of nerve tissue, even in case of low ultrasound throughput of NGC, is the absence of terminal neuroma formation in the area of the proximal fragment of the nerve trunk involved in anastomosis. The indicated abortive regeneration sign (presence of terminal neuroma) does not depend on ultrasound characteristics of NGC materials and can be detected in all cases.

The following were limitations of NGC ultrasound assessment: not the highest degree of ultrasound imaging, and lack of possibility to assess the biotransformation of both the NGCs themselves and the surrounding tissues over time *in vivo*. Finding the optimal material for the NGC is a crucial step towards creating an effective peripheral nerve prosthesis, which can be a good alternative to the autograft. The advantage of using NGC in clinical practice is that the procedure of nerve extraction from the patient (in autografting) and the resulting complications at the donor site are eliminated, surgical time is reduced, and the intervention becomes simpler. Numerous studies show that the possibility of functional recovery when using NGC is equivalent to autotransplantation and direct nerve suturing in an experiment [19, 20].

CONCLUSION

The physical and mechanical properties of NGCs made of PCL and PCL-based hybrid biomaterials were investigated, and the technical feasibility of their surgical use to promote directional nerve growth and repair was studied. NGC samples consisting of hybrid materials showed fundamental biomechanical suitability for use in surgical practice to eliminate peripheral nerve defects.

The authors declare no conflict of interest.

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