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Nerve regeneration using tubular scaffolds from biodegradable polyurethane

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Summary

Introduction. In severe nerve lesion, nerve defects and in brachial plexus reconstruction, autologous nerve grafting is the golden standard. Although, nerve grafting technique is the best available approach a major disadvantages exists: there is a limited source of autologous nerve grafts.

This study presents data on the use of tubular scaffolds with uniaxial pore orientation from experimental biodegradable polyurethanes coated with fibrin sealant to regenerate a 8 mm resected segment of rat sciatic nerve.

Methods. Tubular scaffolds: prepared by extrusion of the polymer solution in DMF into water coagulation bath. The polymer used for the preparation of tubular scaffolds was a biodegradable polyurethane based on hexamethylene diisocyanate, poly(ε-caprolactone) and dianhydro-D-sorbitol.

Experimental model. Eighteen Sprague Dawley rats underwent mid-thigh sciatic nerve transection and were randomly assigned to two experimental groups with immediate repair: (1) tubular scaffold, (2) 180° rotated sciatic nerve segment (control). Serial functional measurements (toe spread test, placing tests) were performed weekly from 3rd to 12th week after nerve repair. On week 12, electrophysiological assessment was performed. Sciatic nerve and scaffold/nerve grafts were harvested for histomorphometric analysis. Collagenic connective tissue, Schwann cells and axons were evaluated in the proximal nerve stump, the scaffold/nerve graft and the distal nerve stump.

The implants have uniaxially-oriented pore structure with a pore size in the range of 2 µm (the pore wall) and 75 × 700 µm (elongated pores in the implant lumen). The skin of the tubular implants was nonporous.

Animals which underwent repair with tubular scaffolds of biodegradable polyurethanes coated with diluted fibrin sealant had no significant functional differences compared with the nerve graft group.

Control group resulted in a trend-wise better electrophysiological recovery but did not show statistically significant differences.

There was a higher level of collagenic connective tissue within the scaffold and within the distal nerve stump. Schwann cells migrated into the polyurethane scaffold. There was no statistical difference to the nerve graft group although Schwann cell counts were lower especially within the middle of the polyurethane scaffold. Axon counts showed a trend-wise decrease within the scaffold.

Conclusion. These results suggest that biodegradable polyurethane tubular scaffolds coated with diluted fibrin sealant support peripheral nerve regeneration in a standard gap model in the rat up to 3 months. Three months after surgery no sign of degradation could be seen.

Keywords: Nerve regeneration; tubular scaffolds; biodegradable; allogenic nerve graft.

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Introduction

Five basic possibilities are described by Millesi to manage a nerve defect [6].

1) Restoration of continuity by end-to-end coaptation. 2) Restoration of continuity by adding tissue. 3) By passing a defect by transfer of synergistic nerve fibers of equal destination as suggested by Millesi and Schmidhammer [3]. 4) By passing a defect by nerve fiber transfer from another nerve if the proximal stump is lacking. 5) Nerve-muscle neurotization if the distal stump is lacking.

The golden standard for bridging nerve defects and in brachial plexus reconstruction is autologous nerve grafting [1, 2]. However, there is a very limited source of autologous nerve grafts. Thus, in the last decades with the rapid advances in biomaterial technology, a number of materials were tested for a nerve guiding function. Different materials, such as polymers, silicone and collagen were used for nerve tubes and nerve scaffolds [1].

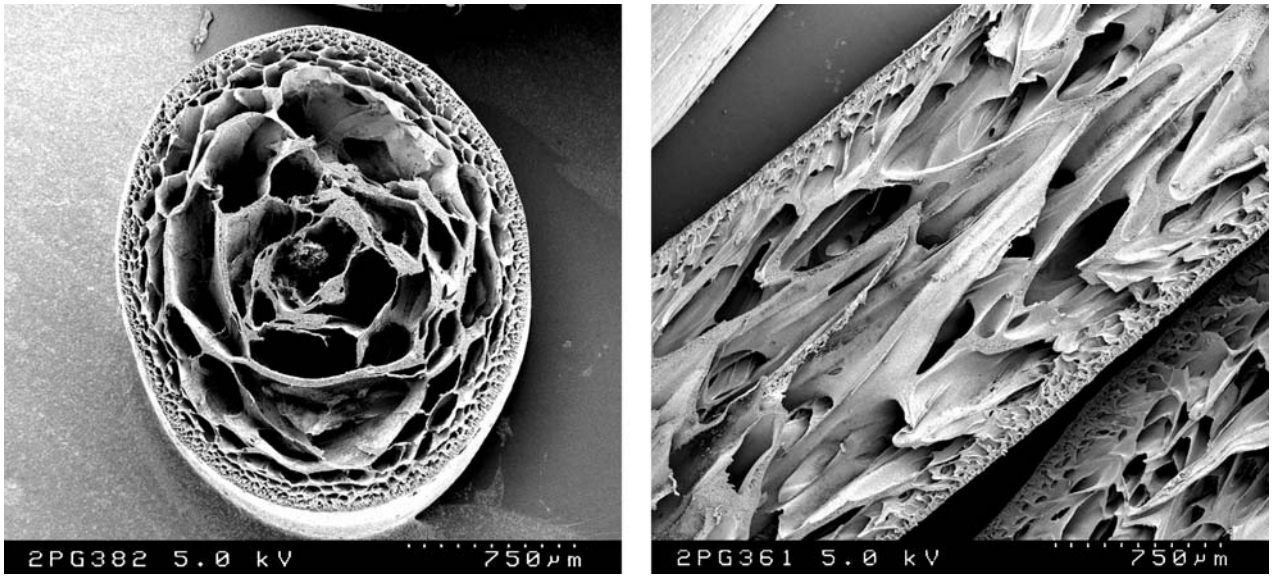

Fig. 1. Scanning electron micrographs of polyurethane tubular scaffolds with uniaxial pore orientation. (A) Cross-section perpendicular to the tube longer axis; (B) Cross-section parallel to the tube longer axis

One major demand to those nerve guiding materials is biodegradability within a short time. Additionally, these biodegradable materials need to be mechanically stable [5] at the site of coaptation to allow movement of the nerve which is especially required in the joint regions.

The aim of this rat study was to investigate the efficacy of a biodegradable polyurethane scaffold with uniaxial pore orientation in functional peripheral nerve regeneration in an 8 mm defect in the sciatic nerve. Focussing on nerve regeneration through the scaffold functional results are discussed in context with electrophysiological and histological data.

Material and methods

Experimental model

We used eighteen Sprague Dawley rats weighing 330–400 g. In each animal the right sciatic nerve was located midthigh. The nerve and its branches were microsurgically dissected from the sciatic notch to the hollow of the knee and isolated atraumatically from the surrounding tissue.

Tubular scaffolds

The experimental biodegradable polyurethane used in the study was based on hexamethylene diisocyanate, poly(ε-caprolactone) and dianhydro-D-sorbitol (K. Gorna, S. Gogolewski, AO/ASIF Research Institute, Davos, Switzerland). The porous scaffolds were prepared by extrusion of the polymer solution in DMF into water coagulation bath.

The implants have uniaxially-oriented pore structure with a size in the range of 2 µm of the pores in the wall and 75 µm × 700 µm of the elongated pores in the implant lumen (Fig. 1). The skin of the tubular implants was nonporous.

Before implantation the tubular scaffolds were coated with 1:8 diluted fibrin sealant.

Groups

The animals were randomly assigned to two experimental groups with immediate repair. Group (1) tubular scaffold. An 8 mm piece of the nerve was cut out and was replaced by a fibrin coated tubular scaffold. The nerve ends were coaptated under the microscope to the scaffold by two epineural sutures.

Group (2) (control group). An 8 mm piece of the nerve was cut out and the segment was rotated for 180° was replaced by a fibrin coated tubular scaffold. The nerve ends of the rotated segment were immediately coaptated under the microscope to the ischiadic nerve by two epineural sutures proximal and two coaptations distal.

Serial functional measurements (toe spread test, placing tests) were performed weekly from 3rd to 12th week after nerve repair. On week 12,

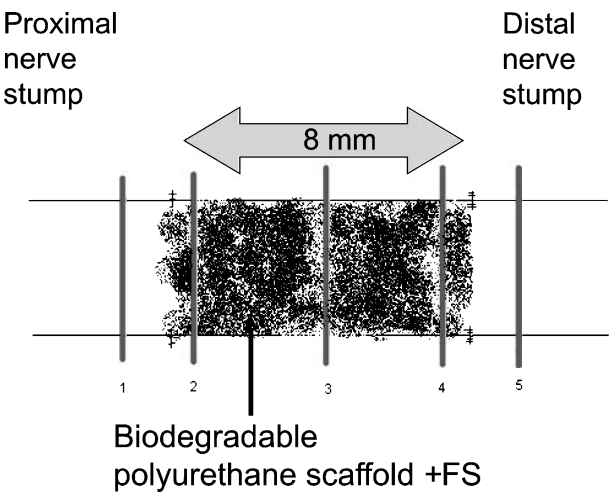

Fig. 2. Specimen zones

electrophysiological assessment was performed. Sciatic nerve and scaffold/nerve grafts were harvested for histomorphometric analysis. Collagenic connective tissue, Schwann cells and axons were evaluated in the proximal nerve stump, the scaffold/nerve graft (Fig. 2) and the distal nerve stump.

Results

Animals which underwent repair with tubular scaffolds of biodegradable polyurethanes coated with diluted fibrin sealant had no significant histological, electrophysiological, or functional differences compared with the nerve graft group.

There was a higher level of collagenic connective tissue within the scaffold and within the distal nerve stump. Schwann cells migrated into the polyurethane scaffold. There was no statistical difference to the nerve graft group although Schwann cell counts were lower especially within the middle of the polyurethane scaffold. In both groups axon counts showed a trend-wise decrease within the scaffold. In the distal zones there were slightly more vessels in the polyurethane scaffold group, but again no statistically significant difference (Fig. 3).

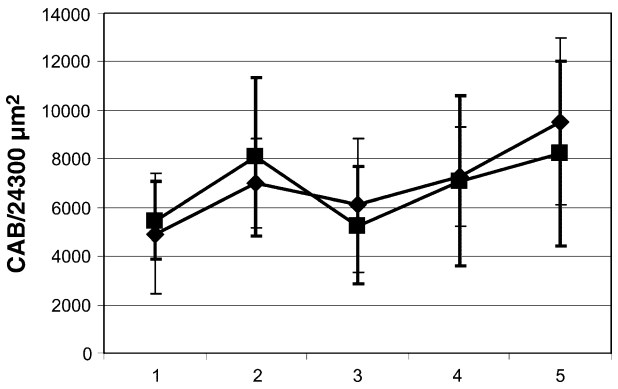

Fig. 3. In the distal zones slightly more vessels could be found (zone 3–5). ◆ PU scaffold FS1:8, ■ control

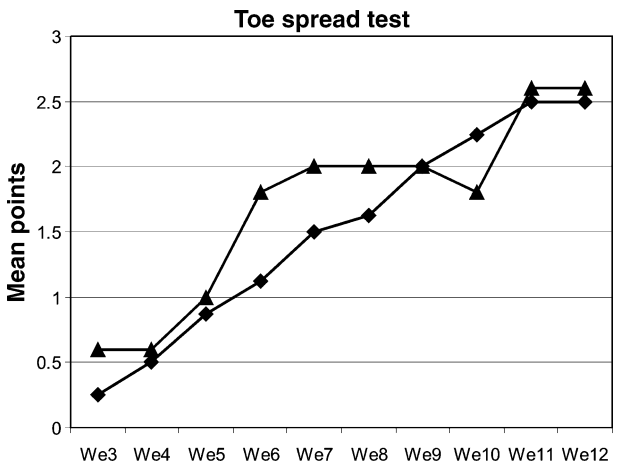

Fig. 4. Functional assessment of nerve regeneration by toe spread test. ◆ PU scaffold + FS, ▲ nerve graft 180°-control

The control group resulted in a trend-wise better electrophysiological recovery but did not show statistically significant differences.

The toe spread assessment as a functional test didn't show any statistically significant difference between both groups (Fig. 4). There was no contact placing in both groups.

Discussion

The results of this animal study demonstrates that using conduits from biodegradable polyurethane to bridge a nerve defect in a standard gap model in the rat leads to histological, functional and electrophysiological improvement up to 3 months.

No statistically significant differences were found compared to the control group with a 180° rotated sciatic nerve segment. We could find that these results were surprisingly good compared to a nerve graft. However, one should notice that the sciatic nerve graft at thigh level was rotated 180° and distally the nerve is divided into the three branches already. Rotation of the nerve graft in longitudinal axis of 180° may result in the major disadvantage of axonal misdirection.

The time of degradation of these polyurethane scaffolds was chosen for more than 6 weeks to create a mechanically stable environment for axonal regeneration and low inflammatory reaction. On the other hand resorption of the polyurethane scaffold should not be too fast, preserving mechanical properties, to keep the regenerative tissue in position.

However, polyurethane material did not show any elementary signs of degradation 3 months after surgery. One may hypothesize that the scaffolds will serve as a block for axonal regeneration at later stages of the regeneration process. We are convinced that using this type of polyurethane scaffolds in peripheral nerve tissue engineering degradation time has to be decreased, in addition to low inflammatory reactions leading to a low level of neural collagenization and improvement of functional results.

Despite the fact that the diameter of the scaffold was about 20% larger than the diameter of the rotated nerve graft no regenerative tissue could be found outside the graft indicating that there was no aberrant innervation. Additionally, it suggests that within the scaffold there is enough space for neural regenerative tissue enabling axonal sprouts advancing distally. Within the scaffold

the amount of collagenic and angiogenetic tissue was slightly higher than in the control group, indicating an increased inflammatory reaction which is essential for biodegradation of the polyurethane scaffolds.

The electrophysiological and functional results finally did not show statistical significant differences three months after surgery referring to high correlation of these data.

However, as a given fact the capacity of nerve regeneration in the rat is much higher compared to humans, the standard gap model has to be proven by a critical gap model.

Conclusion

These results suggest that biodegradable polyurethane tubular scaffolds coated with diluted fibrin sealant support peripheral nerve regeneration in a standard gap model in the rat up to 3 months.

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