CHAPTER 9

Biodegradable p(DLLA-ε-CL) nerve guides vs. autologous nerve grafts: EMG and video analysis

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Chapter 9

Abstract

The aim of this study was to evaluate the functional effects after bridging a gap in the sciatic nerve of the rat with either a biodegradable p(DLLA-ε-CL) nerve guide or an autologous nerve graft. EMG's of the gastrocnemius (GC) and tibialis anterior (TA) muscles were recorded 3¹/₂ and 5 months after bridging the nerve gaps. Furthermore, the rats' gait was recorded on video and the quality of gait was analyzed. EMG patterns of the contralateral non-operated side were essentially normal. The EMG patterns on the operated side were irregular in all animals. A remarkable finding was, however, that the quality of gait was better in the nerve guide group. We conclude that the surgical technique (nerve guide or nerve graft) does not influence the disturbance of the EMG patterns, but gait improves better in rats where the nerve gap was bridged by a nerve guide.

Introduction

The aim of repairing a peripheral nerve gap is to ensure that a maximal number of regenerating axons will cross the site of injury and that the original innervation of target organs is reestablished. Generally, a sensory nerve autograft is used to bridge these gaps also in a mixed nerve consisting of motor and sensory nerve fibers. Clinically, nerve recovery is often compromised, especially when gaps of more than a few centimeters have to be bridged. Obtaining good recovery of nerve function continues to pose a difficult problem in nerve surgery.

Major drawbacks of the use of nerve grafts are sensory deficits at the donor-site, the risk of painful neuroma formation and scarring.¹ It is known that Schwann cells, which normally take care of the myelinisation of axons, produce several growth factors (such as NGF and BDNF). However, Schwann cells do produce myelin-substances that may inhibit growth of axons as well, e.g. myelin-associated glycoprotein (MAG).² A nerve graft consists of a dense structure which may hamper the outgrowth of axons. The graft contains Schwann cells. Sprouts emitted from the proximal nerve stump contact these Schwann cells. As soon as this happens, the collateral sprouts degenerate in an early phase of the nerve regeneration.³⁴ Moreover, two suture lines double the risk of nerve fibers growing outside that suture line and failing to cross the junction between the proximal and distal nerve stump.⁵⁶

Because of these problems, there is considerable interest in alternatives to bridge nerve gaps. Artificial nerve guides, especially biodegradable ones, are a promising alternative to a nerve graft. Regenerating nerve fibers are directed towards the distal nerve stump. Compared to an autologous nerve graft, the nerve guide contains a more open structure through which regenerating nerve fibers can grow more easily. Ingrowth of fibrous tissue into the nerve gap and neuroma formation is
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prevented, and the amount of scar tissue is decreased by using a single suture for each nerve stump to fix the nerve stump into the lumen of the nerve guide. After functioning as a temporary scaffold for axon outgrowth, they degrade in the course of months or years.

Histologically, it was demonstrated that nerve regeneration across a 10 mm nerve gap bridged by a biodegradable nerve guide composed of an amorphous copolymer of DL-lactide and ε-caprolactone [p(DLLA-ε-CL)], was faster than nerve regeneration through a 7 mm autologous nerve graft.\(^7\) The number of (non-)myelinated nerve fibers, the mean axon diameter, the distributions of the (non-)myelinated nerve fibers, the N- and G ratios, and the number of Schwann cell nuclei were investigated. In order to further increase the speed of nerve outgrowth, Meek et al. added modified denatured muscle tissue (MDMT) inside the p(DLLA-ε-CL) nerve guide.\(^8\) The recovery of nerve function after bridging the sciatic nerve with the nerve guide filled with MDMT (15 mm nerve gap) was compared with an autologous nerve graft (12 mm nerve gap).\(^9\) Nerve function was evaluated by walking track analysis and electrostimulation tests. It was demonstrated that the nerve guide induced fast recovery and reinnervation. Recently, recovery of nerve function was reinvestigated using additional methods for evaluation (including video analysis) after bridging 15 mm gaps in the sciatic nerve of the rat with the p(DLLA-ε-CL) nerve guide.\(^10\) Walking track analysis using finger paint and video analysis, the conduction velocity, and the withdrawal reflex recovered to a certain extent but remarkably the EMG of the hindlimb muscles in the operated leg remained highly abnormal.

In the present study, we compare the results in these rats (15 mm nerve gap and nerve guide repair) with rats (12 mm nerve gap and autologous nerve graft repair) that were previously recorded on video-tape in exactly the same way. As nerve regeneration and recovery of nerve function after bridging a nerve gap with a nerve guide is faster or as good as after bridging with a nerve graft,\(^7,9,11-16\) we intentionally compared two different gap lengths in order to find out whether bridging a larger gap with a nerve guide would give similar results. This study concerns the question whether the quality of the walking cycle scored from video recordings between the nerve guide and autologous nerve graft groups differs. Furthermore, we investigated whether repair of a gap in the sciatic nerve with a p(DLLA-ε-CL) nerve guide leads to less disturbed EMG patterns than after an autologous nerve graft. Therefore, EMG’s of the gastrocnemius (GC) and tibialis anterior (TA) muscles during locomotion were recorded in both groups of rats. The results were compared with results obtained from a non-operated control group.

**Materials and Methods**

**Surgical Procedures**

In total, 36 male Wistar rats weighing approximately 250 g (range 225 - 275 g) were studied. In group A (n = 15), the rats were premedicated with atropine (0.25 mg/kg body weight) and anae-
thetized with 1% isoflurane (Forene®) and O₂/N₂O. The left sciatic nerve was exposed by splitting the left superficial gluteal muscle. A gap of 15 mm was made proximal to the tibial and peroneal bifurcation and bridged by a 18 mm biodegradable nerve guide (Polyganics BV, Groningen, The Netherlands). This guide is composed of a copolymer of 50% DL-lactide and 50% ε-caprolactone [85% L-lactide (LLA) and 15% D-lactide (DLA)]. The internal diameter of the nerve guide was approximately 1.6 mm (range 1.57 - 1.67 mm) and the wall thickness 0.3 mm (range 0.27 - 0.37 mm). Both the proximal and distal ends of the sciatic nerve were telescoped into the nerve guide and fixed with a single 10-0 nylon epineural suture. The tube was prefilled with phosphate buffered saline. In group B (n = 15), a 12 mm segment of the sciatic nerve was resected proximal to the tibial and peroneal bifurcation, reversed and implanted as an autologous nerve graft. In group C (n = 6), the rats were not operated, received no anaesthesia and served as a control group. Control rats were studied to exclude the influence of the EMG electrodes in the muscles of the hindlegs on the quality of the walking cycle and

**Table 1. Experimental set up indicating the number of rats per group and assessment tests at different time periods.**

<table>
<thead>
<tr>
<th></th>
<th>Group A (nerve guide)</th>
<th>Group B (nerve graft)</th>
<th>Group C (control)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15 mm gap N = 15</td>
<td>12 mm gap N = 15</td>
<td>no gap N = 6</td>
</tr>
<tr>
<td>3½ months</td>
<td>EMG: N = 3</td>
<td>EMG: N = 3</td>
<td>no intervention</td>
</tr>
<tr>
<td>5 months</td>
<td>video analysis: N = 12</td>
<td>video analysis: N = 12</td>
<td>video analysis: N = 6</td>
</tr>
<tr>
<td></td>
<td>EMG: N = 3</td>
<td>EMG: N = 3</td>
<td>EMG: N = 3</td>
</tr>
</tbody>
</table>

**Table 2. Score list for the evaluation of the rat walking cycle. Phrasing of the parameters was such that “normality” was scored by “yes” and abnormality by “no”.”

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. toe spread during stance phase</td>
<td>yes / no</td>
</tr>
<tr>
<td>2. walking on plantar side</td>
<td>yes / no</td>
</tr>
<tr>
<td>3. absence of dragging</td>
<td>yes / no</td>
</tr>
<tr>
<td>4. absence of exorotation of the foot</td>
<td>yes / no</td>
</tr>
<tr>
<td>5. alternating steps</td>
<td>yes / no</td>
</tr>
<tr>
<td>6. normal swing phase</td>
<td>yes / no</td>
</tr>
<tr>
<td>7. fluent walking</td>
<td>yes / no</td>
</tr>
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to compare these recordings with those obtained in groups A and B. Surgical procedures were performed under an operation microscope (magnification 25 x), and a sterile technique was used throughout the procedure. After surgery, the animals were housed in a temperature- and humidity-controlled room with 12 hr light and dark cycles and had access to standard rat food and water ad libitum. Good laboratory practice (GLP) was maintained, according to the National Guidelines for Animal Welfare, comparable to the international rules for animal experimentation (International Guide on Animal Biomedical Research and Ethical Code for Animal Experimentation of the Council for International Organization of Medical Sciences).

Assessment of Function

The number of rats per group and the different assessment tests that were carried out at different survival times are presented in Table1. The sacrificed rats were used for muscle fiber type distributions and endplate morphology (not in this study).

Electromyography (EMG)

At 3 months EMG electrodes were implanted under general anaesthesia in the hindlegs of 3 rats in group A and of 3 rats in group B. Pairs of electrodes were implanted in the midbelly regions of the GC and the TA muscles of both hindlegs. The animals were allowed to walk freely on a flat surface and they were connected to an amplifier system. The EMG signals were stored and processed off-line on a personal computer. EMG recordings were then rectified and averaged and displayed for visual inspection. After EMG recording, the rats were sacrificed. Detailed information on EMG electrodes, the implantation procedure and the recording technique were described before. Five months after nerve repair, and after walking patterns were recorded on video, EMG electrodes were implanted and recordings were obtained from the GC and TA muscles in another 3 rats of group A and in 3 rats of group B. In group C (control group), EMG electrodes were implanted at an age, matched to that of rats of group A and B five months after sciatic nerve lesioning, and EMG recordings were obtained. The remaining rats were sacrificed for analysis of muscle fiber type distributions and the morphology of the endplates.

Video Analysis of Walking

At 5 months, each of the 30 remaining rats (group A: n = 12; group B: n = 12; group C: n = 6) was placed in a Perspex runway. The lateral view of the animal was recorded directly with a video camera, whereas the ventral view of the animal was visualized by means of an adjustable mirror under the cage, positioned at a 45° angle. In this manner, a split screen image was obtained with the lateral view of the rat in the upper half and the ventral view in the lower half. The runway was illuminated with two 120 W concentric bulbs to improve contrast. Walking movements of the rat were recorded with a video camera containing a stroboscopic shutter (25 frames per sec), creating blur-free stills for analysing the walking pattern. The
movements were recorded on a video tape until at least 4 consecutive and non-hesitant step cycles were collected. The video-tape was then replayed frame by frame. Two investigators, familiar with rat gait analysis, independently scored several aspects of the walking pattern of the rats: toe spread during stance phase, footplacing, the occurrence of dragging of the hindpaw, exorotation of the foot, the swing phase, and the regularity and fluency of walking. Phrasing of the parameters was such that “normality” was scored by “yes” and abnormality by “no” (see Table 2). The positively scored parameters per rat from Table 2 were summarized and the percentages per group calculated. The identity of the rats and their group-allocation was unknown to the investigators. Differences between groups A and B were statistically tested with the Fisher Exact Test and interscorer reliability (correlation of agreement between the dichotome scores) of the two investigators was tested by the Cohen’s Kappa.

**Results**

**Electromyography (EMG)**

In group C, the swing phase started with a shortlasting, brisk burst in the TA. During the stance phase this muscle was electrically silent (Fig. 1; asterisks indicate the onsets of swing phases). The stance phase was accompanied by a tonic burst in the GC muscle and EMG amplitudes in the GC muscle dropped during the swing phase. In rats of group A EMG patterns at the right non-operated side essentially were similar to those of rats of group C. Sometimes, additional peaks occurred during the onset of the swing phases in the TA (compensatory increase in tonic activity). Increases in tonic activity in the GC were sometimes observed as well (Fig. 2). In contrast, the EMG patterns of the GC and TA muscles were highly abnormal at the left operated side in all animals. Burst activity was badly phased in relation to the stepcycle-components and often simultaneous activation of the antagonist (coactivation) was observed (Fig. 2). These phenomena were also observed in group B and any differences could not be detected between the EMG patterns in rats after bridging the sciatic nerve with a graft or a nerve guide (Fig. 3). Particularly, the TA shows abnormal activity during the stance phase which normally does not occur. Results after 5 months essentially were similar to those after 3½ months in both group A and B, and this indicated that no further

![Fig. 1. Averaged EMG records of a normal non-operated rat. Records from the gastrocnemius (GC) and the tibialis anterior muscles (TA) of the right side. Time in seconds, amplitudes in µV; asterisks indicate the onset of swing phases. The alternation of tonic burst activity in the GC and the phasic activity in the TA can be observed.](image-url)
Fig. 2 and 3. Averaged EMG records 5 months after implantation of the biodegradable nerve guide (Fig. 2) and autologous nerve graft (Fig. 3). Records from the gastrocnemius (GC) and the tibialis anterior muscles (TA) of the right side (above) and of the left side (below). Time in secs, amplitudes in $\mu$V; asterisks indicate onsets of swing phases in the right and left hindpaws.

<table>
<thead>
<tr>
<th>parameters</th>
<th>control</th>
<th>nerve guide</th>
<th>nerve graft</th>
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<tbody>
<tr>
<td>toe spread (1)</td>
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<td></td>
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</tr>
<tr>
<td>walking on plantar side (2)</td>
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<td></td>
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<tr>
<td>absence of dragging (3)</td>
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<tr>
<td>absence of exorotation (4)</td>
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<td></td>
</tr>
<tr>
<td>alternating steps (5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>normal swing phase (6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>fluent walking (7)</td>
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Fig. 4. Histogram showing the percentages of rats with positive scores for each of the 7 parameters given in Table 2. For example, in group B (nerve graft), 3 of the 12 rats (25%) showed a normal swing phase.
improvement occurred.

**Evaluation of Gait**

After 5 months, control rats (group C) walked fluently, with regular steps, normal toe spread and swing phase (Fig. 4). None of the rats showed exorotation of the hindfeet, dragging nor walked on the dorsal side of the toes. In group A, normal toe spread was observed in only one rat and in the others no or only slight spreading of the toes was observed. Exorotation of the foot during the stance phase, an abnormal feature in adult rats, was observed in 6 rats. Three rats showed an abnormal swing phase during walking, characterized by exaggerated leg-lifting. The other parameters showed no large deviation from the non-operated rats. In group B, none of the rats showed normal toe spread, and all but one rat walked on the dorsal side of the toes. Respectively, 3 and 4 of the 12 rats showed a normal swing phase and walked fluently.

Statistically (Fisher Exact Test), group A and B differed for footplacing, the swing phase, and the fluency of walking ($p < 0.0001$, $p = 0.012$, and $p < 0.001$ respectively) (see also Table 2 and Fig. 4). The dichotome scores of the video analysis by the two investigators showed a high degree of agreement in both group A and B; $p < 0.001$ (Kappa = 0.94 in the analysis of group A, and Kappa = 0.97 in the analysis of group B).

**Discussion**

A major problem of nerve regeneration after bridging a nerve gap involves the specificity of reinnervation. Regenerating axons have to find the appropriate path leading to their original target and make functional contacts. When entering a target muscle, the axons sprout abundantly leading to hyperinnervation of muscle fibers.\(^{19}\) Regenerating axons may also reinnervate antagonistic muscles (cross-innervation).\(^{20}\) Inaccurate reinnervation leads to abnormal activation patterns in the muscles.\(^{17,21}\) Analysis of the video recordings of walking in the present study showed that walking in both groups of rats in which the sciatic nerve was transected remained disturbed up to 5 months. However, the rats in group A (nerve guide) walked more fluently and had a better swing phase than rats in group B (nerve graft). Clawing and interphalangeal joint contractures, resulting in walking on the dorsal side of the toes was often seen in group B, but not in group A. These results indicate that recovery of walking is better after bridging the gap with a p(DLLA-$\varepsilon$-CL) nerve guide. In a recent study, several methods for the evaluation of sciatic nerve function in the rat were compared after bridging a 15 mm gap in the nerve with a p(DLLA-$\varepsilon$-CL) nerve guide.\(^{13}\) Walking track analysis, conduction velocity, electrostimulation test, and the stance factor showed considerable recovery of nerve functions, but the EMG’s of the muscles in the operated leg of these rats remained highly abnormal.

EMG patterns in the the gastrocnemius and tibialis anterior muscles of rats in the present study of both groups A and B remained highly abnormal; consistent differences between the rats in both
groups could not be detected. The abnormal EMG patterns may be explained by aberrant innervation of the muscles by axons sprouting from the proximal nerve stump after transection.\(^{17}\) It was shown in this study that motorneurons were located outside the original motorneuronal pool (see also Nahm et al.\(^{21}\)). Increased tonic activity and disturbed phasing of burst activity in relation to the stepcycle-components, as were seen in the EMG’s, may also be induced by a disturbance in afferent information after cross-innervation. The interrupted proprioceptive feedback, in addition, may have a negative influence on the stepcycle, for example on the timing of the onset of the swing phase.\(^{23}\)

The surprising finding of the present study therefore was that EMG patterns in both groups were similarly disturbed, whereas several aspects of walking were clearly different. A similar discrepancy was found in a study where the sciatic nerve in rats was transected on the 10th postnatal day. Also in those rats, EMG patterns are disturbed while walking had recovered to a reasonable extent.\(^{24}\) We hypothesize that both after bridging the nerve gap with a p(DLLA-ε-CL) nerve guide and with a nerve graft the axons innervate muscles randomly. Due to subtle adjustments of the recruitment of motorneurons at supraspinal levels, however, walking is relatively normal despite abnormal innervation in group A. Furthermore, we presume that due to faster outgrowth in case of bridging the sciatic nerve gap with a nerve guide this compensational process is enhanced. In the recent past, it was shown that nerve regeneration through a 10 mm biodegradable p(DLLA-ε-CL) nerve guide is faster and qualitatively better compared with regeneration through a 7 mm nerve graft.\(^{7}\)

A possible localization of this compensation by readjustment of motorneuronal recruitment may be the cerebellum. In ongoing experiments this possibility is being studied. Further improvement in nerve function after repair may come from designing methods to improve the accuracy with which axons regenerate to their original targets. The use of gene therapy in nerve regeneration holds great promise. Cells, e.g. Schwann cells, that are transfected with growth factors, can be implanted in the biodegradable nerve guide in order to stimulate (specific) nerve growth and improve the survival capacity.

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