Quality and Extent of Nerve Regeneration through Chitosan Nerve Guides.

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Introduction

The body's initial response to a nerve injury is a process of degeneration known as Wallerian or trophic degeneration. During this process both distal and proximal nerve stumps degenerate after which macrophages phagocytose the degenerated fragments. At this point the distal stump prepares itself to accept regenerating axons from the proximal end. If the distal stump does not receive a regenerating axon, progressive fibrosis will occur and atrophy of the distal stump will occur. First signs of regeneration are seen at the proximal nerve stumps which will start spontaneous sprouting of new daughter axons. Each axon will in turn start to sprout and begin the regenerative process. In such a situation biodegradable nerve guides can enhance the regenerative process by providing directional guidance to the regenerating axons. They can form a protective wall between the regenerating axons and the surrounding milieu. Currently, many biodegradable nerve guides are being fabricated and evaluated for their potential in peripheral nerve regeneration. In our present study we have evaluated the potential of chitosan nerve guides to give directional guidance to regenerating axons.

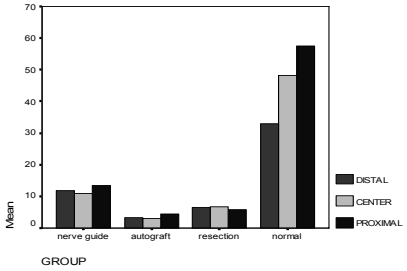
Materials and Methods

Chitosan nerve guides were used to bridge a 10mm sciatic nerve gap, created in experimental Lewis rats (200-225gm) by surgically removing ½ cm of the sciatic nerve. Autografts were implanted in rats as positive controls and nerve resection was performed in negative control rats. 12 weeks post surgery; rats from all three groups were euthanized. Recovered implants were fixed in 10% buffered formalin, dehydrated, and embedded in paraffin blocks. Five-micron sections were cut across the transverse axis starting from the distal to the proximal end. Similar sections were taken from the autograft and control group too. The sections were mounted and stained with Glees & Marshland's Silver Stain and Luxol Fast Blue. Stained sections were examined under a light microscope at 40x magnification using image analysis software (Image-Pro Plus, Media Cybernetics, San Diego, CA). Myelinated and non-myelinated axon counts were collected from the distal, center and proximal regions. The ratio of axon area to the total nerve fiber was also measured. Statistical Analysis was performed by analysis of variance with post hoc pairwise testing, when necessary, using the Scheffé test. (Significance at $p<0.05$)

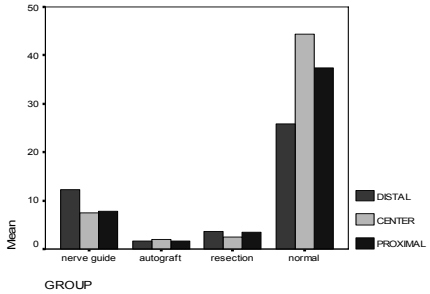
Results and Discussion

At the end of the 12-week post implantation period, the number of axons in the distal, center and proximal region of the nerve guide group were significantly higher than the resection group ($p<0.05$). Whereas, the number of axons in the nerve guide group compared to the autograft group were similar in number. The number of myelinated axons from the center and proximal regions were significantly higher in the nerve guide group compared to the autograft and resection group ($p<0.05$). At the distal end the number of myelinated axons were low compared to both autograft and resection groups. Myelinated and non-myelinated nerve fiber density from the distal to the proximal end was statistically significant in the nerve guide group compared to both autograft and resection groups ($p<0.05$).In our study, chitosan nerve guides have provided directional guidance to the regenerating axons, leading to an increase in axon diameter. As a result an increase in axon area was also seen from the proximal to the distal end. Out of the total axonal area a large amount of area was occupied by myelinated axons in the nerve guide group, indicating successful axonal re-formation at the distal nerve stump. Comparatively, in the autograft group the area of myelinated and non-myelinated axons were lower even though the numbers of axons were higher. This indicates increased axonal sprouting at the proximal end and at the distal end misdirectional connections or slow axonal growth.Even though the numbers of myelinated and non-myelinated axons in the autograft group are similar in number to the nerve guide group, the overall axonal area is larger in the nerve guide group. Thus presence of nerve guide provides faster axonal growth over the experimentally created gap.

Figure1: (a) Non-myelinated and (b) myelinated fiber density for the nerve guide, autograft, resection and normal group.



(a)



(b)

Conclusions

This study suggests that Chitosan represents a novel biomaterial with promising properties to accelerate nerve repair. The quality of nerve regeneration is comparable to the traditional autograft technique.