# Peripheral nerve graft architecture affects regeneration

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# SUMMARY

The primary goal in repairing a peripheral nerve lesion is to guide the outgrowing axon back to its original target organ. If it is not possible to co-apt the nerve stumps directly the defect can be bridged with nerve tissue from the patient itself, a so called 'autograft', or with a synthetic nerve graft. Research in this field aims at improving the properties of a synthetic nerve graft with the primary aim to improve nerve regeneration through such a graft to a level equal to or even better than regeneration through an autograft.

In this thesis an overview is presented of the evaluation methods that are currently used to assess peripheral nerve regeneration. It is demonstrated that analysis of nerve morphometry (i.e. number of nerve fibres, density of nerve fibres, N-ratio [= ratio of the sum of transection areas of myelinated nerve fibres and the total nerve transection area]), success ratio (= ratio of grafts containing a tissue bridge and all grafts that were implanted), CMAP (compound muscle action potential), muscle weight and extended walking patterns all yield sufficiently precise data to resolve small differences in regeneration. There are, however, numerous methods that do possess such resolving power and that consequently should not be used to assess nerve regeneration.

None of the evaluation methods described to assess nerve regeneration through a graft properly evaluates the conduction the action potential, even though this is an important, if not the most important, function of the axon. Therefore we developed an *in vitro* electrophysiological evaluation method that charts the electrophysiological properties of the myelinated Aα- and Aβ-nerve fibres. With the aid of the parameters determined with this method it turned out to be quite possible to discriminate small differences in regeneration as occur after grafting different synthetic nerve grafts. Moreover, the electrophysiological data could be correlated to the morphometrical data, that was likewise broken up into Aα- and Aβ-components. This correlation especially provided new insight in the changes that occur in regenerating nerve fibres.

A panel of evaluation methods (nerve morphometry, *in vitro* electrophysiology and muscle morphometry) was applied to assess whether the presence of pores in and biodegradability of synthetic nerve grafts are indeed beneficial to regeneration, as is generally assumed. We first studied the effect of porosity on nerve regeneration. We demonstrated that, provided the formation of a tissue bridge traversing the graft, regeneration through microporous ε-caprolacton nerve grafts was better in all respects. However, the formation of a tissue bridge, a prerequisite for recovery, did not occur consistenly in these grafts. Hence we assessed the effect of porosity once more, now using synthetic nerve grafts that were made of a copolymer of trimethylenecarbonate and ε-caprolactone. Tissue bridges did form consistently in these grafts. Again the results of the porous nerve grafts were better, evidenced by the shorter refractory period and the higher threshold of the regenerated nerve fibres. A shorter refrac - tory period allows the regenerated axon to accommodate a following action potential faster

and thereby establishes a more adequate contact between the firing central neuron and the target organ. The observation that the refractory period was shorter than predicted based on the smaller nerve fibre diameter, was interpreted as a regeneration strategy aiming at the best possible function in spite of a decrease in diameter. The higher threshold will decrease the probability of the generation of spontaneous action potentials along the axon. Given the fact that hyperexcitability, i.e. a low threshold and much spontaneous activity, is associated with neuropathic pain, a high threshold is considered to be a desirable property. We speculated that the presence of micropores provided the regenerating nerve fibres with a favourable environment resulting in faster maturation of the regenerating nerve fibres. Modifications in the ion channel composition at or near the Ranvier nodes lie, in all probability, at the base of these electrophysiological changes.



The same evaluation methods were used to investigate the influence of the degradability of a synthetic nerve graft on the process of nerve regeneration. Again it were the electrophysiological properties of the regenerated nerve fibres that differed between the degradable and non degradable nerve grafts. Myelinated nerve fibres that regenerated through a degradable graft had a shorter refractory period and a more favourable displaced charge. Theoretically, the advantage of a degradable graft is that there is less compression on the regenerating nerve and that the foreign-body-reaction lasts shorter. Apparently this results in the regeneration of nerve fibres that have electrophysiologically more favourable properties.

In addition the changes that occurred in reinnervated muscles were evaluated. The results helped to gain insight into the preferential architecture of a synthetic nerve graft. Muscles reinnervated by autografted nerves (that actually consist of a collection of very thin tubes formed by the basal lamina scaffolds) displayed a conspicuously different organization compared to muscles reinnervated by (hollow) synthetic nerve grafted nerves. In autografted muscles clusters of muscles fibres of the same type (type I) were observed, in contrast to the individually occurring type I muscle fibres in synthetic nerve grafted muscles.

It is known from literature and from our own observations that regenerating nerve fibres branch. Should branching occurs within a tube (basal lamina scaffold), then the sibling branches will be forced to stay together and consequently the muscle fibres reinnervated by these siblings will tend to be clustered. Since is is surely desirable that all axonal branches of a neuron end up at the same target organ, it is, in our opinion, preferable to develop a synthetic nerve graft that enforces sibling branch cohesion. This could for instance be realized by filling a synthetic nerve graft with numerous tiny littly tubes that contain the regenerating nerve fibre branches.

Finally we evaluated whether the biomaterials used were used were compatible with Schwann cells. Schwann cells will inevitably be present in the synthetic nerve graft because they will migrate in from both the proximal and the distal nerve stumps. Schwann cells produce growth factors that stimulate nerve regeneration, and are necessary for normal nerve function. Studying the interaction of Schwann cells with the biomaterials demonstrated the absence of toxic reactions, that Schwann cells attached well to the biomaterials and that they

proliferated on them. Subsequently it was tested how Schwann cells interact with different coating and matrices. Schwann cells attached to all coatings and matrices tested, but pro - liferated is best on fibronectin, laminin and poly-D-lysin. It thus seems useful to use these biomaterials if the fabrication of a matrix in a synthetic nerve graft is considered.

If anything the findings presented in this thesis bear evidence to the fact that it is hard to say what is good and what is bad with respect to regeneration. It becomes more and more clear that there are lots of phenoma in the field of peripheral nerve regeneration that are insufficiently understood. In our opinion research should aim at the understanding of these phenomena rather than performing 'trial and error' studies aiming at the development and implantation of a continous stream of new synthetic nerve guides.