Summary

This thesis is based on a number of experiments on the application of tubes, processed from various materials compared to epineurial suturing in the healing of peripheral nerves. The results were evaluated by way of a newly developed method of morphometric analysis.

In Chapter 1 an overview of the surgical management of peripheral nerve repair is given. Our present days knowledge on peripheral nerve repair is based on anatomical studies of Sunderland and many others and on Millesi's pursuit of tension-free repair. The main problem still is the repair and management of nerve injuries with a gap. The standard clinical repair procedure is nerve autografting. The disadvantages of this method are loss of donor nerve function and risk of traumatic neuroma formation at the donor site. In order to solve this problem many tubulization experiments in animals have been performed with various materials, such as: various biological fresh or prepared materials (mainly veins and arteries). Experimental nerve reconstructions with a gap have been performed with tubes of bones and muscle autografts in animals. Another development was the application of Millipore tubes and silicone rubber tubes. The results were variable. Recently, application of biodegradable tubes in peripheral nerve reconstruction with gap showed promising results. In order to evaluate the results of experimental peripheral nerve reconstruction in animals, the quality of the axonal outgrowth is tested by electro-
physiology as well as by axon counting in histochemical or immunohistochemical stained sections. Axon counting has been performed by several time-consuming methods and there is hardly any standard procedure.

The favorable results, obtained in animal experiments with the newly-developed "Processed Porcine Collagen (PPC)", in the protection of colonic anastomoses and ligament reconstructions initiated the experimental application of this material with tubulization in nerve reconstruction of injuries with and without a gap. In order to evaluate the results over a longer period, axon counting was performed 3, 6 and 12 months after nerve reconstruction. Moreover we compared the results of the application of PPC in nerve reconstruction with capacities of autologous vein grafts and silicone rubber tubes in the same surgical procedure. For the evaluation of the results of the experiments we developed a new method for the standardization of morphometric analysis.

In **Chapter 2** a new method for morphometric analysis of axons in experimental peripheral nerve reconstruction is described. Twelve adult female rabbits were used. In nine animals the saphenous nerve was transected and stitched epineurially. Three animals functioned as control. After 3, 6 and 12 months the nerves were harvested, fixed in Kryofix and embedded in Histowax. Transverse sections of 6 μm were cut, immunohistochemically stained for NF 90, and counterstained by Sirius Red. Quantification of nerve fibers in cross-sections was performed by using a confocal laser scanning microscope (CLSM), and the images were stored digitally. Data analyzing was performed by the Optimas program (5.2). Calculations were done with Microsoft Excel. The total number of axons, the mean axon diameter and the percentage axon area / fascicle area were evaluated statistically. This method for morphologic analysis provides automatically complete registration of axons and so different methods of experimental nerve reconstruction can be compared in a fast and reliable way.
In Chapter 3 the results of the application of venous graft conduits in experimental saphenous nerve reconstruction are presented. The aim of this investigation was to study the effect of venous tubuli in peripheral nerve reconstruction. In 20 rabbits the saphenous nerves were transected and reconstructed. In ten rabbits (series 1) a venous tubulus was placed around the nerve suture. In another ten rabbits (series 2) a venous tubulus was sutured over a 3-mm nerve gap. Conventional suturing was done in ten contralateral saphenous nerves (series 3, controls). Epineurial stitching was performed. The healing was studied after 3 months and after that histological analysis was performed by means of monoclonal antibody staining. The results of our experiments show that covering a nerve suture by a venous tubulus did not enhance healing in comparison to the conventional end-to-end suturing, but in contrast evoked extensive fibrous tissue, thereby hampering regeneration of axons.

In Chapter 4 the results of experiments, in which we compared the healing of nerve reconstructions with processed porcine collagen (PPC) tubes and conventional end-to-end suturing, are presented. Processed porcine collagen (PPC) is a new inert and biodegradable material with a favorable effect on wound healing, as demonstrated by other tissues. The aim of our study was to compare the healing of nerve sutures with PPC tubes with conventional end-to-end sutures. In our experiments, we reconstructed the saphenous nerves of 27 rabbits. In series 1 (n=12) and 2 (n=12), PPC tubes were slid over an end-to-end nerve suture without or with a 10-mm nerve gap, respectively. In series 3 (n=12), conventional suturing was performed in the collateral saphenous nerves of the animals of the series 1. Epineurial suturing was performed. Three other non-operated saphenous nerves served as controls. The healing was studied after 3, 6 and 12 months in sections stained by monoclonal antibodies and by conventional histologic staining. Morphometric analysis of the regenerating axons was done by using confocal scanning laser microscopy.
Data analysis was carried out by a software program especially developed for this purpose. All results were evaluated statistically. Our results showed that during the healing period in the distal nerve stump the mean number of axons of the PPC procedure with a 10-mm gap was significantly higher than that in the procedure without a gap. After 12 months, the mean number of axons of all procedures was significantly lower than in the non-operated nerve, and the mean axon diameter in all distal stumps did not differ significantly from that of the non-operated nerve. In the distal nerve stump, the ratio total axon area versus total fascicle area in the PPC procedure with a gap was significantly higher than that in the conventional suturing procedure. After 12 months there was no significant difference between the percentages axon outgrowth of the PPC procedure without a gap, the conventional suturing procedure and the non-operated nerve (100%). The percentage axon outgrowth in PPC with a gap was significantly higher than in the other procedures.

In Chapter 5 the results of experiments are presented, in which the healing after nerve reconstruction by using silicone rubber tubes and conventional end-to-end suturing were compared. Silicone rubber (polydimethyl siloxane) tubes are used clinically in peripheral nerve reconstruction. A disadvantage of this procedure is that the material often has to be removed owing to its mechanical properties. The aim of our study was to investigate the healing of reconstructed sensory nerves tubulized by silicone rubber in an animal model. In our experiments, we reconstructed the saphenous nerves in 27 rabbits. In series 1 (n=12) silicone rubber tubes were slid over a nerve suture without a gap. In series 2 (n=12) silicone rubber tubes were slid over a 10-mm nerve gap. In series 3 (n=12) conventional suturing was performed in the collateral saphenous nerves of the animals of the series 1. Epineurial suturing was performed. Three other non-operated saphenous nerves served as controls. The healing was studied after 3, 6, and 12 months. Morphometric analysis of
the regenerating axons was performed by using our new method for quantification of nerve fibers in cross sections stained by immunohistochemistry and using confocal laser scanning microscopy. Data analysis was carried out using a software program especially developed for this purpose. Our results showed in the silicone rubber procedures that at 12 months a significant smaller number of axons per fascicle area were present compared with conventional suturing. However, mean axon diameters in the distal nerve stump of the silicone rubber procedures did not differ significantly compared with the conventional suturing procedure. The ratio of total axon area to total fascicle area in the distal nerve stumps of the silicone rubber procedure without gap was significantly smaller compared with the conventional sutured nerve. The percentage outgrowing axons from the proximal nerve stump into the distal one in the silicone rubber procedure without gap was 57%. This was significantly higher than in the silicone rubber procedure with 10-mm gap (48%). However, in conventional suturing the percentage of outgrowing axons (99%) was significantly higher than in both tubulization procedures. It appeared that tubulization by silicone rubber of sutured nerves without gap did not enhance axon regeneration. Conventional suturing gave significantly better results. If a gap was present, the use of a silicone rubber tube was preferable to non-suturing.

In Chapter 6, firstly the general discussion on the main findings (in the different studies) in this thesis are reviewed. The discussed issues are the application of venous graft conduits in nerve reconstruction, the application of silicone rubber tubes in nerve reconstruction and the application of processed porcine collagen (PPC) tubes in nerve reconstruction. It is concluded that in our hands the application of PPC tubes in peripheral sensory nerve reconstruction give good results and has many advantages in contrast to the application of venous graft conduits and silicone rubber tubes. In the second part of this chapter some recommendations are given for future research.