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Chitosan tubes of varying degrees of acetylation for bridging peripheral nerve defects☆

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ABSTRACT

Biosynthetic nerve grafts are desired as alternative to autologous nerve grafts in peripheral nerve reconstruction. Artificial nerve conduits still have their limitations and are not widely accepted in the clinical setting. Here we report an analysis of fine-tuned chitosan tubes used to reconstruct 10 mm nerve defects in the adult rat. The chitosan tubes displayed low, medium and high degrees of acetylation (DAI: ~2%, DA: ~5%, DAIII: ~20%) and therefore different degradability and microenvironments for the regenerating nerve tissue. Short and long term investigations were performed demonstrating that the chitosan tubes allowed functional and morphological nerve regeneration similar to autologous nerve grafts. Irrespective of the DA growth factor regulation demonstrated to be the same as in controls. Analyses of stereological parameters as well as the immunological tissue response at the implantation site and in the regenerated nerves, revealed that DAI and DAIII chitosan tubes displayed some limitations in the support of axonal regeneration and a high speed of degradation accompanied with low mechanical stability, respectively. The chitosan tubes combine several pre-requisites for a clinical acceptance and DAII chitosan tubes have to be judged as the most supportive for peripheral nerve regeneration.

1. Introduction

In general, peripheral nerves have the ability to regenerate following nerve lesion in contrast to the central nervous system. However, in cases of more complex injuries with substantial loss of nerve tissue and a subsequent defect between the nerve ends, the requirements for axonal regeneration are not sufficiently fulfilled. Such complex peripheral nerve injuries, which include 300,000 cases per year in Europe due to traumatic events, represent a major cause for morbidity and disability. During the last decades considerable efforts have been made to support and improve peripheral nerve regeneration across a nerve defect, but with limited and minor success [1]. Therefore, despite several shortcomings and limitations, such as misdirection of regenerating axons, neuronal cell death, and donor site morbidity, the gold standard for bridging a nerve defect remains grafting of an autologous nerve transplant to overcome such large defects [2]. The purpose is to apply an adequate substrate, including the provision of trophic and tropic factors, for the regrowing axons in order to restore the function after reconstruction of the nerve defect [3].

As a possible alternative for autologous nerve grafts, a variety of materials have been tested with regard to their suitability for fabrication of synthetic nerve conduits for bridging nerve defects [4]. In

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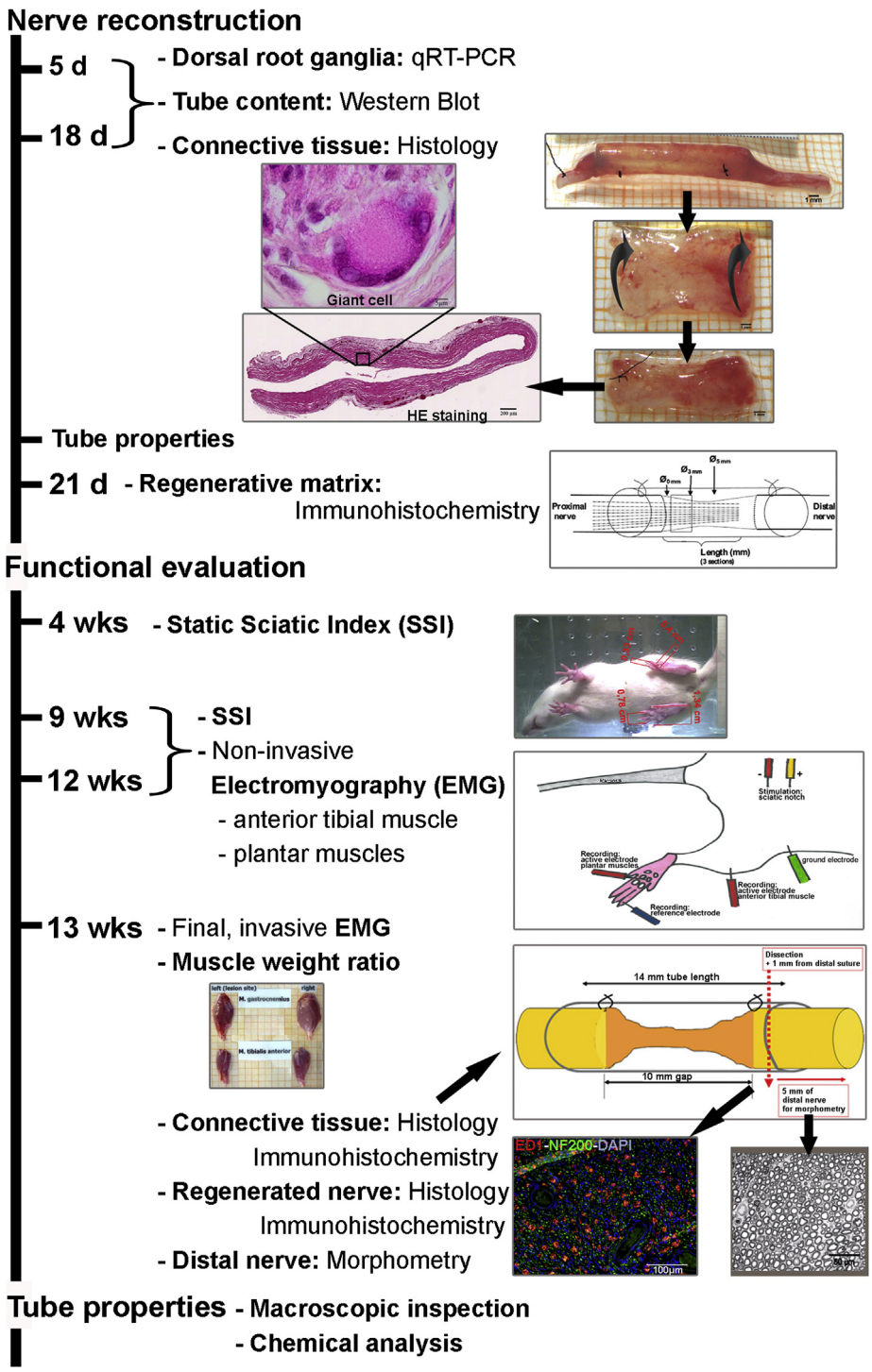

Fig. 1. Illustration of the various components of the comprehensive in vivo analysis of the new chitosan tubes with different degrees of acetylation as performed by several partners of the BIOHYBRID consortium. After the short terms of 5 and 18 days after nerve reconstruction, the connective tissue surrounding the chitosan tubes, the properties of the tubes, tube content as well as the proximal nerve ends and corresponding dorsal root ganglia were assessed with different methods, including RT-PCR, Western Blot, histology and immunohistochemistry. After 21 days in vivo, the newly formed regenerative matrix and the distal nerve segments were evaluated for matrix dimension, length of axonal outgrowth, and numbers of activated and apoptotic Schwann cells and total number of DAPI stained cells in the matrix and in the distal nerve segments. In long term observation, assessments of the motor regeneration were performed including the static sciatic index calculation, non-invasive and invasive electrodiagnostical measurements and muscle weight evaluation. Histological analyses were performed with the connective tissue and the regenerated nerve tissue. Finally, distal nerve morphometry and detailed analyses of the tube properties after nerve reconstruction and explantation were performed.

addition to non-degradable silicone tubes, which were earlier used clinically as an alternative for nerve repair [5,6] several biodegradable materials have been developed in animal experiments and applied for clinical investigation of nerve reconstruction devices [1,4]. Conduits, which are approved by the Food and Drug Administration (FDA) and authorized by the EC respectively, include Neurotube™ poly(glycolide) (PGA) tubes (successfully used in the clinical repair of digital nerves with defects of up to 3 cm in length [7]), Neura-Gen™ collagen nerve tubes [8,9], and Neurolac™ tubes [10,11]. Furthermore, processed, i.e. extracted, nerve allografts have