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Efficient bridging of 20 mm rat sciatic nerve lesions with a longitudinally micro-structured collagen scaffold

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ABSTRACT

An increasing number of biomaterial nerve guides has been developed that await direct comparative testing with the 'gold-standard' autologous nerve graft in functional repair of peripheral nerve defects. In the present study, 20 mm rat sciatic nerve defects were bridged with either a collagen-based micro-structured nerve guide (Perimaix) or an autologous nerve graft. Axons regenerated well into the Perimaix scaffold and, the majority of these axons grew across the 20 mm defect into the distal nerve segment. In fact, both the total axon number and the number of retrogradely traced somatosensory and motor neurons extending their axons across the implant was similar between Perimaix and autologous nerve graft groups. Implantation of Schwann cell-seeded Perimaix scaffolds provided only a beneficial effect on myelination within the scaffold. Functional recovery supported by the implanted, non-seeded Perimaix scaffold was as good as that observed after the autologous nerve graft, despite the presence of thinner myelin sheaths in the Perimaix implanted nerves. These findings support the potential of the Perimaix collagen scaffold as a future *off-the-shelf* device for clinical applications in selected cases of traumatic peripheral nerve injury.

1. Introduction

Much effort has been undertaken to repair clinically challenging peripheral nerve defects using biomaterial-based strategies, but functional recovery has often proved to be inferior to that obtained after autologous nerve grafting. The autologous nerve transplant (ANT) is still regarded as the clinical gold standard to repair physically disconnected nerve stumps, a status which it owes to a cellular, structural, and molecular make-up that is supportive for axon regeneration [1]. However, despite the fact that the ANT represents the best implantable bridging material currently available for the repair of peripheral nerve defects, it is still far from ideal and has a number of drawbacks; the harvesting of autologous nerves is inevitably associated with loss of function loss and the potential risk of neuropathic pain associated with neuroma formation originating from the donor site [2–7]. Moreover, there is a limited availability of suitable nerves in the human body for autologous transplantation. Therefore, a range of novel biomaterials have been developed as alternatives to the ANT, many of which have been tested pre-clinically [8,9].

Abbreviations: AD, axon diameter; ADens, axonal density; ANT, autologous nerve transplantation; DCS, distal coaptation site; DDSC, distal to the distal coaptation site; DRG, doral root ganglia; ECM, extra cellular matrix; EST, epineurial sleeve technique; GFP, Green Fluorescent Protein; ITS, intermediate toe spread; ITSF, intermediate toe spread factor; MST, myelin sheath thickness; PCS, proximal coaptation site; PDCS, proximal to the distal coaptation site; PM, Perimaix; PNI, peripheral nerve injury; SC, Schwann cells; SSI, static sciatic index; TS, toe spread; TSF, toe spread factor.

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Biomaterials in the form of nerve guides should ideally resemble the peripheral nerve structure, as well as its cellular and molecular composition, providing a hospitable microenvironment for regenerating axons and supporting glial cells. The development of such guides has seen major advances over the past decade, starting with hollow tubes [8,10,11] and graduating to more complicated scaffolds that better resemble the macro- and micro-structures of nerves (i.e. following the bio-mimicking concept). These materials are biodegradable, compatible with the host nerve tissue and even mimic the geometric design of natural nerves [12–15]. Topographical cues that are considered to resemble the structure and function of the peripheral nerve extra cellular matrix (ECM) include micro-channels within the nerve conduits [16–19], nano-scale fibrous scaffolds [20,21], Schwann cell (SC)-derived ECM [22], and de-cellularized/sterilised nerve allografts that have been harvested from human corpses [23–25].

We previously described a porcine type-I collagen, micro-structured nerve guide characterized by the presence of numerous longitudinally oriented channels that was designed to resemble the perineurium of peripheral nerves. This nerve guide (Perimaix) was found to be highly compatible with a large range of cell types from neural origin, including astrocytes [26], olfactory ensheathing cells [26], SH-SY5Y human neuroblastoma cells [26], human neural progenitor-derived astrocytes [27], and SC[28]. The compatibility with SC was further supported by the good metabolic activity and the elongated 'Büngner'-like structures these cells formed when cultivated within the 3D framework of the Perimaix scaffold [28]. Moreover, SC grown within Perimaix release factors that augment neurite outgrowth of cultured dorsal root ganglion (DRG) neurons [29]. Recently, we described the effect of Perimaix on axon regeneration after implantation into 20 mm long sciatic nerve defects [17]. Axon counts and myelination at the distal end of the Perimaix graft were high after six weeks, and, depending on the degree of collagen-cross linking and additional SC-seeding, reached values that approached those seen after autologous nerve transplantation in the same model after six weeks [17].

The objective of the present study was to determine whether long-term implantation of the Perimaix scaffold was capable of supporting axon regeneration of distinct neuronal populations (i.e. motor and sensory) as well as encouraging functional recovery that compared favorably with that of the ANT.

2. Materials and methods

2.1. Animal experiments

All experiments were conducted on female inbred Lewis rats (n = 35, 10–12 weeks old, Harlan©, Germany) in accordance with national- and EU regulations regarding animal care. Animals were housed in a temperature- and humidity-controlled environment with a light–dark cycle of 12 h:12 h, and free access to food and water. Every attempt was made to minimize the number of animals used, as well as their pain and discomfort. Animals were separated into three experimental groups (Table 1) and monitored behaviorally from preoperative times up to twelve weeks after surgery.

2.2. Preparation of nerve guides

The Perimaix collagen scaffolds were prepared from highly enriched porcine type-I collagen with typically 10–15% (w/w) of elastin, as indicated by the presence of desmosine and isodesmosine. The purified collagen used, was characterized by low levels of non-collagenous and non-elastin marker molecules such as cysteine (<8 μmol/g), tryptophane (<3 μmol/g) and hexosamines (<8 μmol/g). The scaffold architecture was generated by a patented uni-directional freezing process developed by Matricel GmbH (Herzogenrath, Germany), which resulted in longitudinally orientated-, and continuous micro-channels that were intended to mimic the inner connective tissue layers of the PNS. The orientated micro-pores of the scaffold had a mean pore size varying from 70 to 90 μm, which enables optimal infiltration by SC as well as adhesion and mechanical support for regenerating axons. The fabrication and testing of physical properties and cyto-compatibility have been reported in detail elsewhere [17,26,28,30,31].

2.3. Isolation, purification and seeding of adult rat SC

In vitro isolation of rat Schwann cells (SC) has been described previously [17,28,32]. Briefly, sciatic nerves were harvested from isogenic Lewis rats, cut into 1–2 mm-pieces that were enzymatically and mechanically dissociated through collagenase/trypsin treatment. Single cell suspensions were subsequently plated onto poly-L-lysine/laminin-coated culture flasks (PLL/lam; both Sigma–Aldrich™, Munich, Germany) and kept in Schwann cell growth medium (DMEM containing 10% FCS, 40 μg/ml transferrin, 41.7 μg/ml bFGF, 41.7 μg/ml heregulin, 475 ng/ml forskolin, 10 μg/ml insulin, 0.1% gentamicin, 1% glutamax) for propagation. The magnetic assisted cell sorting (MACS®) system (Miltenyi Biotec, Bergisch Gladbach, Germany) was used in combination with anti-p75-antibody for cell purification yielding highly enriched SC cultures (>99%) as described earlier [17,30]. A cell suspension (20,000 SC/μL) was used to load the Perimaix scaffolds in two steps. Initially, 25 μL cell suspension was applied to the bottom of a culture dish, after which the nerve guide was placed top-down into the cell suspension enabling complete fluid-absorption. Hereafter, a further 25 μL cell suspension was applied directly to the top-end of the scaffold. Subsequently, SC were allowed to adhere to the nerve guide for 24 h prior to implantation in a humidified incubator at 37 °C and 5% CO_2.

2.4. Surgery

Briefly, animals were anesthetized by subcutaneous injection of Buprenorphine (Temgesic®, Essex Chemie AG, Luzern, Switzerland) and sedated by a continuous gas inhalation of 2 ml/h Isoflurane (Abbott GmbH, Wiesbaden, Germany). A skin incision of 30 mm was made over the gluteal region and the right sciatic nerve was exposed from the sciatic notch to the point of trifurcation into the N. suralis, peronealis and tibialis through blunt dissection. For animals in the ANT group, a 20 mm-long segment of the sciatic nerve was excised, then immediately re-implanted with three 10/0 epineurial monofilament sutures (10/0 Ethilon®, Ethicon Inc., Somerville). For the implantation of seeded and non-seeded Perimaix, the epineurial sleeve technique (EST) was used [33]. Briefly, when

Table 1
Experimental groups.

Group	Treatment	Specification	Abbreviation	Number of animals
1	Autologous nerve transplantation (Control)	–	ANT	11
2	Perimaix	Enriched with SC	PM [+SC]	13
3	Perimaix	Without SC (naïve)	PM [−SC]	11