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Functional recovery after peripheral nerve injury and implantation of a collagen guide

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ABSTRACT

Although surgery techniques improved over the years, the clinical results of peripheral nerve repair remain unsatisfactory. In the present study, we compare the results of a collagen nerve guide conduit to the standard clinical procedure of nerve autografting to promote repair of transected peripheral nerves. We assessed behavioral and functional sensori-motor recovery in a rat model of peroneal nerve transection. A 1 cm segment of the peroneal nerve innervating the *Tibialis anterior* muscle was removed and immediately replaced by a new biodegradable nerve guide fabricated from highly purified type I + III collagens derived from porcine skin. Four groups of animals were included: control animals (C, $n = 12$), transected animals grafted with either an autologous nerve graft (Gold Standard; GS, $n = 12$) or a collagen tube filled with an acellular skeletal muscle matrix (Tube-Muscle; TM, $n = 12$) or an empty collagen tube (Collagen-Tube; CT, $n = 12$). We observed that 1) the locomotor recovery pattern, analyzed with kinetic parameters and peroneal functional index, was superior in the GS and CT groups; 2) a muscle contraction was obtained in all groups after stimulation of the proximal nerve but the mechanical muscle properties (twitch and tetanus threshold) parameters indicated a fast to slow fiber transition in all operated groups; 3) the muscular atrophy was greater in animals from TM group; 4) the metabosensitive afferent responses to electrically induced fatigue and to two chemical agents (KCl and lactic acid) was altered in GS, CT and TM groups; 5) the empty collagen tube supported motor axonal regeneration. Altogether, these data indicate that motor axonal regeneration and locomotor recovery can be obtained with the insertion of the collagen tube RevolNerv®. Future studies may include engineered conduits that mimic as closely as possible the internal organization of uninjured nerve.

1. Introduction

Complex peripheral nerve injuries that result in extended neurectomy require reconstructive surgery with nerve grafts [1]. Autologous nerve grafts are currently used in the reconstruction of a peripheral nerve gap. The autologous nerve is a natural substitute, acting as a conduit for axonal regeneration. The surgical procedure, named "Gold Standard" technique, consists of harvesting one nerve from the patient and grafting it in between the two stumps of the transected nerve. However, grafting causes morbidity at the donor

Abbreviations: C, Control; CT, Collagen-Tube; EIF, Electrical Induced Fatigue; GS, Gold Standard; PFI, Peroneal Functional Index; PPD, p-Phenylenediamine; TM, Tube-Muscle.

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site, loss of sensation, painful neuroma formation and scarring. Furthermore, appropriate size and rapid availability of the graft material are necessary to optimize and simplify the reconstruction procedure. Nerve allografts may be used to overcome these problems [2–5] but allogenic transplantations require to assess immuno-compatibility between the recipient and the donor. Therefore, the use of an artificial guide obtained with either biological or synthetic materials can be seen as an alternative [6]. Clinical and experimental investigations have demonstrated that such "nerve conduits" allow nerve regeneration and function recovery [7]. These tubular prostheses are a promising option which may replace the need for nerve grafting [8–10].

Entubulation repair has a lengthy history and many studies have explored the effectiveness of many biodegradable and non-biodegradable materials filled or not with molecules or cells [10–22]. Artificial materials used for such repairs include polylactate/polyglycolate copolymers, acrylic copolymers, polyvinylidene difluoride (PVDF), polyglactin mesh, silicone, Gore-Tex®, arterial cuffs, mesothelial tube, collagen, polylactates and various other synthetic polyesters [23]. Ideally, a nerve guide material should i) provide guidance and support for regenerating axons; ii) have a shelf-life appropriate to the nerve trauma; iii) exhibit biodegradability properties and iv) be cost-effective.

Among the most known nerves guides, Neurolac® [Poliganics B.V. – poly(DL-lactic-co-ϵ-coprolactone)] [24,25], CultiGuide® [Pittsburgh Tissue Engineering Initiative, Inc., – composite poly-caprolactone and porous collagen-based beads] [26,27]ss-refs>, SaluBridge® [Salumedica L.L.C. – polyvinyl alcohol] [28–31], Neurotube® [Synovis Life Technologies Inc. – polyglycolic acid] [26,32–34], Surgisis® [Cook Biotech Inc. – porcine small intestinal submucosa], NeuroMatrix®-Neuroflex® [Collagen Matrix Inc. – collagen] and NeuraGen® [Integra LifeSciences Corp. – collagen] [10,35,36] are marketed for nerve repair. Except for the CultiGuide®, all tubes are approved by the Food and Drug Administration (FDA). Neurolac®, Salubridge®, Neurotube® and NeuraGen®, well known to support axonal regeneration, were granted an EU authorization. Although approved for human use, the efficacy, and thus the indications, for all the tubes marketed to date are limited to the repair of short defects (<3 cm) of the small calibre nerves [37].

In these conduits, the regenerating fibers may be guided by contact guidance, the growth cone displaying numerous microspikes that palpate the local environment. Chemical and physical properties of the environment are crucial for axonal regrowth. Extracellular matrix neurite-promoting molecules guide the nerve fibers. Interactions between regenerating axons and the adjacent substratum are known to be key factors for directing axonal elongation [38].

It was previously shown that collagen filaments guide regenerating axons [39–44]. Furthermore, it has been reported that increased permeability, i.e. extent of exposure to surrounding tissue, improves fiber regeneration [45,46]. The semi-permeable bovine collagen type I tube from *Integra LifeSciences Corp*. used to repair a 5 mm nerve gap in non-human primate, allowed axonal regeneration and functional recovery [35].

In this preliminary study, we used a new EU-approved semi-permeable and bioresorbable porcine collagen type I + III nerve conduit (RevolNerv®) to repair a 10 mm nerve gap in the rat peroneal nerve. Physiological recovery of the animals was assessed using serial behavioral techniques over a period of 12 weeks after the initial surgery. Electrophysiological techniques were also used at the end of the experimental period to measure motor and sensory recovery. The two experimental groups were compared to the standard procedure of direct implantation of an autologous nerve between the severed ends. At the end of the physiological assessments, morphometrics were used to quantify axonal regeneration in the proximal and the distal ends of the injured nerve. The results of the current study are the first data reported for mammalian peripheral nerve repair with RevolNerv®. They suggest its utility for human peripheral nerve repair.

2. Materials and methods

2.1. Animals

Eight-week-old male Sprague Dawley rats, weighting 250–300 g (Charles River®, Les Oncins, France), were housed in smooth-bottomed plastic cages at 22 °C with a 12-h light/dark cycle. Food (Purina®, rat chow) and water were available *ad libitum*. Anesthesia and surgical procedures were performed according to the French law on Animal Care Guidelines and the Animal Care Committee of University Aix-Marseille II approved our protocols. All animals were weighted before each experiment.

2.2. Experimental groups and surgical protocol

Animals were deeply anesthetized (Sodium Pentobarbital® Sanofi Santé Animal, 60 mg kg^{-1}). Surgical procedures were performed aseptically under binoculars. The peroneal nerve from the left limb was dissected free from the surrounding tissues on a length of 3–4 cm. The rats ($n = 48$) were randomized into four groups: 1) Control group (C) ($n = 12$) in which no surgery and no specific treatment was delivered; 2) Gold Standard group (GS) ($n = 12$) in which a 1 cm segment of the left peroneal nerve was removed, reversed, rotated 180°, and then reimplated between the nerve stumps using 10-0 epineural suture (Ethilon® 10-0, Ethicon); 3) Collagen-Tube (CT) group ($n = 12$) in which a 1 cm segment of the peroneal nerve was removed and replaced by a RevolNerv® tube; 4) Tube-Muscle (TM) group ($n = 12$) in which a 1 cm segment of the peroneal nerve was removed and replaced by a RevolNerv® tube filled with acellular skeletal muscle. Female inbred donor rats ($n = 3$) were sacrificed by intra-peritoneal overdose (1 mL) of sodium pentobarbital (Pentobarbital Sodique, Sanofi Santé Animale®). Muscles of lower limbs (i.e. *gastrocnemius*, *tibialis anterior*, and *quadriceps*) were harvested, and bundles were longitudinally sectioned. In order to clear the cells off, muscle bundles were immersed into liquid nitrogen (−196 °C). After thermal equilibrium was achieved, the muscles were transferred into distilled water at room temperature and allowed to thaw for 10 min. This procedure was repeated two times [47]. Then, acellular muscle bundles were stored in a −80 °C freezer until further use. At the time of surgery, the acellular muscle bundles were defrost into sterile water at room temperature and cut under binocular longitudinally into filaments of 3–5 acellular muscle fibers. Then, the muscle fiber filaments were gradually inserted longitudinally into the lumen of the guide with ultra thin forceps (Moria Ultra Fine Tipped Forceps, FST®, Germany) under aseptic conditions.

In these last two groups, the 10 mm nerve gap was bridged with a 12 mm collagen nerve guide, anchored in each nerve stump with a single 10-0 suture (1 mm). For all operated groups, the peroneal nerve was cut proximally, 5 mm away from the ischiatic trifurcation (i.e. starting point of tibial nerve, common peroneal nerve and caudal sural cutaneous nerve), and distally, 10 mm away from the first transection.

Muscles and skin were stitched (Vicryl® 3-0). No clinical sign of pain or unpleasant sensation (i.e. screech, prostration, hyperactivity, anorexia) and no paw-eating behaviour were observed during the study. The animals did not display distress or any secondary complications resulting from the peroneal nerve resection, such as self-mutilation, pressure ulcers, sensory neglect, or infection. The chronological sequence of experimental procedures is presented in Fig. 1.

2.3. Nerve guides

Nerve guides (ID Ø: 500 μm, wall: 50 μm, swelling rate: 1.5–2 – RevolNerv®, International Mark: 914589) were fabricated (Orthomed® S.A. Saint-Jeannet, and Biom'Up® S.A.S, Lyon, France) from highly purified type I + III collagens derived from porcine skin and manufactured according to the patent WO2007147739. Briefly, a smooth mould was coated with a homogeneous solution of collagen, dried, chemically crosslinked and then dried again. The invention relates to collagen tubes which include a wall composed of a succession of continuous, cylindrical and coaxial collagen films. The non-fibrillar structure of the collagen ensures high biocompatibility and full viral safety (unpublished data: Biom'Up®). The innovative fabrication method of the nerve guide is responsible for a particular microscopic structure in which collagen fiber spacing is very narrow (Scanning Electron Microscopy: Fig. 2). As a consequence, the macroscopic structure is smooth and transparent and the nerve guide exhibits i) limited swelling, ii) cell-tight properties and iii) a good mechanical resistance and flexibility that make it easy to handle and to suture. The structural stability and the mechanical strength of the nerve guides were increased by aqueous aldehyde cross-linking which also controlled the rate of *in vivo* resorption. Final resorption was adjusted to meet the technical requirements for nerve regeneration. Nerve guide biocompatibility was evaluated positively using the ISO10993 guideline and this nerve guide reached agreement for UE marking. Biocompatibility was assessed by quantifying tissue inflammation and Schwann cells colonization, survival and migration (unpublished data from Biom'Up®).