Biomaterials 35 (2014) 8970–8982

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

# A comparison of the performance of mono- and bi-component electrospun conduits in a rat sciatic model

Valentina Cirillo [a, b], Basak A. Clements [c], Vincenzo Guarino [a, *], Jared Bushman [c], Joachim Kohn [c], Luigi Ambrosio [a]

[a] Institute of Polymers, Composites and Biomaterials, National Research Council of Italy (IPCB-CNR), Viale Kennedy 54, Naples 80125, Italy
[b] Department of Chemical, Materials and Industrial Production Engineering (DICMAPI), University of Naples Federico II, P.leTecchio 80, Naples 80125, Italy
[c] New Jersey Center for Biomaterials, Rutgers – The State University of NJ, 145 Bevier Road, Piscataway, NJ 08854, USA

ARTICLE INFO

*Article history:*
Received 4 June 2014
Accepted 10 July 2014
Available online 29 July 2014

*Keywords:*
Electrospinning
*In vivo* model
Tubular conduit
Sciatic nerve regeneration

ABSTRACT

Synthetic nerve conduits represent a promising strategy to enhance functional recovery in peripheral nerve injury repair. However, the efficiency of synthetic nerve conduits is often compromised by the lack of molecular factors to create an enriched microenvironment for nerve regeneration. Here, we investigate the *in vivo* response of mono (MC) and bi-component (BC) fibrous conduits obtained by processing via electrospinning poly(ε-caprolactone) (PCL) and gelatin solutions. *In vitro* studies demonstrate that the inclusion of gelatin leads to uniform electrospun fiber size and positively influences the response of Dorsal Root Ganglia (DRGs) neurons as confirmed by the preferential extensions of neurites from DRG bodies. This behavior can be attributed to gelatin as a bioactive cue for the cultured DRG and to the reduced fibers size. However, *in vivo* studies in rat sciatic nerve defect model show an opposite response: MC conduits stimulate superior nerve regeneration than gelatin containing PCL conduits as confirmed by electrophysiology, muscle weight and histology. The G-ratio, 0.71 ± 0.07 for MC and 0.66 ± 0.05 for autograft, is close to 0.6, the value measured in healthy nerves. In contrast, BC implants elicited a strong host response and infiltrating tissue occluded the conduits preventing the formation of myelinated axons.

Therefore, although gelatin promotes *in vitro* nerve regeneration, we conclude that bi-component electrospun conduits are not satisfactory *in vivo* due to intrinsic limits to their mechanical performance and degradation kinetics, which are essential to peripheral nerve regeneration *in vivo*.



## 1. Introduction

Peripheral nerve injuries are very common in clinical practice and often lead to permanent disability. Once the adult nerve tissue is injured, regeneration is often sub-optimal and poor recovery correlates with increasing gap length of the injury [1–3]. Currently, nerve autografts are considered as the “gold standard” for the structural and functional restoration of nerves. However several drawbacks are related to the use of autografts, including secondary surgery, donor site morbidity, limited availability, size mismatch and painful neuroma formation.

Nerve conduits represent a promising alternative for the regeneration of damaged or transected peripheral nerves. In this context, conduits can act as a bridge, providing directional guidance as well as biological support to nerve regeneration [4]. In ensuring the success of neural tissue engineering strategies, material choice plays a crucial role as demonstrated by Ezra et al. [5]. By tailoring the material degradation rates and mechanical properties it is possible to minimize the inflammatory response, thus improving the support and guidance to sustain axon regeneration [6].

A large body of research has been conducted to investigate different kinds of biomaterials for neural tissue engineering including, synthetic materials such as poly(glycolic acid) (PGA) [7], poly(L-lactide-co-glycolide) (PLGA) [8,9], poly(3-hydroxybutyrate) (PHB) [10,11] and natural biopolymers such as gelatin [12–14], collagen [3,8,15–19], chitosan [7,20–23] and silk [24,25]. Synthetic materials are attractive as neural tissue engineering scaffolds because of the ease in tailoring the degradation rate and mechanical properties of these materials to suit the application. On the

* Corresponding author. Institute for Polymers, Composites and Biomaterials (IPCB), National Research Council of Italy, Mostra d'Oltremare, V.le Kennedy 54, Naples 80125, Italy. Tel.: +39 0812425944; fax: +39 0812425932.
*E-mail addresses:* vguarino@unina.it, vincenzo.guarino@cnr.it (V. Guarino).

http://dx.doi.org/10.1016/j.biomaterials.2014.07.010
0142-9612/

other hand, natural polymers, such as collagen and gelatin, offer biomolecular recognition sites [26] but must be stabilized by cross-linking or other methods in order to yield a stable nerve conduit material that can maintain its integrity during the regenerative period.

A considerable effort has been dedicated developing synthetic nerve conduits to bridge long nerve gaps. However, more work needs to be done to improve their efficacy compared to autologous nerve grafts and several designs of nerve conduits have been proposed.

Recently, alternative repair strategies include the use of intraluminal guidance structures and micro-grooved luminal designs [27] to provide additional structure support and topographical guidance to regenerating axons and migrating Schwann cells (SC). However, the presence of fillers clustered in the center of the conduit may reduce axonal regeneration leading to regeneration failure [28]. The addition of a dense collagen sponge within a hollow nerve conduit completely inhibits regeneration [29]. These results illustrate the importance of correct placement of intraluminal fillers within a hollow nerve conduit and the proper choice of materials.

Given the unmet need, investigating new biomaterials that can better interact with cells remains an area of intense research [25]. Equally important is the development of inexpensive processing methods that can create the fine structures necessary to support the regenerative niche. Processing techniques must allow for maintaining fine control of their structural properties, at micro- and nano-meter level, to present to cells topographical cues matching their native environment during the regeneration process [30].

Flemming et al. demonstrated that purely topographical cues offered by nanofibers mimic the conformation of the extracellular matrix thus supporting cell response (i.e., adhesion, the ability of cells to orient themselves, migrate and produce organized cytoskeletal arrangements) [31]. Similar studies on nerve regeneration revealed that in addition to fiber architecture, the combined contribution of biochemical cues can further support the main cellular events triggering cell adhesion and neurite outgrowth over the fiber scaffold [32,33]. Electrospinning represents a promising strategy to produce fibrous conduits able to assure high porosity and large surface areas for cell attachment and nutrient transportation. Furthermore, the chance to process by electrospinning mixtures of synthetic and natural polymers can guarantee the interactions with cultured cells while having suitable mechanical properties and degradation rate to provide longitudinal support for the regenerating nerves [33–35].

Our paper focuses on the production of mono-component (MC) and bi-component (BC) conduits made of PCL and PCL/gelatin by electrospinning. MC and BC tubular conduits have been fabricated with tunable three-dimensional (3D) microarchitecture by electric force driven deposition of fibers onto a rotating mandrel. Previous investigations have demonstrated the biocompatibility of similar fibers in *in vitro* conditions [33–35]. Here we confirm the ability to provide a favorable environment that supports the growth of cells *in vitro*. Moreover, we investigate the response *in vivo* of PCL and PCL/gelatin conduits to stimulate nerve regeneration in rat sciatic nerve defects.

## 2. Materials and methods

### 2.1. Materials

PCL pellets ($M_n$ 45 kDa) and gelatin of type B (~225 Bloom) from bovine skin in powder form, were all purchased from Sigma–Aldrich (Italy), while 1,1,1,3,3,3-hexafluoro-2-propanol (HFP) was supplied by Fluka (Italy) and chloroform ($CHCl_3$) by J.T. Baker (Italy). All products were used as received without further purification.

A 1:1 (weight ratio) polymer solution of PCL and gelatin was prepared by first dissolving the polymers separately in HFP for 24 h under magnetic stirring and then mixing them in order to obtain a solution with a final polymer concentration of 0.1 g/ml.

PCL alone was dissolved in chloroform at a concentration of 0.33 g/ml. The mixture was kept under magnetic stirring at room temperature until a clear solution was obtained.

### 2.2. Preparation of the electrospun conduits

Electrospun fiber membranes were obtained by using a commercially available electrospinning setup (Nanon01, MECC, Japan). The polymer solution was placed in a 5 ml plastic syringe connected to an 18 Gauge needle. At the first stage, fibers were randomly collected over a grounded aluminium foil target in order to obtain flat membranes. Different process parameters were selected to optimize the final fiber morphology: in particular, optimal BC fiber membranes were fabricated by setting a distance of 8 cm, a voltage of 13 kV and a flow rate of 0.5 ml/h while MC fiber membranes were fabricated by adjusting the parameters to 15 cm, 20 kV, 0.5 ml/h respectively. The process was carried out in a vertical configuration at 25 °C and 50% relative humidity, and the deposition time was adequate to obtain the proper thickness (~150 μm) to remove the membranes from the grid. MC and BC conduits (thickness 1 mm) were developed by collecting fibers onto a 1.5 mm diameter metal mandrel, with a rotating rate of 50 rpm.

Flat membranes were cut into 6 mm discs and placed into 96-well tissue culture plates for biological characterization. Prior to the biological assays, MC and BC fiber sheets and conduits were sterilized by immersion in 70% of ethanol (v/v) with antibiotic solution (100 μg/ml streptomycin and 100 U/ml penicillin) for 30 min, washed three times with phosphate-buffered saline (PBS) and air dried.

### 2.3. Morphological characterization

Qualitative evaluation of fiber morphology of the electrospun MC and BC membranes and conduits was performed by field emission scanning electron microscopy (FESEM, QUANTA200, FEI, The Netherlands). Samples were dried in the fume hood for 24 h in order to remove any residual solvent, mounted on metal stubs and sputter-coated with gold–palladium for about 20 s in order to get a 19 nm thick conductive layer. SEM images were taken under high vacuum conditions ($10^{-7}$torr) at 10 kV, using the secondary electron detector (SED). On selected SEM images, the fiber diameter distribution, the mean total porous area and the % porosity were determined by using image analysis freeware (NIH ImageJ 1.37).

### 2.4. Mechanical flattening tests

Transverse compression testing of MC and BC conduits was performed using a dynamometric machine (Instron 5566). The length of samples ($n = 5$/group) was 7 mm and the tube wall thickness was measured before testing. All the tubes were hydrated before testing by soaking them in deionized water for 10 min. The cross-head speed was maintained at 1 mm/min and the cell load was 100 N. The compressive strengths of MC and BC tubes were reported against the displacement values, calculated as the ratio between the outer diameter of the flattened tube and the initial outer diameter. Results are expressed as mean ± standard deviation on five different samples ($n = 5$).

### 2.5. In vitro response of dorsal root ganglion (DRG) neurons

All sterilized samples were immersed in Dulbecco's Modified Essential Medium (DMEM) for 24 h prior to plating undissociated lumbar DRGs dissected from E15 chick embryos and then cultured in DMEM, supplemented with 5% fetal bovine serum, antibiotic solution (streptomycin, 100 μg/ml, and penicillin, 100 U/mL), 2 mM L-glutamine and incubated at 37 °C in a humidified atmosphere with 5% $CO_2$ and 95% air. After 24 and 72 h of seeding, cells were fixed with 4% paraformaldehyde (PFA). Individual neurites extending from the DRG were visualized by staining with NF-200 antibody and fluorescence microscopy. Neurite length was quantified using ImageJ software from randomly selected high magnification images of neurites extending from the DRG periphery. All experiments were run in triplicate.

### 2.6. In vivo implants

Eighteen female Lewis rats weighing 225–250 g were used for this study. Procedures were reviewed and approved by the Rutgers University Institutional Animal Care and Use Committee (IACUC). Rats were randomly assigned to one of three groups ($n = 6$ rats/group). As described elsewhere [36], an incision was made along the left femoral axis on the left leg posterior to the femur. A 5 mm nerve segment was resected 5 mm distal to the internal obturator tendon. 7 mm long nerve conduits were secured to nerve stumps with two 9-0 ethilon sutures (Ethicon, Somerville, NJ) on either side, leaving a 5 mm gap between the stumps (Fig. 1a–b). In the autologous graft group, a 5 mm nerve segment was resected, reversed 180° and subsequently sutured back into the nerve using four 9-0 ethilon sutures on either side (Fig. 1c). Muscle and skin were then closed with 6-0 ethilon sutures. Rats were given on oral acetaminophen for 7 days post-op along with an E-collar (if warranted) to prevent autophagia/autotomy.