Biomaterials 32 (2011) 8077–8086

Contents lists available at ScienceDirect

Biomaterials

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

Comparison of polymer scaffolds in rat spinal cord: A step toward quantitative assessment of combinatorial approaches to spinal cord repair

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ARTICLE INFO

Article history:
Received 22 April 2011
Accepted 8 July 2011
Available online 30 July 2011

Keywords:
OPF
PLGA
PCLF
Axon regeneration
Spinal cord injury
Schwann cell

ABSTRACT

The transected rat thoracic ($T_{9/10}$) spinal cord model is a platform for quantitatively comparing biodegradable polymer scaffolds. Schwann cell-loaded scaffolds constructed from poly (lactic co-glycolic acid) (PLGA), poly(ε-caprolactone fumarate) (PCLF), oligo(polyethylene glycol) fumarate (OPF) hydrogel or positively charged OPF (OPF+) hydrogel were implanted into the model. We demonstrated that the mechanical properties (3-point bending and stiffness) of OPF and OPF + hydrogels closely resembled rat spinal cord. After one month, tissues were harvested and analyzed by morphometry of neurofilament-stained sections at rostral, midlevel, and caudal scaffold. All polymers supported axonal growth. Significantly higher numbers of axons were found in PCLF ($P < 0.01$) and OPF+ ($P < 0.05$) groups, compared to that of the PLGA group. OPF + polymers showed more centrally distributed axonal regeneration within the channels while other polymers (PLGA, PCLF and OPF) tended to show more evenly dispersed axons within the channels. The centralized distribution was associated with significantly more axons regenerating ($P < 0.05$). Volume of scar and cyst rostral and caudal to the implanted scaffold was measured and compared. There were significantly smaller cyst volumes in PLGA compared to PCLF groups. The model provides a quantitative basis for assessing individual and combined tissue engineering strategies.

1. Introduction

The destruction and atrophy of axons in conjunction with glial scar and cyst formation form a physical barrier restricting axonal regeneration after spinal cord injury [1]. In completely destructive injuries, there are two ways to reconnect links and functions below the injured area: bypassing the injured site or rebuilding functional tissue within the cysts and scars. Neuronal survival, axonal growth and remyelination as well as reconnection across the injured site are required for the spinal cord repair with the help of the bridging grafts [2].

Spinal cord repair is complex and will require combining multiple modalities including extracellular architecture, surface or molecular contact guidance cues and appropriate cells. Tissue engineering offers the possibility of bringing polymer chemistry and cellular neurobiology in developing new therapeutic strategies for patients [3–5]. In this context, it is critical to develop model systems that allow deconstruction of the process and quantification of the contributions of individual components to successful regeneration. Polymer scaffolds for neural tissue engineering provide three-dimensional support mimicking the architecture of the extracellular matrix (ECM) for *in vitro* and *in vivo* cell growth and tissue construction [6,7]. We have previously studied a number of different biodegradable synthetic polymers [8–22] and their role as potential scaffolds in nervous system repair in both cell and animal models.

Many cell types have been studied in animal models of spinal cord injury, including schwann cells (SCs) [23]. SCs supported regeneration both in peripheral and central nervous systems, and they have specifically been shown to promote axonal regeneration in the model of spinal cord injury [13,23–26]. Recently we demonstrated schwann cell survival for up to 6 weeks in rats after implantation of a multi-channel polymer scaffold in a complete transection study. We directly compared cell types, and found that schwann cells exhibited a higher capacity than stem cell neurospheres to promote axonal regeneration in the transected spinal

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doi:10.1016/j.biomaterials.2011.07.029

cord [14]. This study used a scaffold made from PLGA, a polymer used clinically in absorbable suture material, which has been studied extensively in our laboratory [17].

While schwann cells are a promising cell type, the optimal polymer type for delivery of these cells to the injured cord remains unknown. PLGA is approved for human use by the Food and Drug Administration (FDA) and is used in many clinical applications. PCLF is a novel biodegradable polymer that has been developed for directed bone and nerve regeneration. This copolymer is self-cross-linkable and biocompatible. Scaffolds fabricated from this material can serve as support for neural tissue engineering applications [16]. OPF is a third polymer type under development within our collaborative group. OPF is a PEG based macromer incorporating a fumarate moiety that is photo-cross-linked to form a biocompatible and biodegradable hydrogel [27]. OPF can be copolymerized with [2-(methacryloyloxy) ethyl]-trimethylammonium chloride (MAETAC) to produce a positively charged hydrogel (OPF+). We have shown that the positively charged substrate enhanced neuronal cell attachment, SC migration and axonal myelination *in vitro* [12], making this an attractive candidate as scaffold material *in vivo*.

In the present study, we describe a model system that allows comparison between four different polymers (PLGA, PCLF, OPF and OPF+) used to construct biodegradable, multi-channel scaffold for implantation with SCs. The degree of axonal regeneration is quantitatively compared across the groups by means of neurofilament staining of transverse sections and counting of axons at multiple levels through the scaffold.

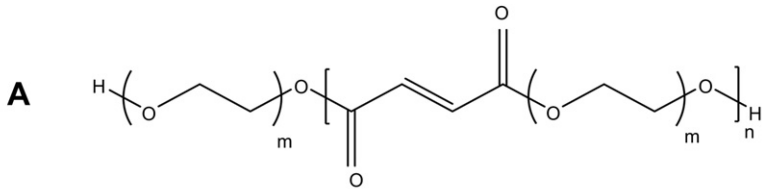
2. Materials and methods

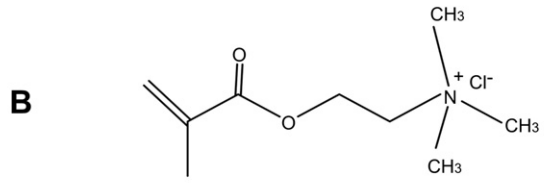
2.1. Scaffold manufacturing

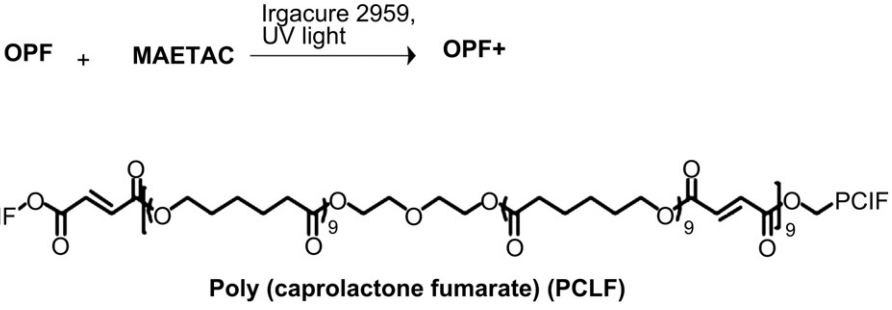
PLGA scaffolds with 7 parallel channels (660-μm diameter) were fabricated by injection molding and solvent evaporation as previously described [17]. OPF and OPF+ were synthesized and fabricated as nerve conduits according to the previously published methods (Scheme 1) [12,16,27–33]. PCLF with 1% Irgacure 819 (Ciba Specialty Chemicals Corp, New York) formulation was injected into scaffold molds that consisted of a glass tube with seven evenly spaced stainless steel rods coated with Ease Release 2000 (Mann Release Technologies, Easton, PA). After treatment under UV light (MRC 58 Multiple Ray Lamp, UVP, Upland, CA) for 1 h the PCLF scaffold tube was cleaned and 'etched' in 3 changes of acetone over 48 h, before being vacuum dried for 24 h [16,34]. Scaffolds were cut into 2-mm lengths and washed in serial dilutions of absolute alcohol for 30 min with mild shaking for sterilization and elimination of residual mold lubricant. PLGA scaffolds were dried by vacuum for 24 h for removing the alcohol, sealed in sterilized glass tubes and then kept dried at 4 °C until further use. PCLF and OPF scaffolds were washed 3 times in sterile PBS over a couple of hours after incubating in 80% ETOH for 30 min.

2.2. Mechanical property of polymer scaffolds

Three-point bending and compression modulus of PCLF, OPF, OPF+ and PLGA conduits were measured using a dynamic mechanical analyzer (DMA2980, TA instruments) at room temperature. Three-point bending in a dynamic mechanical analyzer was used to measure flexural modulus of conduits as previously described [34]. Freshly isolated spinal cord from rat was harvested and compared in the same system within 15 min of isolation. Polymer conduits had an average outer diameter of 2.2 mm for OPF conduits, 2.6 mm for OPF + conduits, 2.4 mm for PLGA conduits, 3.03 mm for PCLF conduits, and a fixed length of 8.5 mm. Six specimens were measured and averaged for each sample. For measurements of compression, modulus was measured on the conduits with thickness of 2 mm as used in transected spinal cord. The test was performed under load control, where load was applied at a rate of 4 N/min. Stress and strain data collected during testing were







Scheme 1. Chemical structure of oligo (polyethylene glycol) fumarate (OPF), Poly (caprolactone fumarate) (PCLF) and OPF+. The OPF (A), composed of repeating PEG and fumarate chains, was crosslinked with [2-(methacryloyloxy) ethyl]-trimethylammonium chloride (MAETAC) (B) in the presence of the photoinitiator Irgacure 2959 and ultraviolet light to form positively charged hydrogels (OPF+).