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The development of electrically conductive polycaprolactone fumarate–polypyrrole composite materials for nerve regeneration

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ABSTRACT

Electrically conductive polymer composites composed of polycaprolactone fumarate and polypyrrole (PCLF–PPy) have been developed for nerve regeneration applications. Here we report the synthesis and characterization of PCLF–PPy and *in vitro* studies showing PCLF–PPy materials support both PC12 cell and dorsal root ganglia (DRG) neurite extension. PCLF–PPy composite materials were synthesized by polymerizing pyrrole in preformed PCLF scaffolds (M_n 7,000 or 18,000 g mol^{-1}) resulting in interpenetrating networks of PCLF–PPy. Chemical compositions and thermal properties were characterized by ATR-FTIR, XPS, DSC, and TGA. PCLF–PPy materials were synthesized with five different anions (naphthalene-2-sulfonic acid sodium salt (NSA), dodecylbenzenesulfonic acid sodium salt (DBSA), dioctyl sulfosuccinate sodium salt (DOSS), potassium iodide (I), and lysine) to investigate effects on electrical conductivity and to optimize chemical composition for cellular compatibility. PCLF–PPy materials have variable electrical conductivity up to 6 mS cm^{-1} with bulk compositions ranging from 5 to 13.5 percent polypyrrole. AFM and SEM characterization show microstructures with a root mean squared (RMS) roughness of 1195 nm and nanostructures with RMS roughness of 8 nm. *In vitro* studies using PC12 cells and DRG show PCLF–PPy materials synthesized with NSA or DBSA support cell attachment, proliferation, neurite extension, and are promising materials for future studies involving electrical stimulation.

1. Introduction

Traumatic injuries resulting in neurological damage to either the central or peripheral nervous system occur frequently. Spinal cord injuries (SCI) affect over 250,000 individuals in the U.S. with 12,000 new cases occurring every year [1]. Peripheral nerve injuries (PNI) are more common, with estimates as high as 5 percent of all patients admitted to level 1 trauma [2]. The frequency and disability associated with PNI injury necessitates the need for therapies to restore the loss of function. The current clinical standard for the treatment of PNI with segmental nerve loss is the use of nerve autografts, which remove a piece of non-critical nerve from a secondary site on the body to replace the missing nerve section. This technique has significant drawbacks including donor site morbidity, insufficient donor nerve length, mismatch of diameter between donor nerve and recipient site, misaligned endoneurial tubes, and mismatched regenerating axons.

The drawbacks associated with autografts motivate the search for alternate treatment options. Synthetic materials have great potential for applications as nerve guidance conduits because they can be fabricated with various dimensions, degradation rates, chemical compositions, mechanical properties, micro-architectures, and external geometries [3–8]. In addition, therapeutic drugs can be loaded into the scaffolds for controlled release over days or weeks, and cellular therapies, such as stem cells [9,10], adipose-derived stromal cells [11], or Schwann cells can be cultured on the scaffolds before implantation [12,13].

Regeneration of damaged nerves faces another obstacle in addition to the above-mentioned challenges. As time passes and nerves extend from the proximal to the distal stump, regenerating axons and the target organs or muscle increasingly lose their regenerative capacity [14–16]. Therefore, increasing the rate of nerve regeneration through stimulation may be a critical step to realizing full functional recovery after segmental nerve loss. Electrical stimulation as a therapeutic treatment is a rapidly expanding

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area in the field of tissue engineering, especially for nerve applications, with numerous reports showing electrical stimulation increases neurite and axon extension *in vitro* and nerve regeneration *in vivo*. Electrical stimulation by either direct exposure to electrical current (AC or DC) or via an electric field has been shown to effect stem cell differentiation [17,18], neurite extension [19,20], and influence directionality of growing axons [21].

Techniques to incorporate electrically conductive materials into biomaterials have included attachment of metal electrodes to proximal and distal nerve stumps [22,23], scaffolds coated with gold nanoparticles [24], and electrically conductive polymers such as polypyrrole [25–36] or polyaniline [37,38]. Schmidt et al. was one of the first researchers to demonstrate that using the conductive polymer polypyrrole and applying an electrical current through the material has a positive effect on neurite extension from PC12 cells [20]. Since then, numerous groups have thoroughly investigated many aspects of polypyrrole including *in vitro* and *in vivo* biocompatibility, stability, conductivity, incorporation of the cell adhesive polypeptide RGD, and more [20,27,29–31,36]. However, most of this work focuses on thin films of polypyrrole.

Although polypyrrole could be very useful for tissue engineering applications, materials composed solely of polypyrrole are not acceptable as biomaterials. PPy has a very low solubility in most solvents that makes it difficult to process into complex three-dimensional structures, poor mechanical properties that make the materials brittle and weak, and is non-biodegradable. Different approaches have been attempted to overcome these limitations and incorporate electrically conductive polymers into biomaterials. Some examples include blending polypyrrole with poly(lactic-co-glycolic acid) [34,39–42], block copolymers of polylactide and polyaniline [37,38], nanoparticles composed of polypyrrole-polyethylene glycol-polylactic acid [35], and the templated synthesis of polypyrrole [26].

Here we report the synthetic method to produce composite materials composed of polycaprolactone fumarate (PCLF) and polypyrrole (PPy). PCLF (chemical structure shown in Fig. 1) is a chemical or photo-cross-linkable derivative of polycaprolactone that can be easily processed into complex three-dimensional structures by injection molding or solid freeform fabrication. PCLF has been shown to exhibit biocompatibility, good mechanical properties, and tunable degradation rates that make it a promising material for application as nerve guidance conduits [8,43]. PCLF has previously been shown to direct nerve regeneration in the rat sciatic nerve defect model [7] and is currently under *in vivo* study as nerve guidance conduits in conjunction with therapeutic drugs, Schwann cells, and adipose-derived stem cells. However, a major issue with polymeric nerve conduits in general is that regenerating nerve tissue grows through the polymer as a cable and is surrounded by a thick wall of fibrous tissue that does not make any contact with the polymer walls [7]. This significantly restricts the available space for regenerating tissue. Therefore, the development of materials that promote neural cell attachment and decrease fibrous tissue ingrowth into the scaffold would represent an attractive improvement to these scaffolds.

To increase cellular compatibility and stimulate nerve regeneration, PCLF was extended to the electrically conductive PCLF–PPy composite materials. PCLF–PPy polymer composites can be easily fabricated into complex three-dimensional structures, such as single-lumen and multi-lumen nerve conduits shown in Fig. 1, and overcome the limitations associated with processing polypyrrole into complex three-dimensional structures. PCLF–PPy materials maintained the physical properties of the host polymer PCLF. This

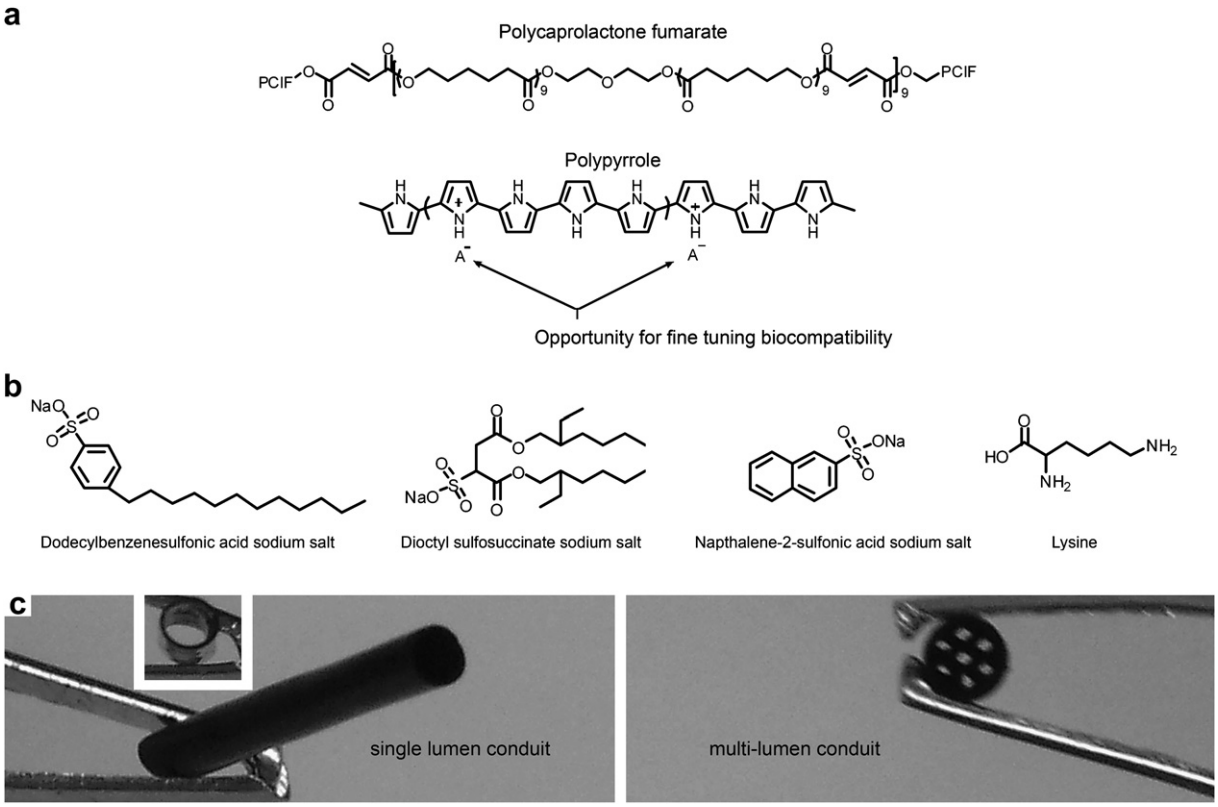

Fig. 1. A) Chemical structures of polycaprolactone fumarate and polypyrrole. B) Anions used in the synthesis of polypyrrole to modify the chemical composition of the resulting PCLF–PPy scaffolds. C) Single-lumen and multi-lumen nerve conduits composed of PCLF–PPy illustrating that these materials can be easily fabricated into three-dimensional structures.