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Electroconductive polymeric nanowire templates facilitates in vitro C17.2 neural stem cell line adhesion, proliferation and differentiation

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ABSTRACT

Stem cells still remain one of the most exciting and lucrative options for treatment of a variety of nervous system disorders and diseases. Although there are neural stem cells present in adults, the ability of both the peripheral and central nervous system for self-repair is limited at best. As such, there is a great need for a tissue engineering approach to solve nervous system disorders and diseases. In this study, we have developed electrically conductive surfaces with controlled arrays of high aspect ratio nanowires for the growth and maintenance of neural stem cells. The nanowire surfaces were fabricated from polycaprolactone using a novel nanotemplating technique, and were coated with an electrically conductive polymer, polypyrrole. The polypyrrole-coated nanowire surfaces were characterized using scanning electron microscopy and X-ray photoelectron spectroscopy. Additionally, the surface resistance of polypyrrole-coated nanowire surfaces was measured. C17.2 neural stem cells were used to evaluate the efficacy of the polypyrrole-coated nanowire surfaces to promote cell adhesion, proliferation and differentiation. The results presented here indicate significantly higher cellular adhesion and proliferation on polypyrrole-coated nanowire surfaces as compared to control surfaces. The differentiation potential of polypyrrole nanowire surfaces was also evaluated by immunostaining key neuronal markers that are expressed when NSCs differentiate into their respective neural lineages.

1. Introduction

Stem-cell-based tissue-engineering therapies incorporating unique scaffold architectures can have significant benefits to patients currently living with neurological diseases and disorders that involve a functional disturbance and/or a pathological change in the spinal cord. There are several causes of these types of disorders. For example, diseases such as polio, measles or herpes may cause myelitis which is inflammation of the spinal cord [1]. Vascular myelopathy is another type of spinal cord disorder and often arises from spinal cord vascular malformations [2]. When spinal cord disorders are caused by trauma, it is referred to as spinal cord injury (SCI) [3]. SCIs represent the most troubling population of individuals with spinal cord disorders. Causes of SCI include motor vehicle accidents (44%), acts of violence (24%), falls (22%) and sports (two-thirds of these are from diving accidents) (8%), with the remainder classified as "other" (2%) [4]. It is estimated that the annual incidence of SCIs, not including those who die at the scene of the accident, is approximately 40 cases per million population in the United States, or approximately 12,000 new cases each year [5]. In 2009, the number of people who were living with an SCI was estimated to be between 231,000 and 311,000 [5]. Further, 52% of individuals with SCI are paraplegic, i.e. their motor or sensory function of the lower extremities is impaired and they only have use of their upper extremities. The other 48% are quadriplegic, i.e. their motor or sensory function of the whole body is impaired and they have completely lost the use of all their limbs and torso. In contrast to other spinal cord diseases and disorders, most people who get an SCI are male (82%) and the injury comes at a young age, the median age at the time of injury is 31.7 years. Unfortunately, there is no clinically proven way to reverse damage to the spinal cord. Current SCI treatment focuses on preventing further injury and empowering people with an SCI to return to an active and productive life as opposed to regaining lost function. However, researchers are continually working on innovative treatments, such as direct stem cell injections [6] and stem cell therapy coupled with tissue engineering [7,8], which may promote nerve cell regeneration or improve the function of the nerves that remain after the SCI.

Nanotechnology offers interesting avenues to explore stem cell based tissue engineering therapies to promote nerve cell regeneration or improve the function of the nerves that remain after SCI. Tissue-engineered scaffolds with a unique nanotopography have

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shown favorable responses to neuronal cells both in vitro and in vivo. Several in vitro studies have reported enhanced functionality of neuronal cells on polymer nanofiber scaffolds [9–11], nanoporous scaffolds [12] and nanowire scaffolds [8]. In addition, there is significant evidence that neural stem cells (NSCs) promote extensive axonal growth when implanted with nanofiber scaffolds into the site of a spinal cord injury [13] and can also reduce the functional deficits in a sciatic nerve injury model in vivo [7]. Thus, it can easily be concluded that nanotopography on a scaffold surface plays a positive role in neuronal cell–surface interactions. However, most of the scaffolds do not have physiologically relevant surface properties. Mammalian cells are known to preferentially adhere and differentiate on electrically charged surfaces [14]. Therefore, while nanotopography has been shown to enhance cellular response, it seems necessary to further functionalize the scaffolds and create an electrically charged surface for growth and maintenance of NSCs.

The electrically conductive polymer polypyrrole (PPy) has garnered substantial interest for applications in neural tissue engineering due its biocompatibility [15,16], ease of synthesis [17] and electrical properties [18]. It has been commonly used in biosensors and polymer batteries for these reasons [19]. Studies have shown that PPy has the ability to promote growth of endothelial cells [20], primary neurons [21] and even mesenchymal stem cells [22]. The need for an electrically conductive polymer for neural tissue engineering applications arises from the fact that uncharged surfaces are less than optimal for promoting normal cellular phenotypic behaviors [14]. Furthermore, the ability to deliver an electrical current to the cells via the scaffold surface may have several advantages. Current research has emerged showing that physiologically relevant electrical stimulation can enhance nerve regeneration [23] and help replenish NSCs in an injury site [24]. It is therefore advantageous to design scaffolds that not only have unique surface nanotopography but also have the ability to provide physiological levels of electrical stimulation for the NSCs to modulate and enhance their cellular response.

In this work, we have developed a solvent-free nanotemplating technique for fabricating polycaprolactone (PCL) nanowire surfaces as templates for growth and differentiation of NSCs. These nanowire surfaces were then coated with PPy to provide an electrically conductive surface for cell–surface interactions. PCL was used to fabricate nanowire surfaces due to its biocompatibility and controlled biodegradability. PPy-coated PCL nanowire surfaces were characterized using X-ray photoelectron spectroscopy (XPS) and scanning electron microscopy (SEM). Further, the stability of PPy coatings on PCL nanowire surfaces in physiological conditions was evaluated using SEM and XPS. C17.2 murine neural stem cells were used as model cells to study the viability, adhesion, proliferation and differentiation on PPy-coated PCL nanowire surfaces. This cell line was derived from the external germinal layer of a neonatal mouse cerebellum [25]. However, the C17.2 cell line is a good NSC model because after isolation they still retain their ability to differentiate into both neurons and glial cells [26]. The cell line has also been shown to express significant levels of several neurotrophic factors, including nerve growth factor, brain-derived neurotrophic factor and glial cell line-derived neurotrophic factor [13].

2. Experimental methods

2.1. Fabrication of PCL nanowire surfaces

PCL nanowire (NW) surfaces were fabricated according to a protocol previously developed in our laboratory and are illustrated in Fig. 1 [27]. Briefly, nanowire surfaces were fabricated via template synthesis from PCL using commercially available nanoporous aluminum oxide membranes with a 20 nm pore size (ANOPORE™, Whatman). Polymer discs (10 mm diameter and approximately 2 mm thick) were placed on the surface of the membrane (Fig. 1A) and the NWs were extruded through the membranes in an oven at 115 °C for 3 min (Fig. 1B and C). The aluminum oxide membranes were dissolved in 1 M NaOH for 75 min to remove the membrane, thus releasing the extruded NWs (Fig. 1D). The NW surfaces were then soaked and rinsed in DI water, dried and stored in a desiccator until further use.

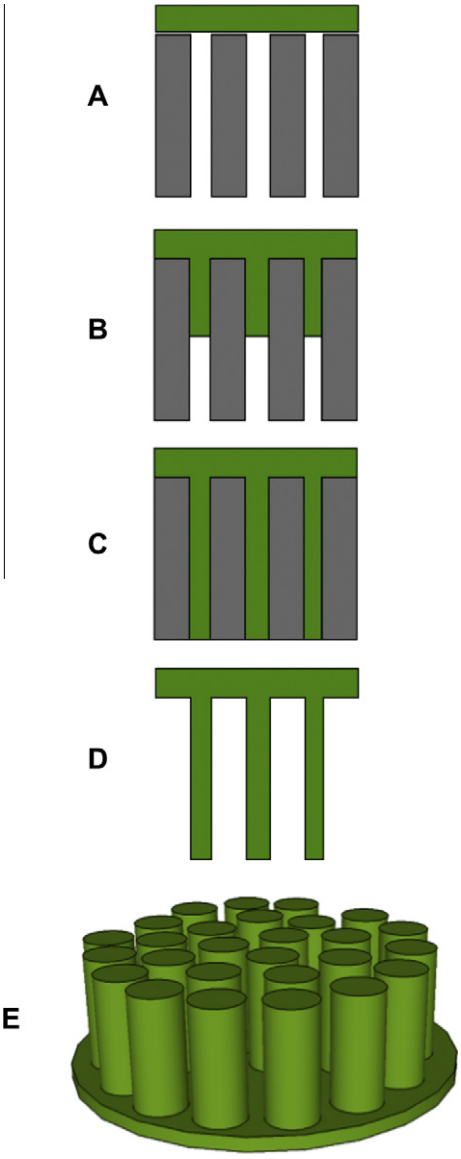

Fig. 1. Schematic of PCL NW fabrication: (A) a PCL pellet is placed on top of the alumina nanoporous membrane. (B and C) The polymer is extruded through the nanoporous membrane in a vacuum oven. (D) The alumina nanoporous membrane is dissolved in NaOH to release the NWs. (E) PCL NW surfaces.

2.2. PPy coating on PCL NW surfaces

The as-fabricated NW surfaces were coated with PPy using a polymerization technique described elsewhere [17,21]. Briefly, the NW surfaces were sonicated in a solution of 14 mM pyrrole and 14 mM sodium *para*-toluene sulfonate for 30 s to saturate the surfaces with pyrrole. This was followed by gentle shaking and incubating the NW surfaces for 1 h at 4 °C. Next, 38 mM ferric

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