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Effect of topography of an electrospun nanofiber on modulation of activity of primary rat astrocytes

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HIGHLIGHTS

- PCL nanofiber topography enhanced cell adhesion but alleviated activity of astrocyte.
- Astrocyte on nanofiber has down-regulated GFAP.
- Rough topography is helpful for neuronal regeneration by alleviating astrogliosis.

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ABSTRACT

Several biomaterials for neural tissue engineering have recently been proposed for regeneration of damaged tissue and promotion of axonal guidance following CNS injury. When implanted into damaged nerve tissue, biomaterials should favorably induce cell infiltration and axonal guiding while suppressing inflammation. Nanofiber scaffolds are regarded as adequate materials to meet the above requirements; however, most studies of these materials conducted to date have targeted neuronal cells, not glial cells, despite their important function in the injured CNS. In this study, an electrospun nanofibrous scaffold of polycaprolactone (PCL) was investigated with respect to its topographic effects on astrocyte behavior and expression of GFAP. The results revealed that the PCL nanofiber topograghy promoted adhesion, but GFAP expression was down-regulated, leading to reduced astrocytes activity. Taken together, these results indicate that the topographic structure of electrospun nanofibers provides a scaffold that is favorable to neural regeneration via alleviation of astrogliosis.

1. Introduction

The central nervous system (CNS) plays an important role in the in vivo regulation of information. When the CNS is injured, an unfavorable environment for nerve regeneration is induced. As a result, damaged nerve cells cannot be regenerated and permanent functional impairment follows [13,16]. In recent years, tissue engineering for the regeneration of damaged tissues in the CNS has been investigated [20].

Astrocytes are glial cells that play critical roles in the CNS by supporting and supplying nutrients to nervous tissue [14,19]. When the CNS is damaged, the activated astrocytes induce glial scarring at the site of injury, which prevents neuronal regeneration [23]. However, it has also been reported that reactive astrocytes could actually help regenerate injured neurons [15,20]. The astrocytes at the site of injury express a wide array of cytokines including glial fibrillary acid protein (GFAP), chondroitin sulfate proteoglycans (CSPG), and Syndecan 1 (HSPG), which are responsible for either helping or interfering with the regeneration of injured neurons [4]. Thus, the expression levels of these proteins can be regarded as biomarkers of the extent of CNS injury.

Electrospinning is a well-known and simple technique for fabrication of continuous sub-micro scale fibers. Electrospun nanofibers are used in multipurpose applications because of their structural properties [11]. Notably, electrospun nanofibers are very similar to natural extracellular matrix (ECM), and a highly porous structure is suitable for cell infiltration and enhancement of the axonal guidance by controlling the alignment of the fiber. For these reasons, electrospun nanofiber is a potential scaffold for neural tissue engineering [6,9,21]. Scaffolds for cell culture should meet several conditions. Specifically, suitable polymers for making scaffold should be biocompatible, biodegradable, and non-toxic. Polycaprolactone (PCL), an aliphatic polyester, is biodegradable and biocompatible and thus regarded as an appropriate scaffold for tissue engineering. Studies of adaptation of PCL nanofiber to neural systems have primarily focused on neurons [7,12]. Even though PCL nanofiber has already been studied for astrocyte activity [2], its molecular aspects have rarely been analyzed. Furthermore, the topographic effects of PCL nanofibers on astrocytes have never been discussed.

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In the present study, the topographic effects of PCL nanofiber on adhesion, viability and GFAP expression of astrocytes were studied to assess its possible use as a biomaterial for neural regeneration.

2. Methods

2.1. Fabrication and characterization of electrospun nanofiber

Organic co-solvent composed of THF:DMF (7:3) was prepared, and 15% (w/v) PCL was homogeneously dissolved in the solvent overnight. Electrospinning was then conducted as previously described [10]. Briefly, PCL solution was placed in a 10 ml plastic syringe (Henke Sass Wolf, Germany) connected to a stainless steel needle (22 gauge). The solution was then electrospun using a microsyringe pump (KDS100, KD Scientific USA) at a flow rate of 2 ml/h with a 13 kV potential and the PCL nanofiber was collected on coverslips (Marienfeld, Germany) 16 cm from the needle tip. To prepare the PCL film, PCL nanofiber mats were stored in an oven at 60 °C for 20 min. All samples were stored in a vacuum until used in the experiment. The wettability of the PCL fiber and film was measured using a contact angle analyzer (S-EO Phoenix touch, Korea) with 3 μL aliquots of distilled water applied to five spots on each sample as probe liquids.

2.2. Characterization of cellular morphology

The morphology of the scaffold was characterized using a field emission scanning emission microscope (FE-SEM, Hitachi S-4300, Japan). Briefly, cells on nanofiber and film were dehydrated with ethanol applied at a gradually increasing gradient (25%, 50%, 75%, 90%, and 100%) for 20 min each at room temperature. The samples were then immersed into 100% HMDS (Hexamethyladisilazane, Sigma) for 3 min, after which they were coated with Pt.

2.3. Cell preparation

Rat primary astrocytes were isolated as previously described [1]. After day 1, neonatal Sprague-Dawley rats were placed on ice, their cerebrums isolated from their skulls, the meninges peeled off, and the remaining samples cut into small pieces. Small tissue samples were then suspended in culture medium via pipetting several times. Next, the suspended cells were filtered through a Cell-Strainer (BD Falcon, USA) with a pore size of 70 μm, after which the filtrate was centrifuged at 1000 rpm for 5 min. The cells were then seeded on T-75 polystyrene-coated tissue culture flasks (Corning, USA) at 2×10^7 cells per flask, after which they were cultured for seven days at 37 °C in a 5% CO_2 incubator. The culture medium was composed of 89% Dulbecco's Modified Eagle's Medium High Glucose (Gibco, USA), 10% fetal bovine serum (Gibco, USA) and 1% penicillin/streptomycin (Gibco, USA). After seven days, the flasks were shaken at 200 rpm for 24 h to isolate the astrocytes. The astrocytes strongly attached to the flasks were then subcultured three times.

2.4. Evaluation of cytotoxicity of the electrospun nanofiber

Cytotoxicity was measured to ensure that the nanofiber was non-toxic to the cells. Briefly, astrocytes were seeded into a 96-well plate (1.0×10^4 cells/well) and then incubated in a 5% CO_2 incubator at 37 °C for 24 h. The culture media was subsequently replaced with the test media, which was prepared by incubating the nanofiber in culture media for 24hr. After incubating the cells for 24 h, an MTT assay was carried out to measure the cytotoxicity.

2.5. Adhesion and viability

Astrocyte adhesion to the PCL film and nanofiber was evaluated by MTT assay. Briefly, rat primary astrocytes seeded on the nanofiber and film were incubated under 5% CO_2 at 37 °C for 2, 8 or 24 h, after which the media were removed and the nanofiber and film were rinsed once with PBS. The rinsed cells were then incubated for another hour with 500 μL of MTT solution. After removing the MTT solution, 1 ml of DMSO was added to each well to dissolve the formazan. The OD was then measured at 540 nm by ELISA (Tecan, Austria). For the viability assay, rat primary astrocytes seeded on the substrates were also cultured in a 5% CO_2 incubator at 37 °C, during which time the media was replaced every two days. After incubation for 1, 3 and 5 days, cell viability was evaluated by MTT assay.

2.6. Western blot

Astrocytes cultured on the PCL nanofiber and nanofiber film for five days were washed with PBS (pH 7.4) and then dissolved with RIPA lysis buffer. The total protein concentration was subsequently quantified via a BCA protein assay kit. The quantified protein was then subjected to SDS-PAGE, after which the separated proteins were transferred to a PVDF membrane and blocked with blocking buffer (10% skim milk in TBST) for 4 h. Next, the membrane was treated with rabbit anti-GFAP polyclonal IgG antibodies ab7260 (1:50,000, Abcam, USA) in TBST containing 10% skim milk for 4 h, and the secondary antibody (1:5000, goat anti-rabbit IgG-HRP, sc-2004, Santa Cruz Biotechnology, USA) was then applied. Each step in the antibody treatment was followed by washing with PBS three times for 5 min each.

2.7. Statistical analysis

All samples were tested three times to obtain the average OD ± the standard deviation. A *t*-test was then performed to identify significant differences among groups ($P < 0.05$).

3. Results

3.1. Characterization of PCL nanofiber and film

The nanofiber and film were fabricated as described above. As shown in Fig. 1A and B, the topography of the nanofiber mat differed significantly from that of the film. Specifically, the nanofiber mat was composed of nano-scale fibers, resulting in generation of a porous and rough structure, but the PCL film showed a smooth morphology with slight roughness. These topographic differences resulted in changes in the water drop contact angles between both surfaces, with PCL nanofiber and film showing contact angles of $132.52° \pm 4.10$ and $77.29° \pm 1.11$, respectively.

3.2. Cytotoxicity, adhesion and viability

After incubation with each PCL film and nanofiber, the media were exposed to the astrocytes for 24 h to determine their cytotoxicity. Astrocyte activity did not differ significantly among media immersed in the PCL nanofiber, film or a commercially proven tissue culture plate system (TCPS). Moreover, the difference between PCL film and PCL nanofiber was not statistically significant (Fig. 2A). The results of an MTT assay for cell adhesion are shown in Fig. 2B. The optical density (OD) of PCL nanofiber was higher than that of PCL film at 2 h. Additionally, the results of a *t*-test indicated that the astrocyte adhesion of the nanofiber and film differed significantly. The effect of nanoscale fiber on the viability of astrocytes was measured by MTT assay at 1, 3, and 5 days after the astrocytes