An In Vitro Model of Electrically Stimulated Tissue Culture: Design and Cytotoxicity Assay of a Conductive Nerve Guidance Channel

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Introduction

Transacted nerve can be bridged using a nerve graft that is either an autologous sensory nerve or vein. To avoid using autologous tissues, synthetic nerve guidance channels provide an option for the surgeons to bridge the severed nerves. Many different types of materials have been tested as nerve guidance channels, such as the Neurotube made of polyglycolic acid. In recent years, there is an increasing interest in the stimulation effect of weak direct electric field on the growth of nerve cells both in vitro and in vivo [1,2]. In vitro electrical stimulation (ES) in literatures normally uses neurons. In vivo ES is however applied to the nerve tissue that has complex structures and contains multiple cell types. This work is to build an in vitro model to provide localized electrical stimulation to specimen tissues such as axon. To do this an electrically conductive guidance channel was fabricated with conductive textile. This channel was integrated into a standard cell culture Petri dish and connected to an external power source to form an in vitro tissue culture model. This model was tested for the cytotoxicity of the conductive textile following ES.

Materials and Methods

Poly(ethylene terephthalate) (PET) fabric was cut into specimens of 2 cm × 4 cm in size. The specimens were washed firstly in isopropanol (99.5%) and then in ethanol (100%) for 30 min each. This washing procedure was repeated to remove finishing chemicals and contaminants on the textile. After, the specimens were put into a 5% sulfuric acid (H_2SO_4) water solution for 30 minutes and then washed with distilled water for 10 minutes. Then, the specimens were transferred into a methanol/water (50/50 vol/vol) solution, to which pyrrole monomers (10%, w/v) was added. The specimens were kept in the solution for about 30 min. Then, the specimens were bathed in ferric chloride ($FeCl_3$, 10% w/v) solution of methanol in water (50:50) till chemical reaction completed. After being thoroughly washed in distilled water, the PPy-coated fabric specimens were dried in atmosphere and kept for the following experiments. Light and scanning electron microscopes were used to observe the surface morphology of the PPy-coated fabric.

The conductivity of the PPy-coated fabrics was measured using a standard four-point method to confirm its conductivity. They were then cut into appropriate size and sutured with a 5-0 polypropylene suture into tubular nerve guidance channels that was 10mm long and 1.5mm inner diameter. Each end of the tube has an extension of the fabric to connect to an external electrical circulation through a small piece of platinum (Pt) film. In this way the nerve guidance tube was integrated into an electrical circuit.

For the cytotoxicity assay, a nerve guidance channels was placed in a Petri-dish and incubated for 24h in culture medium at an electrical potential of 1.6V DC (160mV/mm). Five guidance channels were tested. At the end of 24h, their culture medium was collected and used to culture human skin fibroblasts seeded in 96-well plate at 5000 cells per well for 48h without changing the medium. This cell culture experiment was repeated 6 times for each sample medium. MTT assay was performed at the end of the culture to measure cell viability. Cells cultured in normal culture medium were used as controls.

Results

The surface of the fabrics was very smooth, showing the uniform coating of the PPy on the microfibers, without blocking the inter-microfibre space. Figure 1 shows the light microscope picture

of the PPy-coated PET fabric and its control. The resistivity of the conducting fabrics was measured and found in the order of 10^3 ohm/square.

The configuration of the in vitro culture model is showed in Figure 2. The tubular channel was found work properly as expected. A special design at the interface of the conductive fabric and the Pt film prevented the contact of Pt to culture medium, thus eliminating the possibility of generating and releasing any electrochemical reaction products into the culture medium.

As shown in Figure 3, the medium collected from the Petri dishes where the guidance channels were incubated under ES generated average MTT values of 0.26, compared to 0.24 of the control. Because there is no significant difference (p>0.05), it is clear that the culture media conditioned by the electrically stimulated conductive fabrics did not impose any cytotoxic effect to the cultures.

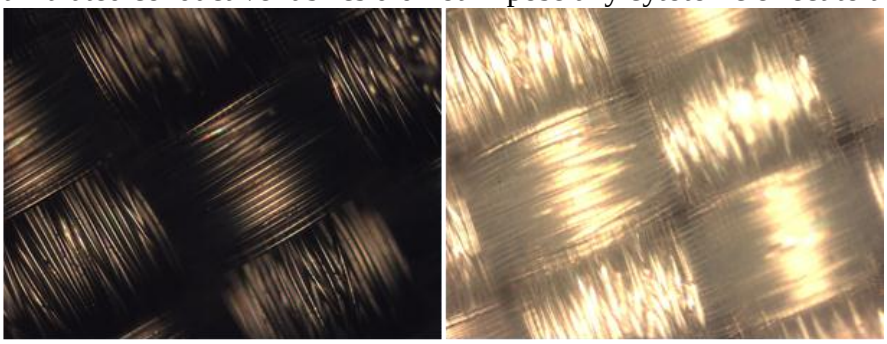

Figure 1. Surface of the PPy-coated PET fabric (black) and the original PET fabric.

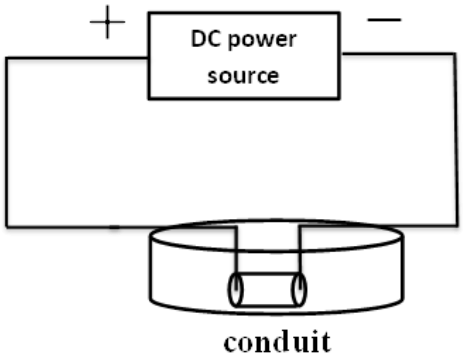

Figure 2. Schematic illustration of the in vitro culture model, showing how the nerve guidance channel is integrated into culture plate and electrical circuit.

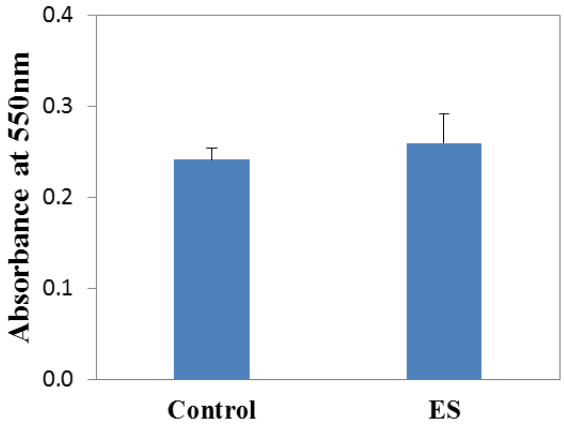

Figure 3. Fibroblast viability after 24h of culture, showing no cytotoxic effect of the conductive textile conditioned culture media.

Discussion

PPy was successfully polymerized onto PET fabrics. The PPy-coated fabrics showed uniform coating of PPy and similar handling property as the virgin fabrics. The electrical resistivity of the conductive fabrics was in the range of semi-conductor and sufficient to mediate ES to cultured cells. This in vitro model was tested and did not show cytotoxic effect on the growth of fibroblasts. This new culture method could be used for the electrically stimulated culture of sciatic nerve or other tissues.

References:

1. Borgens, R.B. J. Neurosci, 1999, 91:251-64.
2. McCaig, C.D. BioEssays, 1997, 19: 819-826.