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Exogenous Nurr1 gene expression in electrically-stimulated human MSCs and the induction of neurogenesis

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ABSTRACT

In this study, synergistic effects of electrical stimulation and exogenous Nurr1 gene expression were examined to induce the differentiation of human mesenchymal stem cells (hMSCs) into nerve cells in *in vitro* culture system. A two-step procedure was designed to evaluate the effects of electrical stimulus and exogenous gene delivery for inducing neurogenesis. First, an electrical stimulation device was designed using gold nanoparticles adsorbed to the surface of a cover glass. Gold nanoparticles, as an electrical conductor for stem cells, are well-defined particles adsorbed to a polyethyleneimine (PEI)-coated cover glass. The nanoparticle morphology was examined by scanning electron microscope (SEM). Second, a plasmid carrying Nurr1 cDNA was complexed with biodegradable poly-(DL)-lactic-co-glycolic acid (PLGA) nanoparticles to support neurogenesis. To evaluate the neuronal differentiation of stem cells mediated by the treatment with either electrical stimulation and exogenous Nurr1 gene delivery, or both, the expression of neuron-specific genes and proteins was examined by RT-PCR and Western blotting. Cells transfected with exogenous Nurr1 genes plus electrical stimulation (250 mV for 1000 s) showed the greatest level of neurite outgrowth with a mean neurite length of 150 μm. Neurite length in cells treated with only one stimulus was not significant, approximately 10–20 μm. These results indicate that electrical stimulation and exogenous Nurr1 gene expression together may be adequate to induce nerve regeneration using stem cells.

1. Introduction

Stem cells have been used in clinical applications by transplantation into damaged organs or tissues. However, stem cell-based tissue regeneration has many limitations, including undesired differentiation of the stem cells into heterogeneous cells. Several approaches have been used to differentiate stem cells into the desired type of cells. In particular, the use of biological stimuli, such as drugs and proteins, has been explored extensively [1–3].

In nerve axonal regeneration, electrical stimulation has played a pivotal role in neurite extension and the regeneration of transected nerve ends, and several types of materials for electrical stimulation have been effective in nerve regeneration [4–7]. Nanoparticle (NP)-modified surfaces have been used to construct homogeneous films for use as an electrical conductor [8]. To construct such devices, negatively-charged particles are complexed with positively-charged surfaces through layer-by-layer (LBL) assembly for the stimulation of cultured cells [9–11]. The construction of NP films by the LBL method has been successfully used to stimulate neurite outgrowth in nerve regeneration [12–15]. Surfaces homogeneously coated with NP-based materials have enabled the spatially controlled construction of conductor layers [16]. Furthermore, both organic and inorganic NPs coated on conventional surfaces have been used as electrical conductors. Electrical charges appear to focus on the stimulation of axonal regeneration; therefore, many electrical stimulating materials have been evaluated to determine if they can be used in the development of effective nerve regeneration. Although the exact mechanisms by which electrical stimulation enhances nerve regeneration are not well understood, it is well known that electrical stimulation enhances both neurite outgrowth *in vitro* and nerve regeneration *in vivo* [9,15].

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