The differentiation of embryonic stem cells seeded on PCL/gelatin scaffolds accompanied by matrigel into dopaminergic neurons

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Abstract:
The repairing process in the nervous system is complicated and brings great challenges to researchers by which the regeneration capability of the human adult nervous system is often limited. In order to facilitate nerve regeneration, Nerve autografts are considered as the ‘golden standard’. However, the shortage of donor grafts, high medical expenses, immunological rejections, chances of disease transfer and the requirement of multiple surgeries are among the reasons that constrain the use of autografts. Tissue engineered scaffolds may serve as an alternative choice for implantation to facilitate neural repair in which Embryonic stem cells are cultured on the scaffolds in vitro and transferred to the body after cell growth and differentiation.

In the present study GPX1/Nurr1 over expressing ES cells were cultured on PCL/gelatin nano-fibers to facilitate the differentiation towards dopaminergic neurons for the treatment of Parkinson’s disease.

Keywords: Scaffold; Electrospinning; PCL/PLGA; Matrigel; Dopaminergic neurons

Introduction:
A common approach in tissue engineering is to mimic the architecture of the natural extracellular matrix (ECM). The three dimensional structure and the high porosity of the scaffolds facilitate cellular penetration resulting in efficient growth and differentiation of ES cells. The ECM plays an important role in regulating cellular behaviors by influencing cells with biochemical signals and topographical cues. By controlling scaffold morphology, architecture and components, suitable topographical and biochemical cues may be provided to promote tissue regeneration. The ECM is variable due to its tissue, so the physical and chemical properties of the scaffolds can be tailored based on applications and the physiology and chemical structure of the specific ECM. A close imitation of the ECM will provide a more conducive environment for cellular functions ranging from adhesion, migration, proliferation to differentiation.

differentiation towards a specific cell line is facilitated, on the other hand it can facilitate grafting and the biodegradable and biocompatible materials that are used reduce the occurrence of immune responses.

Parkinson’s disease is a neurodegenerative disorder associated with the relatively selective loss of nigro-striatal dopaminergic neurons and a reduction in striatal dopamine. The most obvious symptoms are movement-related, including shaking, rigidity, bradykinesia and difficulty with walking. The pharmacological treatment using the dopamine precursor levodopa and other drugs has been demonstrated however, levodopa may hasten the occurrence of dyskinesia, however cell transplantation to replace lost dopaminergic neurons is a new approach

The Nuclear receptor related 1 protein (NURR1) is a member of the nuclear receptor family of intracellular transcription factors. NURR1 plays a key role in the maintenance of the dopaminergic system of the brain which is a gene essential for dopamine neurons differentiation, maintenance and dopamine secretion. Glutathione peroxidase 1 is an enzyme that in humans is...
encoded by the GPX1 gene, Glutathione peroxidase functions in the detoxification of hydrogen peroxide, and is one of the most important antioxidant enzymes in humans which protects dopaminergic neurons against oxidative stress which is associated with Parkinson’s disease.

**Experimental (Modeling):**

Two fiber-fabrication methods have been explored in the field of nerve regeneration: electrospinning and self-assembly. Electrospinning produces fibers with diameters ranging from several micrometers to hundreds of nanometers, self-assembly fibers have diameters of tens of nanometers, the method used in the present study was electrospinning. In this method nanofibers were fabricated via electrospinning using PCL (Sigma–Aldrich, St. Louis, MO) and a specific percentage of gelatin with a ratio of 70:30 and were prepared for cell culture. The gelatin used increased the biodegradability of the scaffolds. The electrospinning setup and the collector used for fabricating and collecting aligned nanofibers consists of five components: syringe pump, syringe, needle, high voltage generator, and collector. The polymer solution used for electrospinning contained 10% PCL (w/v) in a solvent of Hexafluoroisopropanol (HFIP) that were electrospun with a voltage of 14 kv and a flow rate of 1 mL/h.

The ES cells were infected with Lentiviruses carrying NURR1/GPX1 resulting in an overexpression of the two genes. Following that the cells were cultured on the scaffolds and the differentiation towards dopaminergic neurons was promoted using the Mckay standard protocol (Mckay, 2000). Matrigel was also used as a topping coat on the cells to help increase cellular adhesion and differentiation.

**Results and discussion:**
PCL/gelatin nanofibers were electrospun in an aligned manner to promote and guide neurite outgrowth.

The standard protocol for differentiation takes 21 days, after the 21 day period the dopaminergic neuron generation was examined with semi-quantitative Realtime PCR. Levels of neural mRNAs at different stages of ES cell culture was compared to that in the undifferentiated ES cells, the analysis showed high expression of neural mRNAs as an influence of the nanofibrous scaffolds. The production of dopamine is a definitive measure of the identity of a dopaminergic neuron. Reverse-phase high-performance liquid chromatography (RP-HPLC) was used to measure dopamine release directly indicating differentiation. Performing Immunocytochemistry showed the expression of two specific proteins TH and map2 confirming the differentiation of ES cells into dopaminergic neurons.
Conclusions:
Nanofibrous scaffolds can serve as powerful tools in the tissue regeneration processes. The fibrous structure closely resembles the natural environment that cells grow in and provides appropriate physical cues for manipulating cellular function and inducing dopaminergic differentiation. Electrospinning is a versatile and economical way of mass-producing fibrous constructs. It also enables incorporation of biochemicals to provide a synergistic effect in promoting nerve regeneration.

References:


