Evaluation of Human Mesenchymal Stem Cells Differentiation to Neural Cells on Polycaprolactone Nanofiber Scaffolds

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Abstract

Differentiation of human mesenchymal stem cells (hMSC) to neural cells on Nano-scaffolds is a promising method for the treatment of the damaged nervous system through bionanomaterial-cell transplantation. The hMSC’s multipotential features have been discovered in various tissue engineering researches. This investigation shows the in-vitro development and neural differentiation of hMSC in 3D and 2D environments. The 3D environment which used in this study is nanofibrous polycaprolactone (PCL). The differentiation potential of mesenchymal stem cells (MSCs) to neural cells, on the random polycaprolactone (PCL) nanofibrous scaffolds, and tissue plate was examined. Researches have proved that interaction of extracellular nanofibrous matrix with in-vivo cells, gives mechanical maintenance to the cells and plays a functional role in the control of cellular behaviour. Stem cells are developing as a fundamental tool in the evolution of tissue engineering and regenerative medicine. PCL characterization was determined employing scanning electron microscopy (SEM). Agents like, retinoic acid, epidermal growth factor (EGF), fibroblast growth factor (FGF-2), and Ibmx, which they are neural inducing agents, added in DMDM/F12 to differentiate MSCs to neural cells. Reproduction of mesenchymal cells on PCL nanofibrous scaffolds and neural morphology revealed through a scanning electron microscope (SEM) and optical microscope outcomes. The differentiated mesenchymal cells on nanofibrous scaffolds express neural gene markers including; β-tubulin III and Map2 on the day of 14. Our investigation recommends the potential usage of differentiated neural cells from hMSCs on Nano-scaffolds toward the improvement of neural cells. This study conducted in 2011.

Keywords: Human Mesenchymal Stem Cells, Neural Cells, Polycaprolactone Scaffolds, Tissue Engineering, Cell Therapy

1. Background

Repairing the neural damages employing nerve tissue engineering is one of the most promising approaches. In neural tissue engineering, nano-scaffolds use as an extracellular matrix and their efficacy has proved by various researches. Directing role of biomaterial substrates in the differentiation of mesenchymal stem cells (MSCs) has been proven (1, 2). MSCs obtained of bone marrow (BM) are non-hematopoietic stem cells, containing the ability to differentiate into various ectodermal (neural), mesenchymal (adipocyte, chondrocytes), and endodermal (hepatocytes) tissue cells (3). MSC is one of the accessible cell origins of the body, containing the most simple clinical technique of culturing with a high self-regeneration role (4). New researches have demonstrated the capability usages of MSCs obtained of bone marrow, which differentiated on the nano-scaffolds, to tissue renewal (5). Osteoblasts or chondrocytes, which differentiated from MSCs on the 3D nanofiber matrix, were evaluated in the various studies (6, 7). Differentiation of MSCs on the electrosynp nanofiber scaffolds has considered in many studies. Scaffolds for tissue regeneration are essential as they provide suitable requirements for cell resistance regeneration and differentiation and tissues’ expansion for desired tissue engineering goals (8). Contemporary researches have aimed to build and develop suitable scaffold for tissue reconstruction (9). This examination evaluated the efficacy of polycaprolactone (PCL) nanofiber matrices produced via the phase separation technique as a scaffold for neural tissue reforma- tion (10). PCL presented as a biomaterial for medicine and drug delivery method (11, 12). Furthermore, PCL is a fabricated environment-friendly and non-toxic polymer that has considered a biomaterial for nerve tissue engineering as its convenient form appearance. Although, its low hydrophilicity regularly performs in limited cell adhesion on scaffolds.
An admirable scaffold must produce a strong bond for the cell to attach. Therefore, in this research, the scaffolds were coated by oxygen plasma to improve hydrophilic properties. PCL produces by electrospinning which is a technique used to fabricate polymeric nanofibers applying electrostatic power (13). The hydrophilicity of polycaprolactone (PCL) was improved using O$_2$ plasma treatment. Physicochemical and mechanical properties of random PCL nanofibers scaffolds were evaluated by determining tensile strength and contact angle using a scanning electron microscope (SEM) (14).

2. Objectives

This investigation considers the potential application of MSCs for nerve generation on plasma treated polycaprolactone (P-PCL) nanofibrous scaffolds as a system to improve the neural differentiation leading to neural tissue engineering. In this study, neural gene expression, involving β-Tubulin III and Map2, were examined by immunocytochemistry aim to detect the differentiation of MSCs to neural cells (15).

3. Methods

In this study, Stem Cell Technology Research Center (Tehran- Iran) provided us with human mesenchymal stem cells (hMSCs). Dulbecco’s Modified Eagle’s Medium (DMEM), retinoic acid, EDTA growth factor (EGF), trypsin, Fetal bovine serum (FBS), antibiotics, basic-fibroblast growth factor (FGF) were ordered from Sigma (Sigma-Aldrich, USA). Also, chloroform as well as Polycaprolactone with 8000 molecular weight, and dimethyl formaldehyde (DMF) were ordered from Sigma-Aldrich too.

3.1. Electrospinning of Nanofibers

Chloroform /DMF (9:1), with PCL (8 wt %) which dissolved in is required to perform the electrospinning process. Polymer-solution was loaded to a syringe with needle 21G. A syringe pump was used to feed the polymer solution to the needle tip with a flow speed of 0.5 ml per hour. A high voltage power supply was used to provide the needle with a 25 kV positive voltage. To achieve random nanofibers, a rotating disk with 100 rpm linear rate was applied. The collector was placed in 23 cm from the needle tip. In high-level voltages, Taylor cone was formed, and an electrically jet of the melt (polymer solution) was spatetered on the collector. Before using, the nano scaffold was dried overnight.

3.2. Surface Change of Nanofibers

Plasma modification on PCL nanofibrous was conducted employing a plasma cleaning equipment (Germany). Nano-scaffold was located on the chamber of the plasma cleaner. Radiofrequency with electricity power of 30w, following vacuum mode, was applied to plasma va-cate.

3.3. In Vitro Culture of Mesenchymal Stem Cell

Eagle’s medium (DMEM), which contains antibiotics (6% penicillin/streptomycin ), antifungal (amphotericin-B), and 10% fetal bovine serum (FBS), is a suitable medium to keep mesenchymal stem cells, which were taken from Stem Cell Technology Research Center (Tehran Iran).

Cells nourishing with a new medium every three days, and they were kept in a humid incubator at 37. Trypsin was used to isolate cells from the bottom of the plate before feeding. Neubauer lam was employed to enumerate the cells. For culture MSCs on the scaffold, the cells in the second passage were employed.

3.4. Morphology of Mesenchymal Stem Cell

After culturing the MSCs on tissue plate, the MSCs were isolated from the bottom of the culture plate using trypsin and were cultured on P-PCL nano scaffolds. After seven days of cell feeding, both the MSCs cultured on the scaffolds and the culture plate were prepared for SEM and Optical images. The morphology investigation was conducted for in vitro cultured mesenchymal stem cells on P-PCL scaffold and the plate. PBS was used to rinse the scaffold for twice, as well as, 2.5% glutaraldehyde was used to fix the scaffold for three hours. Scaffold was then dried in 15 minutes with different ethanol concentrations (60%, 70%, 80%, 90%, and 100%). Eventually, the scaffold was coated by gold, and then scrutinized with SEM.

3.5. Neural Induction of MSCs

To neural inducing, human mesenchymal stem cells (hMSCs) developed on electrospun nanofibrous and the plate were induced with substrates, including EGF (10 ng/ml), bFGF (10 ng/ml), retinoic acid (0.5 Mm), and IBMX (0.5 Mm) for 14 days.

3.6. Immunocytochemistry

Induced hMSCs, which were developed on electrospun P-PCL nanofibrous, and also in the plate, were prepared for immunocytochemistry analyses. In the first step, 4% paraformaldehyde was ordered from Sigma-Aldrich, and was used for 20 minutes in 4°C to fix the cells. PBS was used
to wash the cells. As well as, Trion-x100 was used for five minutes to block the cells. Then, 5% goat serum was added to the wells containing cells for 45 minutes. In the second step, Primary antibody, anti (1: 500, Bioscience) was added to wells at 4°C overnight. As well as, primary antibody, β-Tubulin (205: 200, Bioscience), was further conducted. In the third step, PBS was applied to rinse the cell-scaffold, and anti-mouse Fluorescein Isothiocyanate secondary antibody (FITC, 1: 500, Sigma) was added to the wells for three hours. Additionally, 4,6-diamidino-2-phenylindole (DAPI 1: 10000 in PBS, Invitrogen, USA) was used for 1 minute to stain the nuclear. The final wash was done with PBS before imagine with the fluorescence microscope (Nikon, Germany). For negative controls of FITC, we considered a cultured cell without primary antibody incubations.

4. Results

During this research, SEM micrograph of electrospun nanofibrous scaffold exhibited beadles, porous nano-scale fibrous construction that developed following suitable spinning situation. Figure 1 shows P-PCL nanofiber scaffold which formed by electrospinning process (Figure 1).

In terms of hydrophilicity, the study of PCL and plasma-treated PCL (p-PCL) nanofibers contact angle exhibited substantial alteration on the surface characteristics of PCL nanofibers. The fiber diameters of PCL nanofibers with a scale of 400-1500 nm were achieved. After plasma treatment, SEM pictures did not reveal any differences in the surface morphology of PCL nanofiber. Investigations of Contact angle in PCL and P-PCL nanofibers scaffolds disclosed the hydrophilic surface characteristics. These scaffolds were highly hydrophilic and nonabsorbent to water with a contact angle of 134°. The PCL treatment with plasma (P-PCL) nanofibrous scaffolds was highly hydrophilic, giving 100% humidity by the water droplet, and with contact angle less than 80°, implying the appearance of the hydrophilic scaffold’s surface. Differentiation between PCL and P-PCL random nanofibrous scaffolds' fiber characteristics has been shown in Table 1. The random P-PCL nanofibrous scaffold presented a decrease in mechanical toughness. The random P-PCL nanofibrous scaffold displayed a reduction in mechanical toughness. It is suggested that the hydrophilicity of P-PCL scaffold lowered their mechanical toughness. tensile strength of PCL and P-PCL nanofibers has shown in Table 2.

Table 1. Differentiation Between PCL and P-PCL Nanofibers

| Properties      | PCL | P-PCL |
|-----------------|-----|-------|
| Porosity        | 99  | 99    |
| Contact angle   | 134 | < 80  |
| Wettability     | Highly hydrophilic | Highly hydrophilic |

Table 2. Tensile Strength in Poly Caprolactone (PCL) and Plasma-Polyacaprolactone (P-PCL) Nanofibers

| Nanofiber Scaffolds | Tensile Stress | Tensile Strain |
|---------------------|---------------|---------------|
| Random PCL          | 1.85          | 363.79        |
| Random P-PCL        | 1.68          | 247.39        |

4.1. Morphological Studies of Mesenchymal Stem Cells

Figure 2 reveals typical morphology of culture mesenchymal stem cells on plate (2D environment) and P-PCL scaffolds (3D environment) after one day of cell culture. The image of MSCs cultured on the plate was taken by an optical microscope, and the image of MSCs cultured on the nano scaffold was taken by an electron microscope. Mesenchymal stem cells have a flat fibroblast-like form. Figure 3 shows a typical morphology of neural cells differentiated from MSCs using neural induction medium. Differentiation of MSCs was conducted on both 2D environment (plate) and 3D environment (PCL nano scaffold).

4.2. Immunostaining of Cultured Scaffolds

Figure 4 shows the Immunocytochemistry outcomes of differentiated MSCs on the P-PCL nano scaffold and also
on plate. Differentiated MSCs were immune stained to detect \( \beta \)-tubulin III and Map-2 genes which they are neural gene markers. Expression of \( \beta \)-tubulin III and Map-2 genes were observed in cytoplasm and nucleus of neural cells differentiated from MSCs on the P-PCL scaffold. Also, the expression of \( \beta \)-tubulin III and Map-2 genes were observed in cytoplasm and nucleus of neural cells differentiated from MSCs on the plate. These neural gene markers were not observed in the control culture containing non-differentiated MSCs. The bulk of differentiated MSCs displays bipolar configuration with two elongated neurites. The differentiated MSCs on the random nanofibers were determined with multiple processes. These outcomes recommend that random nanofibers are not able of managing the orientation of neurons.
5. Discussion

Stem cell therapy is a promising strategy for the healing of the injured CNS. MSCs have been examined in many studies aimed at stem cell-based transplantation in restoring damaged spinal cord (16, 17) because of easy access, low immunogenicity, paracrine (18, 19) and immune-modulatory effects (20). MSCs of bone marrow operate an essential function in tissue reconstruction. Though different from embryonic stem cells (ESCs) in some features, MSCs have self-renewing ability and multilineage differentiation but are free from ethical concerns and tumorigenesis danger (21). Consequently, these cells are more suitable than other cell references for medical usages. Illustrating the impacts of mesenchymal stem cells on peripheral nerve rehabilitation have been recommended by numerous desirable mechanisms. Generally, mesenchymal stem cells build a desirable condition for nerve rehabilitation. They influence nerve reconstruction development by cell transplantation induction, growth factors production, construction of extracellular matrix, anti-inflammatory release as well as immune system regulators (22). Mesenchymal stem cells are recommended to differentiate into bone, cartilage, cardiac myocytes, and neural cultures (23, 24). The neural differentiation of MSCs was notified as a method of cell therapy.

A biocompatible scaffold, useful cell source, and suitable biochemical circumstances are essential for improving biological replacements that can repair, sustain, or enhance tissue function (5). The idea of employing the MSC-bio nanomaterial method for transplantation in nervous system damage was examined (25, 26). This method includes the transplantation of electrospun polymeric nanofibrous scaffolds, including differentiated neuronal cell from MSCs. Electrospun nanofibers structures give construction resistance, architectural direction and facilities in cell connection to reply desirable response to the in-vivo alterations to improve the ECM (27). The P-PCL nanodimensional scaffold, which mimics the ECM, can improve tissue reform in vitro comparable to how the original
In this research we determined that the P-PCL nanofibrous scaffold was capable of supporting neural differentiation of MSCs. The neuronal cells were differentiated and induced to exhibit multipolar elongation and β-tubulin and Map2 gene expression, two standard gene markers of neural cells.

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Footnotes

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