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Electrospun Poly Caprolactone-Carbon Nanotube Scaffold for Nerve Regeneration in Dental Tissue Engineeringg

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ABSTRACT

Regeneration and engineering of functional new tissues containing the neural network have great importance. Progression of neural network into the dental tissue has a crucial role in dental tissue regeneration. In the present study polymer-ceramic blended scaffolds containing different weight percentages of carbon nanotube in poly caprolactone nanofiber matrix were fabricated. Morphological, mechanical and electrical properties of the prepared scaffolds have been characterized. Results showed that the sample containing 5 weight % of carbon nanotube had the smallest mean fiber diameter (50 - 300 nm) and the highest mechanical behavior. Also, its electrical conductivity was suitable to be used in nerve tissue scaffolds. mical properties as scaffold for neural tissue engineering. The static culture of the Schwann cells on the prepared scaffolds indicated that increasing weight percentage of carbon nanotube into the polycaprolactone matrix up to the 5 wt. % enhanced cell viability.

Keywords: Nerve Tissue Engineering; Carbon Nano Tube; Polycaprolactone; Nano Fiber; Schwann Cells; Characterization

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INTRODUCTION

Nerve autografting is the most common surgical procedure currently used to repair facial nerve gaps caused by car accidents or tumor resectioning [1–5]. However, there are several drawbacks to this procedure, including loss of sensation and causalgia of the donor region, and the patient must wait for a long period of time till the muscles contract. Although recent studies have proven the effectiveness of tabulation as an alternative treatment procedure for peripheral nerve gaps, 6,7 this strategies still requires better approach to promote nerve regeneration and decreasing recovery time to improve the patient's quality of life. There is some proposed root to regenerate nerve structure in dental tissue engineering, but using dental pulp stem cells provides many benefits and better confluence than many traditional procedures. Transplanted dental pulp cells would promote peripheral nerve regeneration [8].

Regeneration of the disrupted human nervous system from disease or trauma is a challenge for stem cell-based therapeutic paradigms [9]. Thus, the goal of neuroregeneration may entail a complex process of neuroprotection, immunomodulation of inflammation or gliogenesis, neuroplasticity, and neurogenesis. Understanding the underlying cellular and molecular mechanisms that may facilitate this complex process is paramount to achieve an improvement in neurological function [10].

Regenerative pulpal therapy and dentinogenesis may have concurrent beneficial effects on nerve regeneration. It should be noted that progression of neural network into the dental structure have crucial role in dental tissue engineering. The field of stem cell-based regenerative dentistry is complex and multidisciplinary by nature. Progress will depend on the collaboration between clinicians and researchers from diverse fields (e.g., biomaterials, stem cell biology, endodontics) working together toward the goal of developing biological approaches to regenerate dental and craniofacial tissues. Human adult dental pulp stem cells reside within the perivascular niche of dental pulp and are thought to originate from migrating cranial neural crest cells. During embryonic development, CNC cells differentiate into a wide variety of cell types, including neurons of the peripheral nervous system [11]. Scaffolding in nerve tissue engineering play an important role for nerve cells type function. It has been shown that Adhesion, proliferation, migration and cell-cell interaction depend strongly on the cell's template. Among different scaffold preparing process electrospining gained much attention because of many benefits [12].

Electrospinning is being used to an increasing extent to produce ultra-thin fibers from a wide range of polymer materials[12] high surface area to volume ratio helps especially neural cell type to regenerate and spread on the structure to regenerate and proliferate13.

In this research nanofiber scaffolds containing different wt. % of carbon nanotube (CNT) agent in polycaprolactone matrix were fabricated and characterized. Characterization and conductivity measurement of the prepared structures have been carried out to evaluate their nerve regeneration potential in dental matrix.

MATERIAL AND METHODS

Materials

In this research poly caprolactone was purchased from Sigma-Aldrich Company, multi wall carbon nanotube powder was purchased from Iranian chemistry engineering institute, N,N dimethyl formamide as solvent prepared form Merck chemical company, Dulbecco's modified Engel Medium(DMEM) and Penicillin-streptomycin antibiotic, fetal bovine serum, lysozyme and trypsin enzyme also had been used and purchased from Biowest Company. All of the chemical reagents were in analytical grade.

Scaffold preparation

At first, in order to dissolve carbon nanotube powder in polycaprolactone solution, surface modification process of the powder was carried out [14]. Briefly specific amount of carbon nanotube was washed in a 5 M solution of nitric acid and 5 M solution of sulfuric acid was added and the obtained solution was palced for 2 h in ultrasonic bath to create carboxyl group on the surface of the CNTs. Different wt. % of CNT (3, 4, 5 and 7) was dissolved in the solution containing 13 wt. % of polycaprolactone in N,N dimethyl formamide and the final solutions were used for electrospining in acceleration voltage of 15 KV, solution feeding rate of 2 milliliter per hour and 10 cm distance between syringe nozzle and collector. Finally the fabricated Electrospun membrane structures were used for cellular and physical characterization.

Scanning electron microscopy

Surface morphology and microstructure of scaffolds were investigated by means of scanning electron microscope (S-3200N, Hitachi, Japan). Four different samples of electrospun polycaprolactone-CNT were coated in a vacuum chamber with a gold layer for 200 seconds using a sputter coater (Desk-II, Denton Vacuum Inc.) at 20 or 15 kV [15]. To evaluate fiber diameter distribution of electrospun membrane, the image analysis software was used.

Electrical conductivity measurement

Conductance of the samples was measured using a two-point method in accordance with ASTM 4496-04. An Oltronix D400-007D voltage supply was used to create a voltage, and the current through the sample was measured with a 602 Solid State Electrometer (Keithley Instruments).

Mechanical characterization test

The mechanical properties of the samples were measured using an Instron 0091 device. Samples were cut in rectangular shape and were fixed in the mechanical testing machine. 2 $mm.min^{-1}$ strain rate of compression was selected and a force-displacement curve was obtained for each sample [17].

Cell culture and MTT assay

At first, cells were seeded on to 96 well plates at a density of 1 × 104 cells per well and were incubated under standard culturing conditions.

Schwann cell was purchased from Pasteur institute of Iran. Samples with different CNT content were cultured by Schwann cells. Cells were used for culturing on the scaffolds after 3th passage. The cell viability of the cell-seeded scaffolds was measured using MTT (3-(4, 5dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) assay. The cells were incubated on the scaffolds for 72 h. After the incubation, the scaffolds were removed and the media containing 10 % of MTT solution was added. Then, the plates were incubated at 37 °C for 4 h. the medium was then removed and 100 µl of solubilization buffer (Triton-X 100, 0.1N HCl and isopropanol) were added to each well to dissolve the formazan crystals. The absorbance of the lysate was measured in a microplate reader at a wavelength of 570 nm [18].

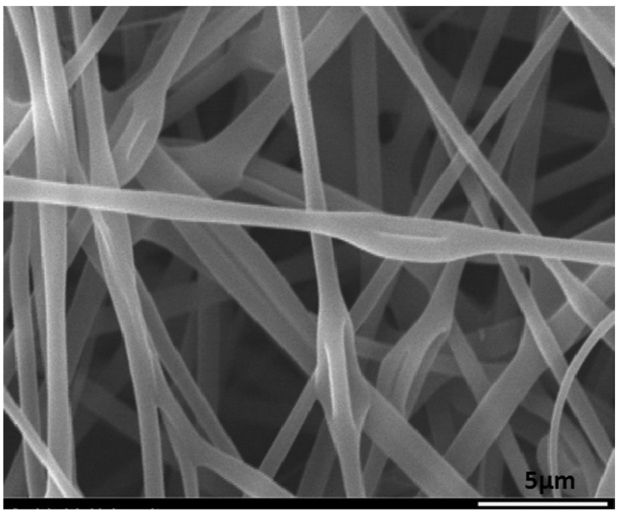
RESULTS AND DISCUSSION

SEM observations

As can be observed in Figure 1-4, SEM micrographs and mean diameter distribution of the prepared electrospun polycaprolactone-CNT are shown. Generally with increasing the CNT content of the samples the mean diameter of the obtained fiber has decreased that could be due to more charge carrier in the electrospun solution. But in the sample containing 7 wt. % of CNT, the mean fiber diameter has increased that it may be regarded to agglomeration of CNT particles which adversely has affected fiber diameter.

Electrical conductivity

Surface electrical conductivity of the prepared structures strongly depends on the fiber diameter (affects specific surface area) and the content of CNT particles. As it is shown in the Figure 5 with increasing in the CNT content up to the 4 wt. %, the surface electrical



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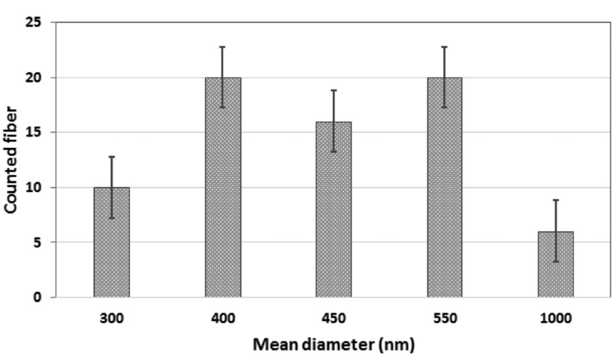

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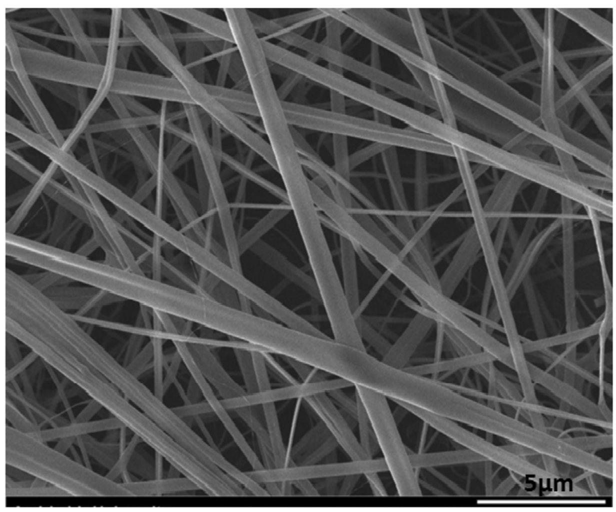
Figure 1. (A) SEM micrograph and (B) Fiber mean diameter distribution of the prepared sample with 3 wt. % of CNT.



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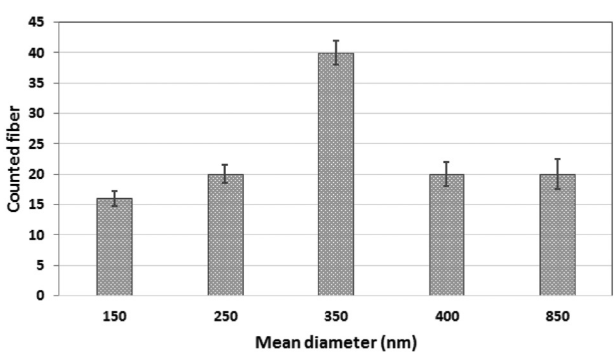

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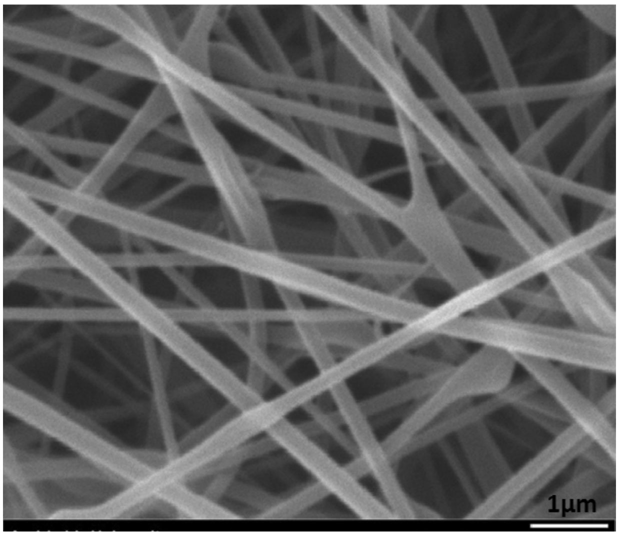
Figure 2. (A) SEM micrograph and (B) Fiber mean diameter distribution of the prepared sample with 4 wt. % of CNT.



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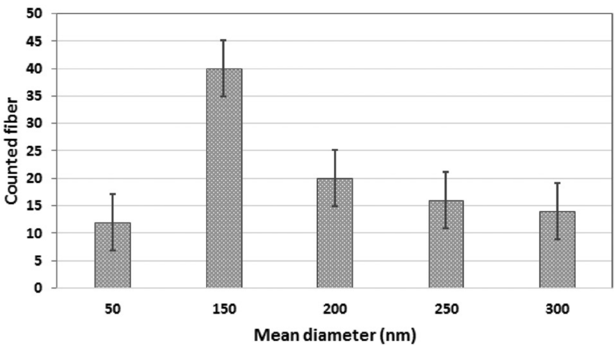

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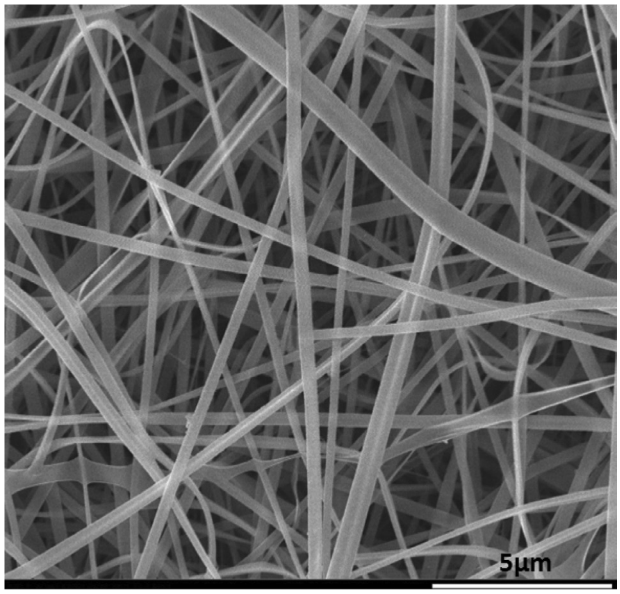
Figure 3. (A) SEM micrograph and (B) Fiber mean diameter distribution of the prepared sample with 5 wt. % of CNT.



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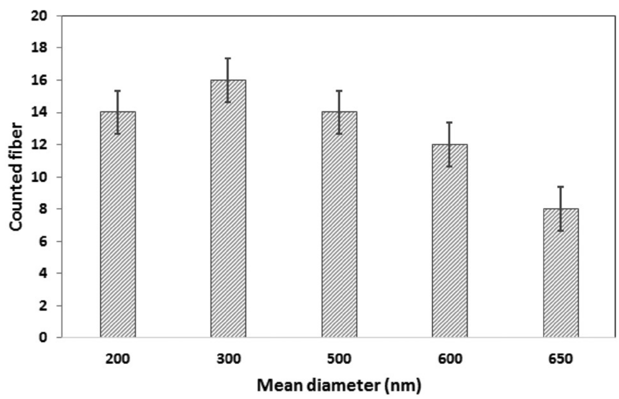

(B)

Figure 4. (A) SEM micrograph and (B) Fiber mean diameter distribution of the prepared sample with 7 wt. % of CNT.

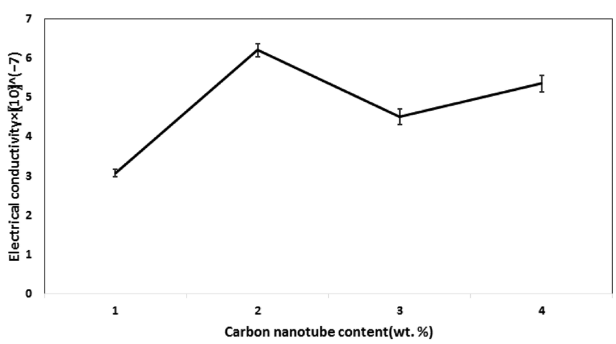

Figure 5. Electrical conductivity of the prepared scaffolds with different CNT content.

conductivity increases but more increase in the CNT content in the samples has decreased the conductivity. In fact due to increase in surface area of the nonconductive polycaprolactone fibers, the conductivity of the whole prepared structure decresed. But in sample with 7 wt. % of CNT, due to increase in charged particles (CNT) an increase in conductivity is observed.

Mechanical properties of the scaffolds

Mechanical behavior of the fabricated scaffolds after the tensile test is shown in Figure 6. The highest elongation (more than 300%) achieved in the sample with 3 wt. % of CNT content. The sample containing 4 wt. % of CNT had higher young modulus and lower tensile strength than the sample with 5 wt. % of CNT content. In the sample containing 7 wt. % of CNT, the rupture strain is very low and a brittle mechanical behavior is observable. In fact the strength of material has increased, but the elongation has decreased. In general, the sample with 5 wt. % of CNT content showed optimum mechanical properties than the other samples to be used in nerve tissue scaffolds.

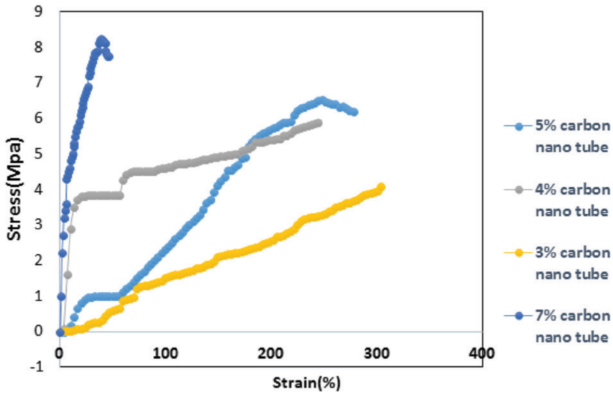

Figure 6. Stress-Strain curve of the prepared scaffolds.

Cell culture and MTT assay

In Figure 7 the MTT-assay results for the Schwann cells cultured on electrospun polycaprolactone-CNT samples is illustrated. Compared with the control sample, the viability of cells cultured on the prepared scaffolds was higher that indicated there are no significant toxic leachables in the prepared samples. These scaffolds have provided suitable situation for cell adhesion and proliferation. Although increase in CNT content more than 5 wt % has decreased the proliferation of the grown cells.

CONCLUSION

Tissue engineering in dentistry needs the development of nerve regeneration strategies to regenerate new tissues. In this study electrospun poly caprolactone nanofiber -CNT scaffolds were prepared and then were characterized. The results showed that the sample with 5 wt. % of CNT content had the optimum mechanical, morphological and biological properties. Finally, this research ascertained that the synthesized scaffolds are biocompatible materials for nerve tissue engineering.

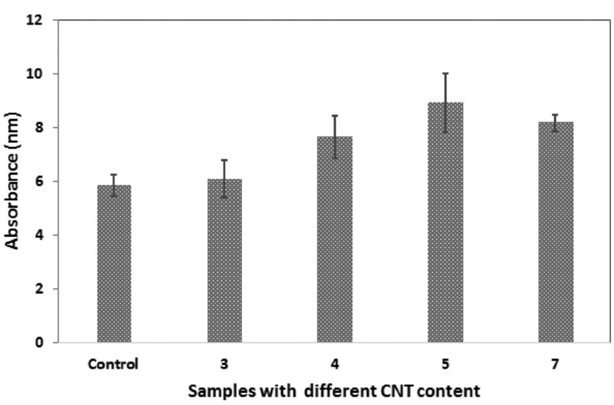

Figure 7. Cell proliferation of the Schwann cells grown on the prepared scaffolds and the control sample after incubation for 3 days.

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