Electrospinning of polymeric scaffold containing conductive nanoparticles

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ABSTRACT

Nervous system damage caused by physical trauma or degenerative diseases can result in loss of sensory and motor function for patients. Intrinsically conducting polymers nanoparticles have shown promise in tissue engineering, as they can be manipulated non-invasively, are easily functionalized, and can be used to mechanically and electrically stimulate cells. By combining different types of biomaterials (hydrogels, nanoparticles, electrospun fibers) and incorporating electrical conducting elements, materials can provide multiple physical and chemical cues to promote regeneration

The major objective of this work was to fabricate a conductive fibrous scaffold to imitate extra cellular matrix (ECM) for differentiation of neural stem cells. In this work, polypyrrole (PPy)/poly(vinyl alcohol) (PVA) conductive fibrous scaffolds were prepared via electrospinning. Using PVA/PPy conductive scaffolds with electrical stimulation can potentially improve cellular response and neural differentiation through mimicking the properties of native neural tissue. Electrical stimulation is another factor we have considered in this study. Presence of electrical current can be substantially effective in mimicking the electrochemical cues surrounding neural cells and differentiation of MSCs into the neural cell lines.

In this research the electrospinning properties of the scaffolds were optimised.. Morphology, porosity and fiber diameters of electrospun scaffolds were investigated. Chemistry of scaffolds were studied using Fourier transform infrared spectroscopy (FTIR. The prepared conductive PPy/PVA fibrous scaffolds showed suitable conductivity to deliver electrical signals.

OBJECTIVES

The compound mixture (Ppy-PVA) was used to build conductive nanofiber scaffolds in this study, resulting in a composite nanofiber scaffold with unique biological and physiochemical features for tissue engineering applications. Also, the effect of different percentages of electrically conductive polymer (polypyrrole) on the morphology, fiber diameter, and porosity was investigated. PVA was chosen as the nanofiber scaffold's backbones, while polypyrrole was used as a conductive polymer to fabricate a conductive composite with unique biological characteristics. Electrospun conductive nanofiber scaffolds with a unique composition of these different polymers were created for cell growth and proliferation.

MATERIALS & METHODS

Materials

Polyvinyl alcohol (MW=70000-85000, 99% hydrolyzed),Pyrrole monomer (0.97 g/cm3), ethanol (0.79 g/cm3), glutaraldehyde (GA, 0.50%), Anhydrous iron (III) chloride, also called ferric chloride ($FeCl_3$) 98%,sodium dodecyl sulphate ($C_{12}H_{25}NaO_4S$) were purchased from Sigma.

Synthesis of polypyrrole

Polypyrrole (PPy) was synthesized by chemical oxidative polymerization technique. The polymerization was carried out in a beaker with 100 ml distilled water by mixing certain amount of pyrrole monomer, oxidant and surfactant as shown in table 1. A given volume of pyrrole monomer was quickly added to the distilled water with the required amount of oxidant and surfactant; vigorously magnetic stirring was maintained to facilitate the pyrrole monomer dispersion. The polymerization reaction was carried out for 4h at temperature of 5°C .after prescribed time, PPy particles was filtered from solution with filter paper and washed with distilled water and ethanol several times. Finally, the PPy powder dried in an oven at about 40°C overnight.

Table 1: All materials used in PPy polymerization

Name	Designated	Concentration (M)
Pyrrole monomer	mPPy	0.05
Ferric Chloride (oxidant)	FeCl3	0.1
Sodium dodecyl sulphate (surfactant)	$C_{12}H_{25}NaO_4S$	0.0225

Electrospinnig of scaffolds

Electrospinning solutions were prepared according to the following procedures: PVA aqueous solutions (10% w/v) were prepared by dissolving PVA powder in distilled water at 80°C with constant stirring for 4 hours. For the preparation of PVA/Ppy electrospinning solution, firstly, PVA solution was prepared according to the above procedure. Then PPy was added to the solution with 1, 3, 5 wt% concentrations of the total polymer mass. At the end to obtain a homogeneous solution for the electrospinning process these mixtures were stirred for 24 hours.

The single nozzle electrospinning apparatus employed in this study was manufactured by Fanavaran NanoMeghyas Co. (Tehran, Iran). The prepared electrospinning solution was transferred to a 5ml syringe with a 0.8 mm OD needle and then placed on a syringe pump throughout the electrospinning process. The used voltage for preparing all samples was 9.3 KV and the distance between the needle tip and the collector was set to 14 cm. The fabricated nanofiber composites were collected in a rotating drum covered in an aluminum sheet. The rotational speed was set to 1800 revolutions per minute. The different flow rate was adjusted for various electrospinning solutions with different polypyrrole concentrations as shown in Table 2.

To prevent the dissolution of nanofibers in aquatic environment electrospun nanofibers were crosslinked via glutaraldehyde by placing them in a sealed desiccator saturated with glutaraldehyde vapor. On the bottom of the desiccator, a petri dish containing 20 mL of aqueous glutaraldehyde solution (50 percent v/v) was placed, while the nanofiber mats were placed on a mesh plate in the upper layer for 5 hours at 38°C. In the next step crosslinking continued for 38 hours at 55°C. To remove the glutaraldehyde vapor residues, the fibers were removed from the desiccator and rinsed with 1% wt/wt aqueous glycine for 45 min and rinsed with PBS.

Table 2: Electrospinning parameters.

Sample	Voltage (KV)	Flow Rate (ml/h)	Nozzle diameter (mm)	Needle-to-collector distance (cm)	Temperature(°C)	Rotation drum (rpm)
PVA	9.3	0.6	0.8	14	30	1800
PVA-1%Ppy	9.3	0.6	0.8	14	30	1800
PVA-3%Ppy	9.3	0.6	0.8	14	30	1800
PVA-5%Ppy	9.3	1	0.8	14	40	1800

Fourier transform infrared spectroscopy (FTIR) studies were carried out on compressed films containing KBr pellets and samples using a FTIR spectrophotometer.

The morphology and microstructure of the synthesized samples were evaluated using scanning electron microscopy (SEM). The average diameters of fibers and the porosity of various layers were calculated by ImageJ (US National Institute of Health, Bethesda, MD)

RESULTS

FT-IR analysis

The chemical characteristics of composite scaffolds and interactions between their elements were determined using Fourier-transform infrared spectroscopy (FTIR). Fig 1 shows FTIR result for Ppy. All spectra reveal the characteristic bands reported for Ppy. [1]. N–H bond stretching is related with the broad absorption band by 3440 cm^{-1}.The C=C stretching vibration bending bond in the pyrrole ring is responsible for the peaks at 1536 and 959 cm^{-1} [2]. The symmetric stretching vibration for the C—N bond is shown by the observed peaks at 1155 and 1439 cm^{-1}[2]. The peak at 2919 cm^{-1} and 2854 cm^{-1} could be attributed to C–H bonds in the backbone[10]. The FTIR spectrum of PPy shows the typical peaks that can be attributed to the C–H in-plane deformation vibration at 1032 cm^{-1}[3].

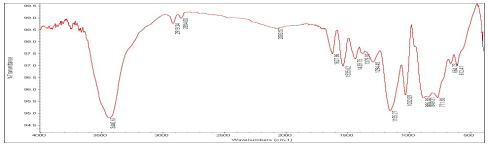

Figure 1: FT-IR result of Ppy

Morphology and porosity of nanofibrous scaffolds

Electrospun PVA and PVA/PPy scaffolds were observed by SEM in 25000 magnification. Fig 2 indicates SEM images of electrospun scaffolds after crosslinking. The fabricated fibers possess a uniform and cylindrical structure with no beads and As shown in Fig2 the electrospun fibers form a highly interconnected web, and the surfaces of fibers are relatively smooth.The fiber diameter distribution for PVA lies between 342-543 nm with an average diameter of 434±44 nm. The fiber diameter distribution for PVA-1% Ppy, PVA-3% Ppy, and PVA-5% Ppy, lies between 362 - 578nm, 374-647 nm, and 424 -752 nm with an average diameter of 447±41 nm, 460±51 nm, 550±72 nm respectively. The diameter of the fiber is determined by the surface tension, flow rate, and electrical conductivity of the solution, as previously demonstrated[4]. Fiber diameters of electrospun fiber matrices had a Gaussian type distribution. (see Figure 3)

The addition of polypyrrole conductive polymer increases both viscosity and electric charge of the solution. It is demonstrated that viscosity has a direct relation to fibers diameter, and when the viscosity is increased, the diameter is increased proportionally[5].The surface to volume ratio and porosity of the scaffolds are both reduced when the average diameter of nanofibers is increased.

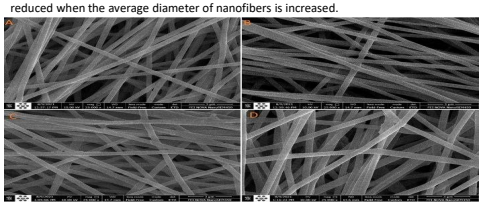

Figure 2: SEM images of Ppy/PVA fibrous scaffolds with different polypyrrole concentrations. A)PVA-0%Ppy B)PVA-1%Ppy C)PVA-3%Ppy D)PVA-5%Ppy

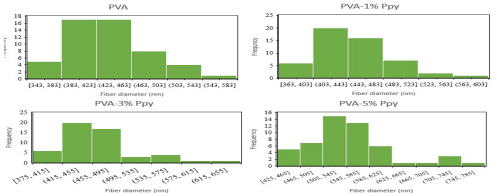

Figure 3: Fiber diameter distribution of electrospun fibers.

Analysis was performed on various layers of nanofibers by application of threshold (see Figure 4)For the calculation of porosity original images were converted to binary images and the porosity of each scaffold was calculated using the mean intensity of micrographs using equation 1

$$P = \left(1 - \frac{n}{N}\right) \times 100 \qquad (1)$$

Where n is the number of white pixels, N denotes the total number of pixels in a binary picture, and P denotes the binary image's porosity. Table 3 shows the results of porosity calculations on different binary pictures.

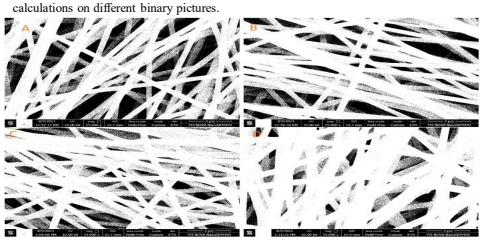

Figure 4 Various binary images for PVA and PVA/Ppy with different polypyrrole concentrations. A)PVA-0%Ppy B)PVA-1%Ppy C)PVA-3%Ppy D)PVA-5%Ppy

Table 3: Porosity measurement of binary images of PVA and PVA/Ppy with various concentration of Ppy

Sample	Magnification	Porosity (%)	Fibers diameters (nm)
PVA	25000	53%	434±44
PVA-1%Ppy	25000	48%	447±41
PVA-3%Ppy	25000	42%	460±51
PVA-5%Ppy	25000	36%	550±72

CONCLUSIONS

The conductive scaffolds developed in this work had distinct properties, making them a good fit for neuronal, vascular, or cardiac muscle tissue scaffolds with higher electrical signals. Furthermore, electrical signals can be effectively transferred due to scaffolds low electrical resistance which comes from the presence of PPy. Taken together, these electrospun nanofibers comprising of biodegradable polymers have the potential to be used in neural tissue engineering.

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