FABRICATION AND CHARACTERIZATION OF CONDUCTIVE SCAFFOLD BASED ON PVA/PPY

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ABSTRACT

Nanotechnology has a wide range of medical and industrial applications. Polypyrrole particles are one of the most promising types of conductive polymers for biomedical applications. Facile synthesis approaches, good electrical conductivity, ease of surface modification, made PPy attractive for use in tissue engineering(TE) applications. PPy have promising prospects in this field due to good conductivity, stability and biocompatibility characteristics and have been of great interest during the last few years.

Tissue engineering is an interdisciplinary field integrating engineering, material science and medical biology that aims to develop biological substitutes to repair, replace, retain, or enhance tissue and organ-level functions. Tissue engineering scaffolds with magnetic or conductive properties may conduct electric or magnetic signals and bring out synergetic promoting effect to cells growth. Fabrication and optimization of conductive scaffolds capable of inducing proper intercellular connections through electrical signals is critical for neural tissue engineering.

The aim of this study was the design of a 3D conductive biocompatible and biodegradable scaffold composed of poly(vinyl) alcohol (PVA)/polypyrrole (PPy) microparticles via hybrid method to investigate effect of electrical stimulation for neural tissue engineering. The scaffold was fabricated using a combination of gas foaming and freeze-drying processes. The physio-chemical properties of the PVA/PPy scaffolds were investigated by Fourier transform infrared spectroscopy (FT-IR), Scaffolds morphology observed by using scanning electron microscopy (SEM).

OBJECTIVES

In this study, we developed 3D porous scaffolds based on PVA and PPy using combination of gas-foaming and freeze-drying methods for nerve tissue engineering applications. From the best our knowledge, the current combination of materials and methods that used to produce porous conductive scaffolds applied for the first time. Polypyrrole particles was synthesis via chemical polymerization method. Mechanical stirring of PVA/PPy solution followed by freeze-drying was employed to fabricate porous conductive scaffolds with interconnected pores. Porosity, microstructure and chemical bonds of scaffolds were characterized.

MATERIALS & METHODS

PVA (average molecular weight of 70000 g/mol), Triton X-100, pyrrole monomer(mPPy) 98%, Anhydrous iron (III) chloride, also called ferric chloride ($FeCl_3$) 98%,sodium dodecyl sulphate ($C_{12}H_{25}NaO_4S$) and glutaraldehyde(crosslinking agent) were obtained from Sigma. The polymer and surfactants were used without further purification.

Synthesis of Polypyrrole

Polypyrrole (PPy) was synthesis 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

Fabrication of scaffolds

A 14%wt PVA solution was prepared by dissolving PVA in distilled water at 80°C under constant stirring until to reach a homogeneous solution. To fabricate the different samples, Triton X-100 and Polypyrrole powder were added to 5ml PVA solution in certain proportion(table 2) and mechanically agitated for 45sec with stirring speed 1000 rpm. Following the mechanical stirring, the generated foams immediately placed at -70°C for 24h. following freezing, the samples were freeze dried for 48h. Finally, prepared foams were exposed to glutaraldehyde vapor for 24h at 50°C in order to crosslinking reactions.

Table 2: Prepared Foams combination

Sample	PVA (ml)	Triton X-100 (ml)	PPy (g)
PVA	5	0.25	0
PVA/ PPy 1%	5	0.25	0.007
PVA/ PPy 3%	5	0.25	0.021
PVA/ PPy5%	5	0.25	0.035

FTIR spectra of synthesized Polypyrrole were obtained with FTIR spectrometer in wave number range 4000 to 500 cm^{-1}.

The scaffolds microstructure characterization was done by scanning electron microscopy (SEM).

The scaffolds porosity was studied as following equation:

$$\text{Porosity}(\%) = 1 - [(\text{weight/volume}) / \rho \text{ polymer}] \times 100 \qquad \text{(eq.1)}$$

RESULTS

FTIR Analysis

FTIR analysis were carried out to confirm the polymerization of the pyrrole monomer to Polypyrrole during the Polymerization process. FTIR spectra of synthesized Polypyrrole shown in figure1. The FTIR spectra of the synthesized PPy were recorded in the range of 4000 to 500 cm^{-1} to confirm the polymerization. The wide peak in 3400 cm^{-1} is related to presence of N-H stretching vibrations of pyrrole ring. The presence of a band near 2100 cm^{-1} could be due to C-H stretching bonds. The peak in 1600 cm^{-1} is due to C=C bonds. The peaks at 1400 cm^{-1} and 1200 cm^{-1} correspond to C-N and C-H plane deformation bonds, respectively and the peak near 1100 cm^{-1} is corresponding to C-C stretching. All the peaks observed in this study, confirm the polymerization of pyrrole monomer to PPy and match well with available results in literature[1,2].

Figure 1: FTIR spectra of synthesized PPy

Morphology

Nerve tissue scaffolds should have a highly porous structure with interconnected pores to allow to cell growth, flow nutrients and elimination of waste product. The use of gas foaming with freeze-drying technique led to fabricate a suitable highly porous scaffold with desirable characteristics for nerve tissue engineering.

SEM images (figure 2) shows scaffolds morphology with different PPy ratio. The PVA and PVA/PPy scaffolds had porous structure. The pores were pseudo spherical shape with diameter range of 27 to 111 μm, 34 to 260 μm, 25 to 150 μm and 21 to 79 μm for PVA, PVA/PPY 1%, PVA/PPy 3% and PVA/PPy 5%, respectively with an average diameter of $62\pm20\mu m$,102 ± 55 μm,71.88 ± 26 μm and 45 ± 14 μm. The pores size distribution graph for different scaffolds shown in figure 3(50 pores size were measured).

The pores size and porosity of the scaffolds with various ratio of PPy was different, indicates that PPy proportion has significant impact on pores size and porosity of the scaffolds. There was a relationship between porosity and PPy content in scaffolds. as increasing in PPy content, the porosity was increased, but the pores size changes was irregular and apathetic to PPy contents.

The pore size and porosity of the scaffolds was suitable for neural cells. Small pores can support cells adhesion, medium sized pores could provide enough space for cell expansion and large pores allow nutrients and oxygen delivered to cells located throughout the 3D scaffold[3].

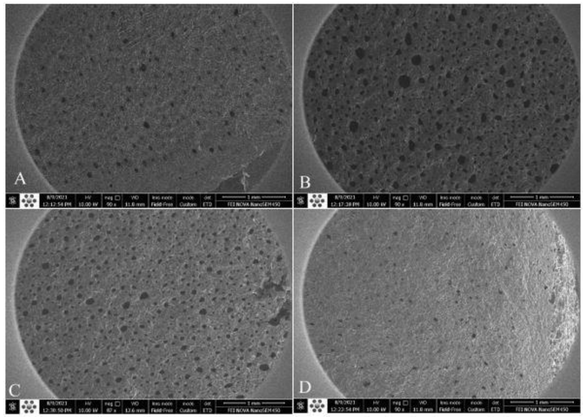

Figure 2: SEM images of scaffolds with different PPy ratio A) PVA B) PVA/ PPy 1% C) PVA/ PPy 3% D) PVA / PPy 5%

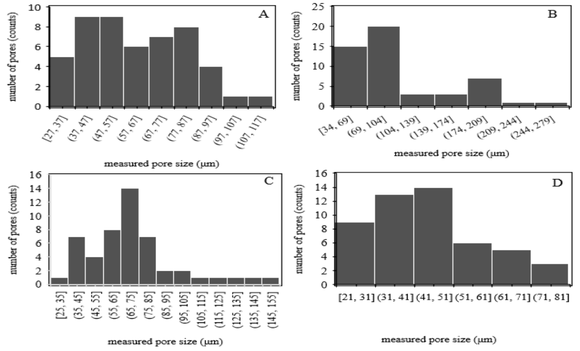

Figure 3: Pores size distribution of A)PVA, B)PVA/ PPy1%, C) PVA/ PPy3%, and D)PVA/PPy5% scaffolds

Porosity

Porosity is one of the most important parameters in tissue engineering scaffolds. The free spaces in scaffolds will serve as cellular path during growth and permit nutrient and oxygen flow in and out of the scaffolds. Moreover, the porosity provide enough space for cell adhesion, migration and proliferation.

The weight and volume of the scaffolds were measured. ρ is the theoretically density of the scaffolds components (1.19 g/cm^3 for PVA and 1.05 g/cm^3 for PPy). The porosity of the samples was measured using (eq .1) and shown in table 3(at least 3 samples for each scaffold).

Table 3: Quantitative porosity of the samples

Samples	Porosity (%)
PVA	79 ± 11
PVA / PPy 1%	87 ± 2
PVA / PPy 3%	90 ± 1
PVA / PPy 5%	88 ± 3

CONCLUSIONS

The present study demonstrated the development of 3D porous scaffold with interconnected pores using combination of gas foaming and freeze-drying technique. PPy was synthesized by chemical oxidative polymerizaion technique. The scaffolds morphology, Different ratio of PVA/PPy and porosity of the scaffolds were totally investigated. Overall, we demonstrated that the conductive scaffold based on PVA/PPy may be considered as a favourable substrate with suitable characteristics for nerve tissue engineering applications.

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