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Fabrication and characterization of graphene oxide and polyaniline doped 3d-printed neural scaffold

Songul Ulag[1]*, Armaghan Moradi[2], Sabereh Nouri[3], Sema Seren Karapehlivan[1], Beyza Sonmez[1], Zehra Kanli[4]

[1]*Department of Metallurgical and Materials Engineering, Marmara University, Istanbul, Türkiye, songul.ulag@marmara.edu.tr*
[2]*Department of Bioengineering, Marmara University, Istanbul, Türkiye*
[3]*Department of Cell and Molecular Biology and Microbiology, University of Isfahan, Iran*
[4]*Department of Biophysics, Marmara University, Basic Medical Sciences Building, Istanbul, Türkiye*

**songul.ulag@marmara.edu.tr*

Abstract

One innovative way to incorporate biological components with materials used in damaged tissue regeneration is through tissue engineering. The main goal of neural tissue engineering is to find ways to reduce fibrosis and inflammation after foreign materials that can act as a scaffold for cell development are implanted. With great potential, neural tissue engineering offers a compelling technological advance in restoring brain function. On the other hand, creating implantable scaffolds for brain culture that meet all the requirements is an incredible challenge for material science. These materials require numerous desirable properties, such as support for cellular survival, proliferation, and neuronal migration as well as the reduction of inflammatory reactions. In addition, they ought to support electrical communication between cells, exhibit brain-like mechanical characteristics, mimic the complex structure of the extracellular matrix, and ideally permit drug release under control. In this study, the initial composition and geometry of 3D-printed neural scaffolds were produced by extrusion-based 3D printing technology. These 3D-printed scaffolds were based on polycaprolactone (PCL). The graphene oxide (GO) and polyaniline (PANI) were added separately to provide electrical conductivity. In addition, the study aims to observe the characteristic difference between 0.1 wt.% PANI and GO-added 25% PCL scaffolds. The morphological analysis was performed with a scanning electron microscope (SEM) and chemical characterization was carried out with fourier-transformed infrared spectroscopy (FTIR). SEM images showed the successful printing of the scaffolds with pores. The biocompatibility test was performed with human neuroblastoma cells (SH-SY5Y) and the results demonstrated that all scaffolds had high biocompatibility.

Keywords: 3D printing, polymer, production, graphene oxide, polyaniline.

1. Introduction

Tissue engineering aims to develop functional replacements for damaged tissues and organs. Neural tissue engineering focuses on repairing, maintaining, and enhancing neural tissue function using biomimetic scaffolds and cells. Successful tissue-engineered neural scaffolds must support cell adhesion and proliferation, exhibit biocompatibility with low cytotoxicity, degrade into cytocompatible metabolites, possess high porosity for nutrient exchange, and maintain three-dimensional mechanical stability [1]. In recent decades, various techniques, notably 3D printing, have been explored to create functional neural tissue scaffolds. 3D printing enables precise and highly controlled spatial architectures tailored to patient requirements. Conventional methods for fabricating 3D scaffolds have shown promise in nerve regeneration, but 3D bioprinting offers a distinct advantage by producing patient-specific scaffolds. This capability enhances customization and potentially improves treatment outcomes [2]. In this study, 3D printing technology was used to fabricate neural scaffold using PCL, GO and PANI. The GO and PANI was selected to provide electrical properties to the scaffolds and their performances was compared each others.

2. Materials and Method

PCL (80.000 MW) and PANI (5.000 MW) was bought from Sigma-Aldrich. The GO was pruduced with Hummers' method which has ~4.2 nm thickness value and ~10-20 layers.

2.1. Preparation of the solutions and scaffolds

The 25% PCL was dissolved in 10 mL chloroform at room temperature for a hour. The 0.1 wt.% GO was added into the 25% PCL scaffold and mixed for 20 minutes. On the other hand, the 0.1 wt.% PANI was added into the 25% PCL scaffold and was stirred for 20 minutes. The model was designed using a three-dimensional drawing program (Solidworks) . The model was converted to G codes by Slic3r software. The scaffold configuration was designed to be square with dimensions of 20 mm×20 mm×0.1 mm. The 3D scaffolds were produced with an extrusion 3D printer (Hyrel 3D, Engine HR, SDS-5 Extruder, GA, USA). Polymer solutions were loaded into a 10 ml syringe and placed into the chamber of the device. The needle which has 0.2 mm diameter was used. The printing speed and flow rate were adjusted to the value of 10 mm/s and 1 ml/h, respectively. Other parameters were filling density = 60%, filling rate = 96%, total layer = 7 during the printing process. The fabrication process was shown in Figure 1.

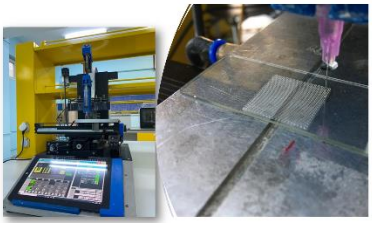

Figure 1. The fabrication process of the 3d-printed neural scaffolds. (note: larger figures can be set over 2 text columns)

2.2. Characterization of the neural scaffolds

To obtain the morphological properties of the scaffolds, scanning electron microscope (SEM) was used (Evo LS 10, ZEISS). The samples were sputter-coated with gold for 60 seconds using a Quorum SC7620 coating equipment. The chemical properties between the components were examined with fourier-transform infrared (FT-IR) spectroscopy. The analysis was performed with 4 cm^{-1} resolution and 4000-400 cm^{-1} wavenumber range. The SH-SY5Y cells cultured as previously mentioned were exposed to scaffolds for the specified durations of time in order to perform DAPI labeling. Following treatment, the cells underwent a 30-minute fixation in ice-cold acetone after being rinsed with phosphate-buffered saline (PBS). Following two PBS washes, the cells were stained for ten minutes in the dark using DAPI (300 nM). A Leica DMI 6000B fluorescent microscope (Wetzlar, Germany) was used to take the pictures.

3. Results and Discussions

3.1. SEM analysis

Figure 2 shows the SEM images of the 3D-printed neural scaffolds. In Figure 2a, 25% PCL had homogeneous pore distributions. Figure 2b shows the SEM images of the 0.1% GO added 25% PCL scaffolds. It can be said that by the addition of 0.1% GO into the 25% PCL scaffold, the pore size decreased compared to the pore structure of the 25% PCL scaffold. The 25% PCL/0.1% PANI scaffold showed a homogeneous pore distributions. The results demonstrated that both GO and PANI addition did not change the homogeneous pore structures of the 25% PCL scaffold.

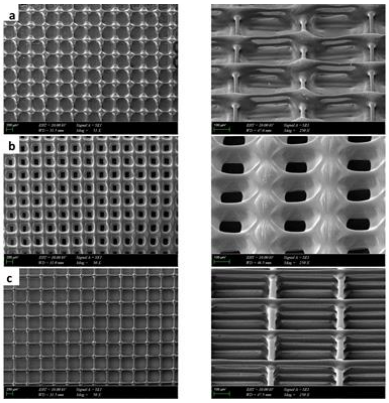

Figure 2. SEM images of the 25% PCL (a), 25% PCL/0.1% GO (b), and 25% PCL/0.1% PANI (c) scaffolds.

3.2. FTIR analysis

Figure 3 shows the FTIR spectrums of the components and 3D-printed scaffolds. Figure 3a represented the FTIR spectrum of the 25% PCL and the existence of peaks at ~2900 cm^{-1} indicating the stretching and vibration of $–CH_2–$ functional groups linked to alkyl long chains. Because of the bands of O–C–O, C–O–C, and C–O, respectively, this peak, together with others seen at ~1100, ~1200, and ~1800 cm^{-1}, shows the presence of ester bonds [3]. In Figure 3b, GO had main peaks at ~1600 cm^{-1} (C=C bond), 1750 cm^{-1} (C=O), and 1020 cm^{-1} (C-O) [3]. The specific peaks for PANI (Figure 3c) detected at 3400 cm^{-1} (N-H stretching) and the C-H stretching detected at 2950 cm^{-1} [4]. The FTIR spectrums of the 25% PCL/0.1% GO (Figure 3d) and 25% PCL/0.1% PANI (Figure 3e) were similar with the spectrum of the 25% PCL scaffold. This is related with the high amount of PCL in the composites [5].

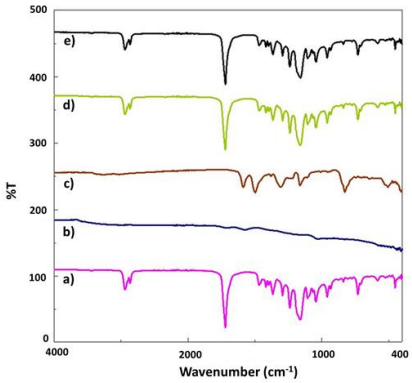

Figure 3. FTIR spectrums of the 25% PCL (a), GO (b), PANI (c), 25% PCL/0.1% GO (d), and 25% PCL/0.1% PANI (e) scaffolds.

3.3. Biocompatibility properties of the scaffolds

Figure 4 shows the fluorescence images of the scaffolds after culture with SH-SY5Y cells. It can be said that 25% PCL/0.1% GO and 25% PCL/0.1% PANI scaffolds had more cells on their surfaces after 1, 3, and 7 days of incubation. This demonstrated that GO and PANI addition increased the viability of the cells.

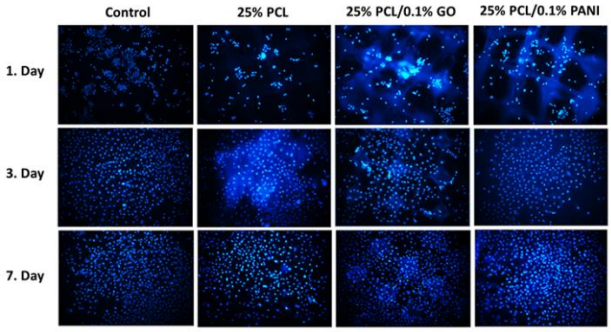

Figure 4. The flourescence images of the neural scaffolds after 1, 3, and 7 days of incubation.

4. Conclusion

This study is aim to fabricate 3D-printed neural scaffold which has electrical properties by doping with GO and PANI. The SEM images proved the homogeneous pore distribution of the scaffolds. The FTIR results showed that GO and PANI are homogeneously mixed in solution. The DAPI staining demonstrated that all scaffolds had high biocompatibility properties. In the future, with the completion of electrical measurements, neural tissues with electrical properties will be produced.

References

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