Special Issue of the 6th International Congress & Exhibition (APMAS2016), Maslak, Istanbul, Turkey, June 1–3, 2016

Preparation, Characterization, Mechanical Properties and Electrical Conductivity Assessment of Novel Polycaprolactone/Multi-Wall Carbon Nanotubes Nanocomposites for Myocardial Tissue Engineering

S. Ghaziof[a] and M. Mehdikhani-Nahrkhalaji[b,*]

[a]Department of Science and Nuclear Engineering, Islamic Azad University, Najafabad Branch, Najafabad, Iran

[b]Department of Biomedical Engineering, Faculty of Engineering, University of Isfahan, Isfahan, Iran

Cardiac tissue engineering aims to create functional tissue constructs that can reestablish the structure and function of injured myocardium. In this study, nanocomposite scaffolds composed of polycaprolactone and multi-walled carbon nanotubes, containing different amounts of carbon nanotubes, were prepared via solvent casting and vacuum drying technique, for myocardial tissue engineering. Characterization techniques such as Fourier transform infrared spectroscopy and scanning electron microscopy were used. Furthermore, mechanical properties of the prepared polycaprolactone and nanocomposite scaffolds were determined. The results have revealed that the scaffolds contain sufficient porosity with highly interconnected pore morphology. Addition of carbon nanotubes to the polycaprolactone matrix has improved conductivity of the prepared scaffold. The desired distribution of carbon nanotubes with a few agglomerates was observed in the nanocomposite scaffolds by scanning electron microscopy. Polycaprolactone/multi-walled carbon nanotubes nanocomposite scaffold containing 1 wt% of carbon nanotubes has shown the best mechanical behavior and electrical conductivity. In conclusion, the electrically conductive and nanofibrous polycaprolactone/1 wt% multi-wall carbon nanotubes scaffold could be used as an appropriate construct for myocardium regeneration and it deserves further investigations.

DOI: 10.12693/APhysPolA.131.428

PACS/topics: 87.68.+z

1. Introduction

The heart is a fascinating engineering marvel of nature. In the heart, cardiac muscle contraction pumps nutrient and oxygen rich blood to supply the entire body, including the cardiac muscle itself [1]. When damaged, adult heart muscle has poor capability to repair itself due to a minimal regeneration potential of cardiomyocytes [2]. Myocardial infarction causes several changes to the structure and proper function of the tissue, which can lead to arrhythmias and eventual heart failure [3]. In the past decade, great interest has arisen for the possibility to regenerate lost tissue by implanting therapeutic cells, biomaterials, and cardiac patches. Selection of a scaffold with appropriate mechanical and electrical properties is critical for inducing functional cardiac tissue, in vitro or in vivo [2]. In cardiac tissue engineering, the ideal scaffold should mimic the structure of the extracellular matrix, which is very important for the proliferation and differentiation of the seeding cells [4]. Biomaterials play a critical role in the success of tissue engineering approaches, as they guide the shape and structure of developing tissues, mechanical stability, and present opportunities to deliver inductive molecules to transplanted or migrating cells [5]. Roughly, the bioresorbable polymers can be divided into natural-based and synthetic polymers. Polycaprolactone (PCL) is an FDA approved polymer for clinical use. It is a semicrystalline, aliphatic polyester which is synthesized by ring-opening polymerization of ε-caprolactone. It displays good mechanical characteristics, such as biocompatibility, high elongation and strength [6]. Since cardiac muscle is an electroactive tissue, this research sought to create and characterize an electroactive scaffold. Electroactive biomaterials are a part of a new generation of "smart" biomaterials that allow the direct delivery of electrical, electrochemical and electromechanical simulation to cells. The family of electroactive biomaterials includes conductive polymers, electrets, piezoelectric and photovoltaic materials [7]. Multi-walled carbon nanotubes (MWCNTs) and carbon nanofiber are mechanically strong, flexible, lightweight, heat resistant, and they have high electrical conductivity [8]. Their unique electrical and mechanical properties can be exploited to create biomimetic tailored scaffolds. Initially, a range of MWCNTs concentrations were screened to determine the optimal electrical properties [9]. In recent years, the combination of natural and synthetic polymers with various other materials has shown improvement in their chemical, physical, and biological properties [5]. For preparation of porous scaffolds several methods have been reported including electrospinning technology, separation technique, spin coating (casting), solvent casting/ particulate leaching (SC/PL), solvent casting/vacuum drying (SC/VD) and so on [10].

*corresponding author; e-mail: m.mehdikhani@eng.ui.ac.ir

In this research, we have tried to investigate nanocomposite scaffolds consisting of PCL and MWCNTs with unique qualities, to make a promising construct for repairing defective heart tissue.

2. Materials and methods

2.1. Materials

PCL (Mw ε 80 000 by GPC), was purchased from Sigma, St. Louis, MO. MWCNTs ($D \times L$ = 10−20 nm × 30 μm; w/v) were purchased from Research Co, Spain. Dichloromethane (DCM-CH_2Cl_2, M=84.93 g/mol) and Polyethylene glycol 400 (PEG-HO(C_2H_4O)nH, M = 380–420 g/mol) were purchased from Merck Inc.

2.2. Methods

2.2.1. Preparation of PCL/MWCNTs scaffolds

Nanocomposite scaffolds were prepared by SC/VD technique. Polymer solution was prepared by dissolving 1 gr of PCL in 10 ml of DCM solvent. Then, 0.5 ml of PEG was added to the solution. The solution was stirred for 15 min at 25 °C. Multi-wall carbon nanotubes (three different amounts of 1, 0.75 and 0.5 wt%) were added to PCL solution and mixed together. Finally, PCL/CNT solution was cast in glass molds (10 cm in diameter) and the scaffolds were dried in vacuum oven at 37 °C for 24 h.

2.2.2. Characterization of the samples

Prepared samples were characterized using scanning electron microscopy (SEM) (JEOL JSM 6400, Japan) and Fourier transform infrared spectroscopy (FT-IR) (FT infrared spectroscope, JASCO, FT/IR-6300 (500–4000 cm^{-1}), Japan). Mechanical properties of the PCL and nanocomposite scaffolds were determined using electromechanical universal testing machine (CB6-K006, Hounsfield, Korea) at 25 °C. Mechanical properties of the nanocomposite and PCL scaffolds were measured on standard disc-shaped specimens according to the ASTM F451-86 standard. The height-to-diameter ratio of the samples was 3:10. The distance between the gripping points was 15–16 mm. Mechanical testing was conducted with the grips moving at stretching speed of 25 mm/min, the load was 60 N. The reported data on tensile strength and elongation represent the average results of three samples. The mechanical properties were calculated at 50% strain.

2.2.3. Porosity measurement

In order to calculate the porosity of the PCL scaffold, liquid replacement method was used. The precise method was described elsewhere [11]. In brief, weighed PCL scaffold was emerged in a graduated cylinder containing ethanol with specific volume of V_1 for 2 h. Sequential evacuation-repressurization cycles at room temperature and vacuum conditions were performed to allow penetration of the ethanol into the construct. Then, the total volume V_2 of the soaked scaffold was determined. Then $V_2 - V_1$ is the volume of the PCL scaffold. Therefore, V_3 was recorded by removing the ethanol-soaked scaffold and measuring the residual ethanol volume. The pore volume of the scaffold recorded by measuring the ethanol volume remained in the PCL structure, was defined as $V_1 - V3$. The porosity percentage of the scaffold was calculated using equation:

$$\text{Porosity}(\%) = 100(V_1 - V_3)/(V_2 - V_3).$$

The porosity was also measured using the Matlab-R2013 software. The porosity percentage of the samples was determined by calculating the porosity of the most superficial three layers of the nanocomposite scaffolds.

2.2.4. Electrical conductivity

The unit of commonly used electrical conductivity is one millionth of a Siemens per centimeter (micro-Siemens per centimeter or μS/cm) [12]. In order to measure the electrical conductivity the JENWAY 3540 pH & conductivity meter was used. In brief, PCL solution and PCL solution containing different amounts of MWCNTs were located between two electrodes under the voltage of 9 VAC, frequency of 50/60 Hz, and 6 VA power at 25 °C.

3. Results and discussion

3.1. Characterization of the prepared nanocomposite scaffolds

The SEM images of PCL/MWCNTs scaffolds showing there porous architecture are presented in Fig. 1a–c. The porosity of the scaffolds was determined to be approximately 50–52%. This amount of porosity is necessary and suitable for this kind of scaffolds. it is actually needed for cell seeding, supplying of oxygen and for cells survival.

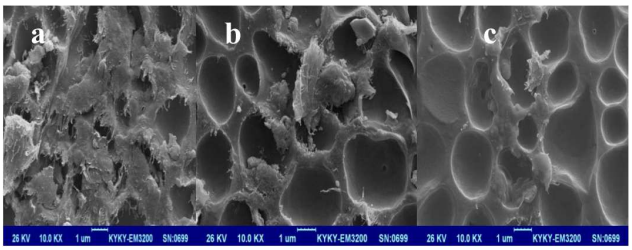

Fig. 1. SEM photo micrographs of the nanocomposite scaffolds: (a) S1, (b) S2, (c) S3.

The macropore structure should host seeded cells and allow cell adhesion to the inner surfaces, while the network of microchannels is important in allowing diffusion of water-soluble substances through the scaffold and also to produce quite rough pore wall topography adequate for cellular adhesion. The size of the micropores can be controlled during scaffold fabrication, since it is highly dependent on the concentration of PCL solution before the vacuum process [11].

3.2. Electrical conductivity

Due to addition of MWCNTs the electrical conductivity was increased. This means that the MWCNTs can

be used as providers of conductivity in the nanocomposite scaffold, in accordance with the nature of myocardial structure. Thus we can state that this smart synthetic biomaterial is one of the best choices for use in cardiac tissue engineering (Table I).

TABLE I

The electrical conductivity of pure PCL polymer and PCL/MWCNTs nanocomposite thin films.

Samples	MWCNTs [wt%]	Conductivity ratio [μS/cm]
PCL	0	2.59
S_1	1	11.32
S_2	0.75	8.15
S_3	0.5	5.23

3.3. FT-IR study

FT-IR spectra of the PCL, MWCNTs, and PCL/ MWCNT nanocomposite scaffolds are presented in Fig. 2. Prominent peaks can be observed at 2944, 2864, 2150, 2022, 1710, 1418, 1365, 1293, 1240, 1174, 1107, 1046, 961 and 732 cm^{-1}. Of these, in PCL spectra, 2944, 1710, 1471, 1240, and both 961 and 732 are related with C–H bond stretching, carbonyl stretching, C–H scissoring, C–O stretching, C–C stretching, respectively. Characteristic peaks of MWCNTs at 3500–1000 cm^{-1} (aromatic ring) appeared in spectra of nanocomposite scaffolds. An extra peak at 2864.74 cm^{-1}, which is actually a background of CO_2, was observed in the nanocomposite samples. Furthermore, 1465 cm^{-1} peak of pure MWCNTs was observed in the nanocomposite scaffolds, Fig. 2 (samples). For better detection of the MWCNTs, the expanded FT-IR spectra between 3000 and 1500 cm^{-1}, caused by C=O and C–O stretching vibrations of of the PCL/MWCNTs, were inspected. The results were in accordance with other studies on PCL and PCL/MWCNTs nanocomposites [13]. According to FT-IR spectra of MWCNTs, the presence of the broad band

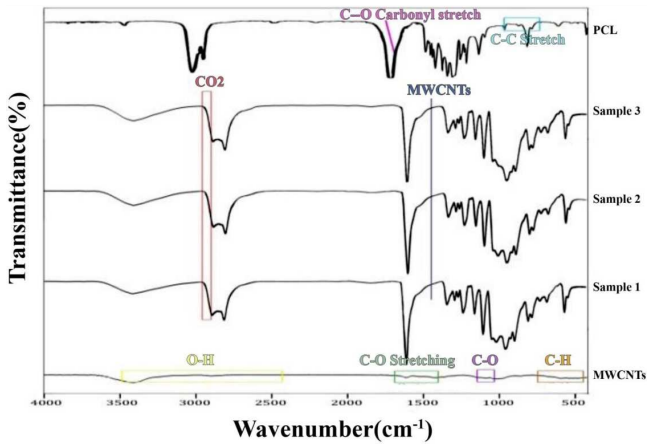

Fig. 2. FT-IR spectra of the PCL, MWCNTs and nanocomposites.

in the range 2400–3500 cm^{-1} confirms the presence of hydroxyl group and carboxyl group. The appearance of a peak approximately at 1450–1650 cm^{-1} corresponds to the C–O stretching, which indicates the introduction of carboxylic groups due to surface oxidation of nanotubes. The peak at 1035.59–1158.04 cm^{-1} indicates C–O vibration. Peak at 465.725–694.248 cm^{-1} may be corresponding to a bending vibration of C–H bond [14].

3.4. Mechanical properties

Figure 3a, and b shows the value of tensile strength and elastic modulus of the PCL and nanocomposite scaffolds. The averages of elastic modulus and tensile strength of PCL thin film scaffold were 20.33 MPa and 13.56 MPa, respectively, see Fig. 3a and b. Elongation of PCL was about 1089%. By addition of MWCNTs to the nanocomposites (1, 0.75, and 0.5 wt.%), the elastic modulus has increased by about 85%, 65% and 45%, respectively. This is due to the nature of MWCNTs which have better mechanical properties and elastic modulus than single walled CNTs and mimic those of myocardial structure. In contrast, the tensile strength and elongation have decreased due to the poor dispersion of the MWCNTs in the PCL matrix and due to formation of the MWCNTs agglomerates at high concentrations [15]. In fact, agglomeration of MWCNTs has occurred due to weak bonding between the agglomerates and the matrix. During the tensile test their interface was broken. The broken agglomerates could act as stress concentration points, that may be resulting in the formation of microcracks and also in high microporosity and macroporosity levels in its microstructure, that caused the reduction of the nanocomposite strength (Fig. 1). In fact, in tissue engineering, tunable mechanical properties (structural integrity after implantation) are necessary requirements. It was confirmed that the mechanical properties were not deteriorated when the amount of MWNCTs in the nanocomposite scaffolds was raised.

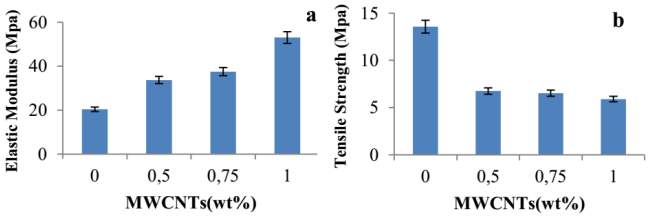

Fig. 3. Mechanical properties of the samples (a) elastic modulus, (b) tensile strength.

4. Conclusions

According to the results of this study, with addition of the MWCNTs in the nanocomposite scaffolds, the mechanical properties and electrical conductivity of the construct were enhanced. In conclusion, the electrically conductive and nanofibrous PCL/1 wt% MWCNTs scaffold could be used as an appropriate construct for myocardium regeneration and it deserves further investigations.

Acknowledgments

The authors gratefully acknowledge the University of Isfahan and Isfahan University of Medical Sciences (Central laboratory, School of Medicine).

References

[1] Z. Li, J. Guan, *Polymers* **3**, 740 (2011).

[2] A.M. Martins, G. Eng, S.G. Caridade, J.F. Mano, R.L. Reis, G. Vunjak-Novakovic, *Biomacromolecules* **15**, 635 (2014).

[3] H. Han Cui, Ph.D. Thesis, The State University of New Jersey, 2010.

[4] Y. Zhang, Y. Tang, Y. Wang, L. Zhang, *Nano-Micro Lett.* **3**, 270 (2011).

[5] H.E. Davis, J.K. Leach, *Hybrid and Composite Biomaterials in Tissue Engineering*, University of California, 2008.

[6] M. Generali, P.E. Dijkman, S.P. Hoerstrup, *EMJ Int. Cardiol.* **1**, 91 (2014).

[7] R. Balint, N.J. Cassidy, S.H. Cartmell, *Acta Biomater.* **10**, 2341 (2014).

[8] J. Howard, *Intelligence Bulletin 65*, National Institute for Occupational Safety and Health (2013).

[9] J.N. Mackle, D.J.-P. Blond, E. Mooney, C. McDonnell, W.J. Blau, G. Shaw, F.P. Barry, J.M. Murphy, V. Barron, *Macromol. Biosci.* **11**, 1272 (2011).

[10] M. Abedalwafa, F. Wang, L. Wang, C. Li, *Rev. Adv. Mater. Sci.* **34**, 123 (2013).

[11] T.C. Gamboa-Martinez, J.L. Gomez Ribelles, G. Gallego Ferrer, *J. Biaoavtive Compatible Polymers* **26**, 464 (2011).

[12] M. Heyda, *A Practical Guide to Conductivity Measurement*, MBH Engineering Systems, Lynnfield 2008.

[13] K. Saeedfar, L.Y. Heng, T.L. Ling, M. Rezayi, *Sensors* **13**, 16851 (2013).

[14] S. Sagar, N. Iqbal, A. Maqsood, *J. Phys.: Conf. Ser.* **439**, 012024 (2013).

[15] K. Saeed, S.-Y. Park, M. Ishaq, *J. Chil. Chem* **4**, 54 (2009).