Marquette University

e-Publications@Marquette

School of Dentistry Faculty Research and
Publications

Dentistry, School of

11-1-2019

Conductive Nanofibrous Chitosan/PEDOT:PSS Tissue Engineering Scaffolds

Ali Abedi
University of Tehran

Mahdi Hasanzadeh
Yazd University

Lobat Tayebi
Marquette University, lobat.tayebi@marquette.edu

Follow this and additional works at: https://epublications.marquette.edu/dentistry_fac

Part of the [Dentistry Commons](#)

Recommended Citation

Abedi, Ali; Hasanzadeh, Mahdi; and Tayebi, Lobat, "Conductive Nanofibrous Chitosan/PEDOT:PSS Tissue Engineering Scaffolds" (2019). *School of Dentistry Faculty Research and Publications*. 366.
https://epublications.marquette.edu/dentistry_fac/366

Marquette University

e-Publications@Marquette

Dentistry Faculty Research and Publications/School of Dentistry

This paper is NOT THE PUBLISHED VERSION; but the author's final, peer-reviewed manuscript. The published version may be accessed by following the link in the citation below.

Materials Chemistry and Physics, Vol. 237 (November 1, 2019): 121882. [DOI](#). This article is © Elsevier and permission has been granted for this version to appear in [e-Publications@Marquette](#). Elsevier does not grant permission for this article to be further copied/distributed or hosted elsewhere without the express permission from Elsevier.

Conductive Nanofibrous Chitosan/PEDOT:PSS Tissue Engineering Scaffolds

Ali Abedi

Department of Tissue Engineering, Faculty of Biomedical Engineering, University of Tehran, 14399-57131, Tehran, Iran

Mahdi Hasanzadeh

Department of Textile Engineering, Yazd University, P.O. Box 89195-741, Yazd, Iran

Lobat Tayebi

Marquette University School of Dentistry, Milwaukee, WI

Abstract

Design of a proper scaffold is the first step in fabrication of a tissue engineering product, which should be able to support cellular growth in *in vitro* conditions. This study focuses on the fabrication and characterization of chitosan (CS) scaffolds containing PEDOT:PSS, a conductive polymer. The scaffold is primarily designed for cardiac tissue engineering, although it can be used for other applications too. Chitosan scaffolds containing 0.3, 0.6 and 1 wt% of PEDOT:PSS are fabricated through electrospinning. The structure and morphology of scaffolds are characterized by scanning electron microscopy (SEM), 3D Laser Measuring Microscopy and Fourier-transform infrared spectroscopy (FTIR). The electrical and mechanical properties, as well as biocompatibility and

cell viability of scaffolds are also investigated. It is found that addition of PEDOT:PSS to chitosan scaffold not only enhances the mechanical properties and electrical conductivity of electrospun scaffolds, but also improves their biocompatibility and cell viability. Our results have shown that increasing the PEDOT:PSS content up to 1 wt% results in 30–40% reduction of fiber diameter and increase in electrical conductivity by around 100-fold. Additionally, in the scaffold containing 1 wt% of PEDOT:PSS, the tensile strength increases about 9 MPa compared to the neat sample. Results obtained from scaffolds compared with the properties of native myocardium extracellular matrix reveal its potential application for cardiac tissue engineering.

Graphical abstract

Keywords

PEDOT:PSS, Chitosan, Electrospinning, Cardiac tissue engineering, Electrical conductivity

1. Introduction

During the past two decades, cardiac diseases have been one of the most prominent causes of death, and have remained a serious health risk in the 21st century, even in developed countries [[1], [2], [3], [4]]. Cardiac failure happens as a final result of cardiac infarction in left ventricle, where cardiac muscle cells have limited potential for regeneration of damaged tissue [2,4,5]. Heart transplant have not been established as a standard method of treatment, despite being the most applicable treatment method, due to low number of donors and undesirable immunologic responses [2,4]. These limitations have drawn more attention to using cellular therapy and tissue engineering approaches [[4], [5], [6]]. Different *in vitro* and *in vivo* studies have shown promising results in this area [5]. However, one of the biggest challenges with engineering cardiac tissue is the fabrication of a porous nanofibrous scaffold that can mimic the physical and chemical properties of target tissue's extra-cellular matrix (ECM), which can support cellular adhesion, proliferation and nutrition delivery [4,7]. Electrospinning is more adaptable in mimicking ECM features, due to having a better control over surface area, diameter of fibers and incorporation of biological agents [4]. Cardiac muscle tissue possesses electro-conductive properties, making it possible for transmission of beating electrical signals [5]. Due to this feature, use of non-conductive scaffolds for cardiac muscle will result in disturbance of intracellular signaling [1,8].

Chitosan (CS), as a natural polymer provided by deacetylation of chitin, is abundantly used in tissue engineering scaffold fabrication due to its biocompatibility, non-toxicity, hydrophilicity, rapid kinetics and high processability [9,10]. Due to presence of amine groups in its structure, chitosan has a cationic property, allowing a positive charge even in acidic environments. Due to this reason, as well as low conductivity of this material, electrospinning of chitosan is reported to be difficult [10,11]. To solve this problem, several easily electrospinnable polymers have been suggested by researchers to blend with CS, such as poly(vinyl alcohol) (PVA), poly(ethylene oxide) (PEO) and more [12,13]. For example, uniform chitosan/PVA fibers with an average diameter of 99 ± 21 nm were made from a chitosan/PVA solution in 40:60 mass ratio [12]. Also, in the same

research, the results showed the successful construction of Chitosan fibers by adding of PEO in 2:1 or 1:1 mass ratios of chitosan to PEO from chitosan/PEO solutions [13].

Many materials in recent years, including carbon nanotubes, graphene and poly (glycerol sebacate), have been used together with chitosan in order to enhance physical properties [11,14]. Although these efforts could enhance the fabrication and physical properties of scaffolds, some of these materials, such as carbon nanotubes and graphene, cause the loss of biocompatibility and biodegradability of scaffolds simultaneously with the proper upgrade in mechanical and electrical properties [[15], [16], [17]]. For instance, measurement of graphene's and carbon nanotubes' toxicity on neuronal PC12 cells show that adding 100 µg of graphene to 1 ml of cell culture medium reduced the metabolic activity of cells by 50% in 24 h, along with significant LDH (cell membrane damage marker) release was noted after 24 h. Addition of 100 µg carbon nanotube to neuronal PC12 cells induce a dramatic release of LDH and reduced the metabolic activity of cells by 70% in 24 h [18]. Among the conductive polymers, PEDOT:PSS has been widely used in biomedical applications. It is a poly(thiophene) derivative formed during the polymerization of bicyclic 3,4-ethylenedioxythiophene [19]. Due to proper bond length and chemical properties, PEDOT:PSS has higher chemical and thermal stability than other commonly used polymers, including poly(pyrrole)(PPy) and poly(aniline)(PANI) [[19], [20], [21]]. There was no report of toxicity when using PEDOT:PSS as a coating layer for platinum, gold and iridium oxide implants, and results show acceptable immunologic response and high biocompatibility from this polymer [22,23].

To the best of author knowledge, there is no comprehensive study on the effect of PEDOT:PSS on electrospinning of CS base scaffolds in order to obtain conductive fibers with proper mechanical properties. Hence, in this study, PEDOT:PSS has been used together with chitosan to fabricate electrically-conductive scaffold with superior mechanical properties and biocompatibility through electrospinning, mimicking physical properties of myocardium ECM.

2. Materials and methods

2.1. Materials

Chitosan (medium molecular weight, DDA = 80%), polyvinyl alcohol (PVA, 99% hydrolyzed), poly (3,4-ethylenedioxythiophene)–polystyrenesulfonic acid (PEDOT:PSS) dispersion (1.3 wt% in water, conductive grade) were all purchased from Sigma-Aldrich (Germany). Glutaraldehyde, glycine, ethanol and dimethyl sulfoxide (DMSO) were purchased from Merck Co. (Germany). Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum(FBS), trypsin and phosphate buffer saline (PBS)were purchased from Invitrogen (USA).

2.2. Electrospinning of scaffolds

In order to fabricate scaffolds, 4 wt% of chitosan solution was dissolved overnight in 7% v/v acetic acid at room temperature by magnetic stirring (150 rpm). Afterwards, different amounts of PEDOT:PSS dispersion was added to reach electrospinning solutions, including pure chitosan and 0.3, 0.6 and 1 wt% chitosan-PEDOT:PSS solutions (Table 1). Then, 12 wt% PVA was dissolved overnight in deionized water at 80 °C using magnetic stirring. Upon completion of dissolving, PVA was cooled down to room temperature and added to chitosan-PEDOT:PSS solutions at a weight ratio of 3:1, followed by magnetic stirring (200 rpm for 1 h) to reach homogenous solution.

Table 1. Properties of chitosan, PVA and PEDOT:PSS solutions.

Sample	Chitosan-PVA	PEDOT:PSS (wt.%)	Electrical conductivity of electrospinning solution (µS/cm)
CS/PVA	1:3	—	215 ± 14
CS/PVA/PEDOT:PSS (0.3)	1:3	0.3	366 ± 29
CS/PVA/PEDOT:PSS (0.6)	1:3	0.6	801 ± 11

CS/PVA/PEDOT:PSS (1)	1:3	1	912 ± 7
----------------------	-----	---	---------

Details of the solid content of all samples are presented in Table 2. This information will help to understand which factor has a major impact on the diameter of the fibers.

Table 2. Solid content of all samples.

Sample	Chitosan(g)	PVA(g)	PEDOT:PSS(g)
CS/PVA	0.8	2.4	0
CS/PVA/PEDOT:PSS (0.3)	0.8	2.4	0.0096
CS/PVA/PEDOT:PSS (0.6)	0.8	2.4	0.0192
CS/PVA/PEDOT:PSS (1)	0.8	2.4	0.032

Electrospinning of the prepared solution was carried out using a double nozzle electrospinning apparatus (Nano Azma Co., Iran) for 10 h at 0.5 ml/h flow rate, 2500 rpm speed of aluminum wrapped collector, 20 kV applied voltage, and 18 cm nozzle-to-collector distance. These parameters were optimized using pure PVA-chitosan solution and applied to all samples. Upon completion of electrospinning, aluminum foil was removed and cut for subsequent characterization methods.

In order to crosslink polymer scaffolds, electrospun nanofibrous mats were removed from aluminum foil and subjected to 0.2 M Glutaraldehyde solution vapor in desiccator placed inside oven at 80 °C for 1 h. Then, the samples were kept in vacuum oven for 24 h at 50 °C for completion of crosslinking. Afterwards, scaffolds were washed with 1% glycine solution in deionized water for three steps of 30 min for removal of residual crosslinking agent.

2.3. Characterization

Morphology of electrospun scaffolds were evaluated using scanning electron microscope (SEM). The fiber diameter and average fiber diameter of electrospun scaffolds were calculated using image J software. The surface morphology and roughness of electrospun scaffolds were performed using a 3D Laser Measuring Microscope (LEXT OLS4000, Olympus, Japan). Surface characterizations and roughness of scaffolds were measured using OLS4100 offline V3.11 software.

In order to study the chemical composition and present functional groups in electrospun scaffolds, Fourier transform infrared (FTIR) spectra were recorded on a Perkine-Elmer.

Electrical conductivity of electrospinning solutions was measured before electrospinning at 25 °C using a conductometer device (WDA-CMD510). Electrical conductivity of solutions was measured with 5 repeats and results were reported as average conductivity.

Tensile properties of electrospun scaffolds were characterized using Instron-3367 universal uniaxial tensile testing machine. Electrospun scaffolds were cut into 5 × 30mm specimens. Test was carried out with three repeats for each specimen at grip speed of 1 mm/min until their failure.

Electrical conductivity of electrospun scaffolds were also measured using Kithley-2361 multimeter. Electrospun scaffolds were removed from aluminum sheet and cut into 15 × 1 mm sample. Test was carried between two electrodes with length of 10 mm. Voltage was ranged between 10 and -10 V and current was measured at least three times. The electrical conductivity was obtained from current-voltage (I–V) curve.

MTT assay was used to evaluate the influence of physical and chemical characteristics of scaffolds on metabolic activity and proliferation of cells on electrospun scaffolds. Accordingly, rat bone marrow mesenchymal stem cell was seeded at density of 4000 cells/cm² in 24 plates. MTT assay was performed at day 1, day 3 and day 7, and

optical density of formazan in DMSO for each specimen was measured with plate reader instrument to evaluate metabolic activity of cell populations by use of equation (1).

(1)

$$OD = \frac{\text{absorbance of the samples} - \text{absorbance of blank wells}}{\text{absorbance of TCP samples} - \text{absorbance of blank samples}}$$

3. Results and discussion

3.1. Morphology of scaffolds

There are several factors involved with electrospinning that can be manipulated to change the diameter of fibers. Conductivity of electrospun solutions is one of the key factors in determining the diameter of fibers [24]. SEM images and distribution of fiber diameters of scaffolds are shown in Fig. 1.

Fig. 1. SEM images and fiber diameter distribution of (a) CS/PVA, (b) CS/PVA/PEDOT:PSS (0.3), (c) CS/PVA/PEDOT:PSS (0.6), and (d) CS/PVA/PEDOT:PSS (1) scaffolds.

As shown in Table 1, the increase in PEDOT:PSS content increases the electrical conductivity of solution. During electrospinning process, higher conductivity of electrospun solution will facilitate pulling of polymeric jet toward the collector [25,26]. Our results have shown that increasing the PEDOT:PSS content up to 1 wt% results in 30–40% reduction of fiber diameter. Reduction of fiber diameter can also increase the fiber-fiber cross attachments. PCL was used before together with chitosan and has been shown to decrease the diameter of electrospun fibers [25]. PANi was also electrospun as a conductive polymer in electrospinning of cellulose and has been shown to decrease the diameter of fibers. PANi additionally was utilized for facilitating electrospinning of collagen and reduction of fiber diameter [27]. Addition of polyelectrolytes, including PAH, PAA and poly (ethylene oxide) (PEO), has been shown to increase the conductivity of solution and lower fiber diameter in electrospinning [[28], [29], [30]]. Increase of carried electrical current from electrospinning nozzle due to increased conductivity has been stated to be the main factor decreasing the fiber diameter [28].

Equation (2) shows the parameters affecting fiber diameter in electrospinning [30].

(2)

$$d = \sqrt[3]{\frac{2\gamma\epsilon Q^2}{l^2\pi(2\ln X - 3)}}$$

where d is the diameter of fibers, γ is the surface tension of solution, Q is the flow rate of the solution, l is the current carried by the jet, X is the initial jet length divided by diameter of the nozzle and ϵ is the dielectric constant of the solution. It is clear that at a constant electrical field, higher conductivity of the solution will result in a lower diameter of fibers. Our results on changes of fiber diameter in different samples also follow this trend, where scaffolds with highest amount of conductive polymers, and therefore with highest conductivity of solution, have the lowest diameter of fibers (Fig. 1). Another factor influencing the diameter of the fibers was the addition of PEDOT:PSS aqueous solution. According to Table 2, the highest added amount of PEDOT:PSS solution was in CS/PVA/PEDOT:PSS (1) sample, which resulted in a 6% increase in volume. Adding the volume of water to the solution, according to equation (2), will result in an increase in the flow rate and consequently, an increase in the diameter of the fibers.

3.2. FTIR analysis

In order to check the presence of different functional groups in scaffolds, Fourier transform infrared spectroscopy (FTIR) analysis was performed. Results of FTIR spectroscopy of different scaffolds are shown in Fig. 2. It should be mentioned that PVA and chitosan share a large number of functional groups, such as O–H and C–H, therefore peaks identifying these functional groups might overlap, making it more difficult to detect these functional groups. However, in FTIR data of CS/PVA scaffold, the peak between (3300-3400 cm^{-1}) is related to the stretch of O–H bond present in both PVA and chitosan [31]. Peak at $\sim 2950 \text{ cm}^{-1}$ and peak at (1370 - 1450 cm^{-1}) are related to the respective stretch and bending of C–H bond in both PVA and chitosan [32]. Peak at $\sim 1650 \text{ cm}^{-1}$ and peak at $\sim 1260 \text{ cm}^{-1}$ are related to N–H bond and acetyl group in remaining chitin inside chitosan polymer, respectively [33]. Peak at $\sim 1740 \text{ cm}^{-1}$ is related to C=O bond in remaining vinyl alcohol in PVA, which does not participate in tautomeric reaction [34]. In pure PEDOT:PSS sample, peak at $\sim 3100 \text{ cm}^{-1}$, $\sim 1730 \text{ cm}^{-1}$ and $\sim 1450\text{-}1600 \text{ cm}^{-1}$ are related to the stretch of C–H bond in PSS aromatic ring, stretch of C=C bond in thiophene ring and C=C bond in aromatic bond, respectively [35,36]. Peak at $\sim 800 \text{ cm}^{-1}$, which is in fingerprint zone, is related to bending of C–H bond in aromatic ring [37]. C–S bond in thiophene rings of PEDOT:PSS can be detected with the peak at $\sim 700 \text{ cm}^{-1}$ [35].

Fig. 2. FTIR spectrum of PEDOT:PSS, CS/PVA, and CS/PVA/PEDOT:PSS (1).

Detection of functional groups in CS/PVA/PEDOT:PSS (1) scaffold was problematic due to the variety of peaks. Bending of C–H bond and C–S bond in thiophene ring have increased the number of peaks in lower wavenumbers [35,37]. Therefore, wavelength is inversely proportional to frequency of the wave, so lower wavenumber will reduce the frequency of bond resonance within the nanocomposite, meaning that materials inside the composite have higher stability as compared to pure samples.

3.3. Electrical conductivity of scaffolds

Electrical conductivity of fibers was measured with a Kithley-2361 multimeter. Data was in the form of current as a function of voltage, where the reciprocal of the slope gives us the resistance (R). L/A ratio (where L is the distance between 2 electrodes and A is the cross-sectional area of the fiber) was kept the same through all samples. Conductivity of scaffold were measured using Equation (3).

(3)

$$\sigma = \frac{L}{R \times A}$$

where, R is resistance of scaffolds, collected from their relative I–V curve (Fig. 3).

Fig. 3. The current-versus-voltage curve of (a) CS/PVA, (b) CS/PVA/PEDOT:PSS (0.3), (c) CS/PVA/PEDOT:PSS (0.6), and (d) CS/PVA/PEDOT:PSS (1) scaffolds.

After calculating the resistance of each scaffold using their I–V curve, electrical conductivity of each scaffold was measured, which is shown in Table 3.

Table 3. Mechanical properties and electrical conductivity of CS/PVA and its PEDOT:PSS nanocomposites.

Sample	Electrical conductivity of scaffold (S/m)	Elongation at break (%)	Ultimate strength (MPa)	Toughness ($J \cdot m^{-3}$)
CS/PVA	$6 \cdot 10^{-5}$	6.4 ± 0.3	9.59 ± 0.63	$34.56 \cdot 10^6$
CS/PVA/PEDOT:PSS (0.3)	$1.5 \cdot 10^{-3}$	5.6 ± 0.2	13.07 ± 1.09	$33.54 \cdot 10^6$
CS/PVA/PEDOT:PSS (0.6)	$5.02 \cdot 10^{-3}$	5.3 ± 0.1	16.45 ± 1.13	$42.38 \cdot 10^6$
CS/PVA/PEDOT:PSS (1)	$7.63 \cdot 10^{-3}$	5.6 ± 0.3	18.78 ± 0.95	$48.87 \cdot 10^6$

Conductive polymers, due to the presence of conjugated π -electron backbone in their structure, have high conductivity that provides a high variety of applications from electronic industry to medical devices [20,[38], [39], [40]]. Conductive polymers, including PPy, PANi and PEDOT:PSS and polythiophene derivatives, have already been used in engineering of cardiac, nerve and bone tissue [41,42]. Range of conductivity for non-

conducting materials and semi-conductors are 10^{-16} - 10^{-5} S/m and 10^{-5} - 10 S/m, respectively. Conductive polymers usually lie in the semi-conductive region. Any material with conductivity value higher than 10 S/m is considered a conductive material [43]. As shown in Table 3, adding PEDOT:PSS to CS/PVA increases the conductivity of samples from the non-conducting range of the CS/PVA sample by around 100-fold, to the semi-conductive range, where the scaffold containing PEDOT:PSS lies. It is worth mentioning that conductivity of scaffolds containing PEDOT:PSS is close to the conductivity of native neural tissue in gray and white matter, which is between 2×10^{-3} - 3.3×10^{-3} S/m [44]. Moreover, highest conductivity value reported for cardiac tissue is between 1.9×10^{-2} - 2×10^{-2} S/m, which was measured in native tissue [45]. Conductivity values of scaffold were measured in dry condition. Considering the placement of these scaffolds in wet condition of *in vitro* or *in vivo* condition, scaffolds will show higher values of conductivity, close to that of cardiac tissue. Conductive scaffolds have been used previously as additive materials in tissue engineering. PPy was added to hyaluronic acid, which increased the conductivity to 3.8×10^{-5} S/m where scaffolds were used for wound healing [43,46]. Also, adding PPy to cellulose nano-composites have shown to increase the conductivity of scaffolds up to 10^{-2} S/m [43,47]. Moreover, PU-PEDOT composite have shown to increase the conductivity to 10^{-2} S/m [43,48]. However, PU-PPy composite fabricated for cardiac and neural tissue engineering have shown conductivity of 10^{-4} S/m [43,49]. Influence of conductivity of scaffolds on cell behavior have been studied. Semi-conducting PANi/gelatin scaffolds were used for cardiac tissue engineering and conductive PCL/PPy, PANi/PG and SIBS/PPy were used for neural tissue engineering, where these scaffolds showed to be promising for growth, proliferation and arrangement of cells [19,50,51].

3.4. Mechanical properties of scaffolds

Mechanical properties of electrospun nanofibers depends on different parameters, including chemical composition of polymer, type of solution, collector properties, etc. [52]. Reduction in fiber diameter will reduce the structural imperfections in fiber formation and will increase the tensile strength and stiffness of electrospun fibers. In a similar study, it was shown that in electrospinning, crystallinity and molecular orientation are increased, which in turn reduce the structural imperfections and increase the strength of fibrillar mats [52,53]. In our study, mechanical properties were evaluated using tensile testing, and results are shown in Fig. 4.

Fig. 4. Stress-strain curve of different nanofibrous scaffolds.

Tensile test results show that initially with the displacement of jaws, arrangement of fibers will change with no significant force. However, applied force will be distributed into fibrillar structure, causing fibers to get aligned with displacement direction. Afterwards, applied force will cause deformation in fibers. Lower fiber diameters, due to higher crystallinity and molecular orientation, will show higher resistance to displacement [53,54]. This is confirmed by higher Young's modulus in samples with higher PEDOT:PSS content and lower diameter. Similar studies on PVA/MWCNT composites have shown a decrease in structural imperfections and higher mechanical

strength as a result of lower fiber diameter in polymeric network [54]. Also, it has been reported that a 5-fold reduction of fiber diameter in PCL electrospun fiber will increase the mechanical strength by 20 MPa [53]. Also, another reports on electrospinning of PCL showing that an increase in fiber diameter from 0.1 μm to 3 μm will decrease the Young's modulus and tensile strength from 30.6 to 7.6 MPa and 6.3 to 0.9 MPa, respectively, as well as 4-fold increase in strain at the point of failure [55]. Another study on electrospinning of Polyvinylidene fluoride have also confirmed the influence of fiber diameter reduction on enhancing molecular arrangement and mechanical properties [56]. In our study, as shown in Table 3, CS/PVA/PEDOT:PSS (0.6) and CS/PVA/PEDOT:PSS (1) have been shown to have higher elasticity compared to other samples, which is related to less imperfections in fibrillar structure and higher crystallinity. In CS/PVA and CS/PVA/PEDOT:PSS (0.3) samples, higher fiber diameter have resulted in more imperfections in the structure, which will increase the deformation of fibers in lower stress values. CS/PVA/PEDOT:PSS (0.6) and CS/PVA/PEDOT:PSS (1) scaffolds have shown to withstand up to 16.45 MPa and 18.78 MPa, respectively, where fibers have shown to break together in a short time, leading to failure of samples. However, in CS/PVA and CS/PVA/PEDOT:PSS (0.3) samples, fibers start to break gradually from the start of deformation, which is due to high imperfection in structure of fibers. Therefore CS/PVA and CS/PVA/PEDOT:PSS (0.3) samples show lower elasticity and tensile strength and, therefore, a lower toughness (Table 3), which is the most important parameter in describing the mechanical properties [55]. Fig. 4 clearly depicts that CS/PVA/PEDOT:PSS (0.6) and CS/PVA/PEDOT:PSS (1) samples have a toughness about 35%–45% more than of CS/PVA/PEDOT:PSS (0.3) samples. Another factor that can affect the mechanical strength of scaffolds containing PEDOT:PSS can be the presence of hydrogenic bonds between OH groups in PVA and chitosan and SO_3^- groups in PSS of the conductive polymer dispersion [35,57,58].

3.5. Cell proliferation

Scaffolds were checked using MTT assay to check the cell viability on scaffolds. Optical Density (OD) results on day 1, 3 and 7 of culture are shown in Fig. 5. All scaffolds containing PEDOT:PSS have shown a better support of cellular viability and growth compared to CS/PVA sample and tissue culture plate (TCP) samples.

Fig. 5. MTT assay for mesenchymal cell proliferation on different nanofibrous scaffolds and TCP.

At day 1, TCP samples have shown a better OD compared to other samples. This may be due to a better attachment of cells to TCP substrates, as well as applied stress to cells on scaffold samples, due to presence of surface impurities from fabrication processes. It was shown in previous studies that upon seeding, mesenchymal stem cell need at least 48 h to form proper attachment for spreading and division [59]. On day 3, CS/PVA/PEDOT:PSS (0.3) and CS/PVA/PEDOT:PSS (1) scaffolds, among other scaffold samples, have shown to have better support for cell division marked by higher OD values. In contrast, cells on CS/PVA/PEDOT:PSS (0.6) scaffold have shown to have a higher cell death rate than cell division and number of live cells in the CS/PVA/PEDOT (0.6) sample on day 3 was less than the other two samples, which could be due to the poor surface properties of the scaffold, as shown in Fig. 8. The surface and linear roughness of the CS/PVA/PEDOT:PSS (0.6) sample weakness leads to a decrease in cell adhesion. This will reduce the amount of cell proliferation on

the third day. Attachment, migration and division of cells highly depends on material surface properties and their interaction with transmembrane proteins, including integrin [60,61]. In this regard, chemical and topographical feature will embrace the fibrillar structure of electrospun mats and interact with them through flexible ligands. In order to provide balance between elastic energy of cell membrane and curved structure of fibers, ligand bonds between fiber surface and cell surface receptors will form in a highly stable form. In fact, reduction of fiber diameter and increase in local curvature will cause stronger bonds in between surface receptors and curvatures to maintain the balance in elastic energy of cell membrane [61,62]. Therefore, reduction of fiber diameter will result in higher cell attachment as shown Fig. 6 and higher cell division, specifically in scaffolds containing PEDOT:PSS, Although the fiber diameter increase in all samples is evident after 3 days of cell culture, But the diameter of the fibers in the samples that containing the pedot pss is still lower than the pure sample.

Fig. 6. SEM imaging of cell culture on 3 day: (a) CS/PVA, (b) CS/PVA/PEDOT:PSS (0.3), (c) CS/PVA/PEDOT:PSS (0.6), (d) CS/PVA/PEDOT:PSS (1).

Fig. 7. 3D Laser Microscope images: (a) CS/PVA, (b) CS/PVA/PEDOT:PSS (0.3), (c) CS/PVA/PEDOT:PSS (0.6), (d) CS/PVA/PEDOT:PSS (1).

Fig. 8. Linear and surface roughness of different scaffolds.

In a similar study on PGA/Collagen scaffolds, reduction of fiber diameter from 10 μm to 0.5 μm have increased the attachment length from 40 nm to 90 nm [61]. In another study on the impact of scaffolds with sub-micron fiber diameters, on stem cell culture, human adipose derived-MSCs (hASC) have shown to have significantly increased cell attachment and cell division by reduction of fiber diameter [63]. Moreover, in culture of 3T3 fibroblasts on PCL scaffolds, it was shown that reduction of fiber diameter from 1.6 μm to 0.4 μm results in higher attachment and division of cells. However, further reduction of fiber diameter results in bead formation in fibers, which have been shown to negatively affect the attachment of cells [64].

On the other hand, surface roughness, and the factors describing it, can be very effective in describing the impact of surface roughness on cell attachment and spreading [62,65]. Surface and linear roughness of scaffold, which is shown in Fig. 7, have been analyzed by the 3D Laser Measuring Microscope. Higher surface roughness will result in higher cell-surface interaction, affecting cell attachment and division. Scaffolds containing PEDOT:PSS have shown to have higher topographical features as compared to CS/PVA and TCP surfaces, along physical properties, which in turn enhance protein adsorption on scaffolds, cellular interaction with surfaces and a better cellular support provided from scaffolds. These are confirmed by MTT assay results on day 7, where lower fiber diameter, higher surface area and surface roughness and higher conductivity have enhanced cellular metabolism and cell division on scaffold surfaces. Presence of conductive material will enhance the interaction of proteins like fibronectin with surface, which in turn supports cell behavior and metabolism. As mentioned earlier, conductive compartment in polymeric solution will increase the randomized direction of fibers, which should reduce the roughness of surface [25,26]. However, random arrangement of fibers is not the only influential parameter. Complex branching patterns in electrospinning will result in formation of areas with higher geometric patterns. Conductive polymers will influence the formation and shooting patterns of jet in different directions. CS/PVA/PEDOT:PSS (0.3) and CS/PVA/PEDOT:PSS (1) scaffolds have respectively shown the highest surface roughness and linear roughness as shown in Fig. 8, which also show better support and attachment of cells [54,62].

Therefore, considering the impurities on fibrillar structures remaining from fabrication process in initial days of culture, lower cell viability on scaffolds has been observed. Afterwards, cell-surface interactions, facilitated with surface roughness, randomly arrangement of fibers and lower fiber diameter have enhanced cell attachment, metabolic activity and support on scaffolds samples [54,62,64]. To the best of our knowledge, except for a study that reported that addition of PEDOT:PSS of more than 0.6 wt% in gelatin/bio-glass scaffolds have caused toxicity in mesenchymal stem cells, there are no other highly significant reports on toxicity of PEDOT:PSS [66].

4. Conclusion

Cardiovascular engineering requires conductive scaffolds with suitable mechanical properties. Chitosan has unique properties for tissue engineering applications but the lack of mechanical and electrical properties, especially for the purpose cardiovascular engineering, has been a major obstacle to its use. By adding PEDOT:PSS to the CS/PVA scaffolds in this study, causes significant progress in the electrical and mechanical properties of heart tissue. Especially in the CS/PVA/PEDOT:PSS (1) sample, morphological and topology properties of the scaffold are also enhanced, due to the decrease in fiber diameter. In the scaffolds containing the PEDOT:PSS, more cell support was observed than the control samples, without any cell toxicity. In fact, with the presence of PEDOT:PSS, all the properties of the base scaffold have been significantly improved at the same time. According to the results, use of PEDOT:PSS is recommended in order to build scaffolds for cardiac tissue engineering requiring special electrical and mechanical properties.

References

- [1] T.H. Qazi, *et al.* **Development and characterization of novel electrically conductive PANI-PGS composites for cardiac tissue engineering applications.** *Acta Biomater.*, 10 (2014), pp. 2434-2445
- [2] G. Zhao, X. Zhang, T.J. Lu, F. Xu. **Recent advances in electrospun nanofibrous scaffolds for cardiac tissue engineering.** *Adv. Funct. Mater.*, 25 (2015), pp. 5726-5738
- [3] World Health Organization. *Global Status Report on Noncommunicable Diseases 2010*, vol 176, World Health (2010)
- [4] D. Kai, M.P. Prabhakaran, G. Jin, S. Ramakrishna. **Polypyrrole-contained electrospun conductive nanofibrous membranes for cardiac tissue engineering.** *J. Biomed. Mater. Res. - Part A*, 99 A (2011), pp. 376-385
- [5] A.M. Martins, G. Eng, G. Caridade, F. Mano, R.L. Reis. **Electrically conductive chitosan/carbon scaffolds for cardiac tissue engineering.** *Biomacromolecules*, 15 (2014), pp. 635-643
- [6] G. Vunjak-novakovic, *et al.* **Challenges in cardiac tissue engineering.** *Tissue Eng. Part B*, 16 (2010). Number 2
- [7] J. Leor, N. Landa, S. Cohen. **Renovation of the injured heart with myocardial tissue engineering.** *Expert Rev. Cardiovasc. Ther.*, 4 (2006), pp. 239-252
- [8] T. Dvir, *et al.* **Nanowired three-dimensional cardiac patches.** *Nat. Nanotechnol.*, 6 (2011), pp. 720-725
- [9] S.M. Willerth, S.E. Sakiyama-Elbert. **Approaches to neural tissue engineering using scaffolds for drug delivery.** *Adv. Drug Deliv. Rev.*, 59 (2007), pp. 325-338
- [10] E.S. Costa-Júnior, E.F. Barbosa-Stancioli, A.A.P. Mansur, W.L. Vasconcelos, H.S. Mansur. **Preparation and characterization of chitosan/poly(vinyl alcohol) chemically crosslinked blends for biomedical applications.** *Carbohydr. Polym.*, 76 (2009), pp. 472-481
- [11] Saima Naz Khan, Aurangzeb Khan, E. Martin, Kordesch. **Conducting polymer fibers of polyaniline doped with camphorsulfonic acid.** *Mater. Res. Soc. Symp. Proc.*, 948 (2007), pp. 0-5
- [12] Y. Zhang, *et al.* **Preparation of electrospun chitosan/poly(vinyl alcohol) membranes.** *Colloid Polym. Sci.*, 285 (2007), pp. 855-863
- [13] B. Duan, C. Dong, X. Yuan. **Polymer Edition Electrospinning of chitosan solutions in acetic acid with poly (ethylene oxide).** *J. Biomater. Sci.* (2012), pp. 37-41
- [14] M. Kharaziha, *et al.* **Tough and flexible CNT-polymeric hybrid scaffolds for engineering cardiac constructs.** *Biomaterials*, 35 (2014), pp. 7346-7354
- [15] P. Khalid, V.B. Suman, M.A. Hussain, A.B. Arun. **Toxicology of carbon nanotubes - a review.** *Int. J. Appl. Eng. Res.*, 11 (2016), pp. 148-157
- [16] J.R. Lee, S. Ryu, S. Kim, B.S. Kim. **Behaviors of stem cells on carbon nanotube.** *Biomater. Res.*, 19 (2015), pp. 19-24
- [17] X. Huang. **Bioresorbable Materials and Their Application in Electronics.** Cambridge University Press (2017), 10.1017/9781108290685
- [18] Y. Zhang, *et al.* **Cytotoxicity effects of graphene and single-wall carbon nanotubes in neural pheochromocytoma-derived pc12 cells.** *ACS Nano*, 4 (2010), pp. 3181-3186

- [19] Laleh Ghasemi-Mobarakeh, *et al.* **Application of conductive polymers, scaffolds and electrical stimulation for nerve tissue engineering.** *J. Tissue Eng. Regen. Med.*, 5 (2011), pp. e17-e35
- [20] R. Balint, N.J. Cassidy, S.H. Cartmell. **Conductive polymers: towards a smart biomaterial for tissue engineering.** *Acta Biomater.*, 10 (2014), pp. 2341-2353
- [21] A. Peramo, *et al.* **In situ polymerization of a conductive polymer in acellular muscle tissue constructs.** *Tissue Eng. Part A*, 14 (2008), pp. 423-432
- [22] V. Karagkiozaki, *et al.* **Bioelectronics meets nanomedicine for cardiovascular implants: PEDOT-based nanocoatings for tissue regeneration.** *Biochim. Biophys. Acta Gen. Subj.*, 1830 (2013), pp. 4294-4304
- [23] M.H. Bolin, *et al.* **Nano-fiber scaffold electrodes based on PEDOT for cell stimulation.** *Sens. Actuators B Chem.*, 142 (2009), pp. 451-456
- [24] M. Mohammadian, A.K. Haghi. **Systematic parameter study for nano-fiber fabrication via electrospinning process.** *Bulg. Chem. Commun.*, 46 (2014), pp. 545-555
- [25] Z.M. Huang, Y.Z. Zhang, M. Kotaki, S. Ramakrishna. **A review on polymer nanofibers by electrospinning and their applications in nanocomposites.** *Compos. Sci. Technol.*, 63 (2003), pp. 2223-2253
- [26] M. Li, Y. Guo, Y. Wei, A.G. MacDiarmid, P.I. Lekes. **Electrospinning polyaniline-contained gelatin nanofibers for tissue engineering applications.** *Biomaterials*, 27 (2006), pp. 2705-2715
- [27] J. Lee, *et al.* **Synthesis of samarium-cobalt sub-micron fibers and their excellent hard magnetic properties.** *Front. Chem.*, 6 (2018), pp. 1-7
- [28] M. Mohammadian, A.K. Haghi. **Systematic parameter study for nano-fiber fabrication via electrospinning process.** *Bulg. Chem. Commun.*, 46 (2014), pp. 545-555
- [29] W.K. Son, J.H. Youk, T.S. Lee, W.H. Park. **The effects of solution properties and polyelectrolyte on electrospinning of ultrafine poly(ethylene oxide) fibers.** *Polymer*, 45 (2004), pp. 2959-2966
- [30] A. Baji, Y.W. Mai, S.C. Wong, M. Abtahi, P. Chen. **Electrospinning of polymer nanofibers: effects on oriented morphology, structures and tensile properties.** *Compos. Sci. Technol.*, 70 (2010), pp. 703-718
- [31] M. Koosha, H. Mirzadeh, M.A. Shokrgozar, M. Farokhi. **Nanoclay-reinforced electrospun chitosan/PVA nanocomposite nanofibers for biomedical applications.** *RSC Adv.*, 5 (2015), pp. 10479-10487
- [32] E.A. El-Hefian, M.M. Nasef, A.H. Yahaya. **The preparation and characterization of Chitosan/Poly (Vinyl Alcohol) blended films.** *E-J. Chem.*, 7 (2010), pp. 1212-1219
- [33] I.F.M. Rumengan, *et al.* **Structural characteristics of chitin and chitosan isolated from the biomass of cultivated rotifer, *Brachionus rotundiformis*.** *Int. J. Fish. Aquat. Sci.*, 3 (2014), pp. 12-18
- [34] A. Mohamed Saat, M.R. Johan. **The surface structure and thermal properties of novel polymer composite films based on partially phosphorylated poly(vinyl alcohol) with aluminum phosphate.** *Sci. World J.*, 2014 (2014)
- [35] C. Sriprachuabwong, *et al.* **Inkjet-printed graphene-PEDOT:PSS modified screen printed carbon electrode for biochemical sensing.** *J. Mater. Chem.*, 22 (2012), pp. 5478-5485
- [36] L. Jin, *et al.* **A facile approach for the fabrication of core-shell PEDOT nanofiber mats with superior mechanical properties and biocompatibility.** *J. Mater. Chem. B*, 1 (2013), pp. 1818-1825
- [37] K. Gipson, K. Stevens, P. Brown, J. Ballato. **Infrared spectroscopic characterization of photoluminescent polymer nanocomposites.** *J. Spectrosc.*, 2015 (2015)
- [38] T.F. Otero, J.M. Sansinena. **Artificial muscles based on conducting polymers.** *Bioelectrochem. Bioenerg.*, 38 (1995), pp. 411-414
- [39] M. Zambrzycki, A. Fraczek-Szczypta. **Conductive hybrid polymer composites based on recycled carbon fibres and carbon nanofillers.** *J. Mater. Sci.*, 53 (10) (2018), pp. 7403-7416
- [40] L. Bay, T. Jacobsen, S. Skaarup, K. West. **Mechanism of actuation in conducting polymers: osmotic expansion.** *J. Phys. Chem. B*, 105 (2001), pp. 8492-8497
- [41] M.R. Gandhi, P. Murray, G.M. Spinks, G.G. Wallace. **Mechanism of electromechanical actuation in polypyrrole.** *Synth. Met.*, 73 (1995), pp. 247-256
- [42] G. Wallace, G. Spinks. **Conducting polymers - bridging the bionic interface.** *Soft Matter*, 3 (2007), pp. 665-671

- [43] A. Khan, M. Jawaid, A. Khan, A. Asiri. **Electrically Conductive Polymers and Polymer Composites: from Synthesis to Biomedical Applications**. Wiley-VCH Verlag GmbH & Co. KGaA (2018)
- [44] Damijan Miklavicic, *et al.* **Electric Properties of Tissues**. Wiley Encyclopedia of Biomedical Engineering, Copyright & 2006 John Wiley & Sons, Inc (2006)
- [45] J.D. Bronzino. **The Biomedical Engineering Handbook**. (second ed.), CRC Press (1999)
- [46] J.H. Collier, J.P. Camp, T.W. Hudson, C.E. Schmidt. **Synthesis and characterization of polypyrrole-hyaluronic acid composite biomaterials for tissue engineering applications**. *J. Biomed. Mater. Res.*, 50 (2000), pp. 574-584
- [47] Tsubasa Takano, Aya Mikazuki, Takaomi Kobayashi. **Conductive polypyrrole composite films prepared using wet cast technique with a pyrrole–cellulose acetate solution**. *Polym. Eng. Sci.* (2014), 10.1002/pen.23545
- [48] H. Okuzaki, S. Takagi, F. Hishiki, R. Tanigawa. **Ionic liquid/polyurethane/PEDOT:PSS composites for electro-active polymer actuators**. *Sens. Actuators B Chem.*, 194 (2014), pp. 59-63
- [49] C.R. Broda, J.Y. Lee, S. Sirivisoot, C.E. Schmidt, B.S. Harrison. **A chemically polymerized electrically conducting composite of polypyrrole nanoparticles and polyurethane for tissue engineering**. *J. Biomed. Mater. Res. - Part A*, 98 A (2011), pp. 509-516
- [50] A.T.1 Rad, N. Ali, H.S. Kotturi, M. Yazdimamaghani, J. Sma, D. Vashae, L. Tayebi. **Conducting scaffolds for liver tissue engineering**. *J. Biomed. Mater. Res. A*, 102 (11) (2014), pp. 4169-4181
- [51] Aref Shahini, Mostafa Yazdimamaghani, Kenneth J. Walker, Margaret A. Eastman, Hamed Hatami-Marbini, J. Smith Brenda, John L. Ricci, V. Madhally Sundar, Daryoosh Vashae, Lobat Tayebi. **3D conductive nanocomposite scaffold for bone tissue engineering**. *Int. J. Nanomed.*, 9 (2014), pp. 167-181
- [52] Longo Elson, F. de A. La Porta. **Recent Advances in Complex Functional Materials**. (first ed.), Springer International Publishing (2017)
- [53] S.C. Wong, A. Baji, S. Leng. **Effect of fiber diameter on tensile properties of electrospun poly(ϵ -caprolactone)**. *Polymer*, 49 (21) (2008), pp. 4713-4722
- [54] K. Young, *et al.* **Strong dependence of mechanical properties on fiber diameter for polymer -nanotube composite Fibers : differentiating defect from orientation effects**. *Am. Chem. Soc.*, 4 (11) (2010), pp. 6989-6997
- [55] H.H. Kim, M.J. Kim, S.J. Ryu, C.S. Ki, Y.H. Park. **Effect of fiber diameter on surface morphology, mechanical property, and cell behavior of electrospun poly(ϵ -caprolactone) mat**. *Fibers Polym.*, 17 (7) (2016), pp. 1033-1042
- [56] Avinash Baji, Yiu-Wing Mai, Shing-Chung Wong. **Effect of fiber size on structural and tensile properties of electrospun polyvinylidene fluoride fibers**. *Polym. Eng. Sci.*, 55 (2015), pp. 1812-1817
- [57] N. Romyen, S. Thongyai, P. Praserttham, G.A. Sotzing. **Enhancement of poly(3,4-ethylenedioxy thiophene)/poly(styrene sulfonate) properties by poly(vinyl alcohol) and doping agent as conductive nano-thin film for electronic application**. *J. Mater. Sci. Mater. Electron.*, 24 (8) (2013), pp. 2897-2905
- [58] D. Alemu Mengistie, P.C. Wang, C.W. Chu. **Effect of molecular weight of additives on the conductivity of PEDOT:PSS and efficiency for ITO-free organic solar cells**. *J. Mater. Chem. A*, 1 (34) (2013), pp. 9907-9915
- [59] R.H. Lee, *et al.* **Cellular physiology and biochemistry characterization and expression analysis of mesenchymal stem cells from human bone marrow and adipose tissue**. *Cell. Physiol. Biochem. Orig.*, 14 (2004), pp. 311-324
- [60] E.A. Cavalcanti-Adam, A. Micoulet, J. Blümmel, J. Auernheimer, H. Kessler, J.P. Spatz. **Lateral spacing of integrin ligands influences cell spreading and focal adhesion assembly**. *Eur. J. Cell Biol.*, 85 (3–4) (2006), pp. 219-224
- [61] Furong Tian, Hossein Hosseinkhani, Mohsen Hosseinkhani, Khademhosseini Ali, Yoshiro Yokoyama, Giovanni Gomez Estrada, Hisatoshi Kobayashi. **Quantitative analysis of cell adhesion on aligned micro- and nanofibers**. *J. Biomed. Mater. Res. A*, 84 (2) (2008), pp. 291-299
- [62] Robert Chunhua Zhao. **Essentials of Mesenchymal Stem Cell Biology and its Clinical Translation**. (first ed.), Springer Netherlands (2013)

- [63] S.A. Tuin, B. Pourdeyhimi, E.G. Lobo. **Creating tissues from textiles: scalable nonwoven manufacturing techniques for fabrication of tissue engineering scaffolds.** *Biomed. Mater.*, 11 (1) (2016), p. 15017
- [64] M. Chen, P.K. Patra, S.B. Warner, S. Bhowmick. **Role of fiber diameter in adhesion and proliferation of NIH 3T3 fibroblast on electrospun polycaprolactone scaffolds.** *Tissue Eng.*, 13 (3) (2007), pp. 579-587
- [65] Masoud Mozafari, Mehrnoush Mehraien, Daryoosh Vashaee, Lobat Tayebi. **Electroconductive nanocomposite scaffolds: a new strategy into tissue engineering and regenerative medicine.** *Nanocompos. New Trends Dev.* (2012), 10.5772/51058
- [66] M. Yazdimamaghani, M. Razavi, M. Mozafari, D. Vashaee, H. Kotturi, L. Tayebi. **Biomineralization and biocompatibility studies of bone conductive scaffolds containing poly(3,4-ethylenedioxythiophene):poly(4-styrene sulfonate) (PEDOT:PSS).** *J. Mater. Sci. Mater. Med.*, 26 (12) (2015), pp. 1-11