

Biocomposite conductive scaffold based on PEDOT:PSS/nHA/chitosan/PCL: Fabrication and characterization

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Abstract

Biomaterial-based scaffolds with suitable characteristics are highly desired in tissue engineering (TE) application. Biocomposites based on polymer and ceramics increase the chance for modulating the properties of scaffold. In recent years, researchers have considered conductive polymers to be used in TE application, due to their conductivity. This property has a good impact on tissue regeneration. A suitable design for bone substitute that consists of considerations such as material component, fabrication technique and mechanical properties. The previous studies on PEDOT:PSS/nHA/CS showed high wettability rate but low mechanical properties. Polycaprolactone (PCL) is a biodegradable and biocompatible polymer with a low wettability. The incorporation of PCL inside biocomposite can lead to the decrement in wettability and increment in mechanical property. In addition, this paper would examine the feasibility of blending of PCL and chitosan to fabricate PEDOT:PSS/nHA/CS composite scaffold. The fabrication technique of freezing/lyophilization was used in this study. The scaffolds were characterized morphologically using scanning electron microscopy (SEM). Wettability was studied using a contact angle instrument. The attenuated total reflectance fourier transform infrared spectroscopy (ATR-FTIR) spectra interpreted the presence of polymeric ingredients within composite scaffold. Conductivity of the scaffolds was measured using a Digital Multimeter. In-vitro biological evaluation of the scaffolds was studied using human skin Fibroblast (HSF) cell line. The morphological study of biocomposite PEDOT:PSS/nHA/CS/PCL scaffold revealed random pore sizes and 66% porosity. Contact angle of the scaffold was increased and the swelling property and pore sizes were decreased after blending of PCL polymer. The viability of HSF cells on biocomposite PEDOT:PSS/nHA/CS/PCL scaffold was 85%. After 7 days, SEM analysis revealed the presence of cells on the surface of scaffold. In conclusion, the results suggested that PEDOT:PSS/nHA/CS/PCL biocomposite scaffold was non-toxic to cells and has suitable properties.

Keywords: Biocomposite, tissue engineering, conductive polymer

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INTRODUCTION

In recent years, biotechnology has influenced the world by introducing new areas of research, such as tissue engineering (TE). The human body is a complex network that contains various organs and tissues that have a specific function. As the human body ages, it suffers numerous changes, leading to the loss or damage of tissues, or a change in their functions. The tissue engineering approach involves isolating cells from tissue, ideally from a patient, and then seeding them onto a biodegradable scaffold structure for use as an implant for patients. The scaffold should be able to mimic the natural cell environment and activity. The scaffold should possess some important properties, namely, non-toxic to cells, better mechanical properties, optimum surface properties, proper degradation time and rate, porous architecture, good conductivity and high porosity.

There are several techniques to fabricate a suitable TE scaffolds. The freezing/lyophilization technique is known as a novel processing technique for producing porous scaffolds. It is a suitable fabrication technique for both composite and polymer scaffolds. It is found that through this method, scaffold with better morphology and mechanical

properties can be fabricated (Jafari *et al.*, 2017; Lin *et al.*, 2002). This method can produce scaffold with controlled microstructure and porosity by changing the parameters such as quenching temperature, polymer concentration and water phase concentration of solvent (Kasaju *et al.*, 2016; Whang *et al.*, 2002).

In the development of tissue engineering scaffold, the selection of suitable biomaterials is important. The previous study on conductive biocomposite showed good potential of bone tissue engineered scaffolds in biomedical application, but it has high wettability rate which might affect mechanical structure of scaffold after applying in host-body (Lari *et al.*, 2016). In this case, Polycaprolactone (PCL) has suitable properties to be used due to its biodegradability, biocompatibility, low wettability and hence, better mechanical properties. PCL can be blended with other polymers to improve stress crack resistance, degradation rate, and cell adhesion (Jin *et al.*, 2015). By blending PCL with chitosan to form biocomposite scaffold, the mechanical properties are expected to be improved.

On the other hand, poly (3,4-ethylenedioxythiophene)-poly(4-styrene sulfonate) (PEDOT:PSS) is a conductive polymer that used to fabricate new composite scaffold, which is a new approach. Recently,

scaffold-based tissue engineering has mostly focused on the enhancement of bone healing by electrical stimuli. This effect is induced by using various compositions of conductive polymer (CP). Since 1980s, various biocompatible conductive polymers have been used in several medical applications (Guimard *et al.*, 2007) and CP has the potential to be used in composite scaffolds for bone regeneration (Li *et al.*, 2006; Mozafari *et al.*, 2012). PEDOT:PSS copolymer has a moderate band gap and good stability in the doped state (Groenendaal *et al.*, 2000). Studies on conductive polymer composite scaffolds for tissue engineering, particularly PEDOT:PSS, will represent a new tissue engineering approach.

The most commonly studied potential materials in tissue engineering are chitosan (CS) and nano-hydroxyapatite (nHA). The combination of CS and nHA has been shown to possess good biocompatibility and suitable mechanical properties (Lari *et al.*, 2016).

The present study reports the fabrication and characterization of biocomposite PEDOT:PSS/nHA/CS/PCL scaffold. Blending of CS and PCL polymers and incorporation of nHA may improve the properties of the scaffold such as morphology, surface and mechanical properties.

The scaffolds are needed to have proper microstructure which has a significant influence on the cell attachment and proliferation (Chen *et al.*, 2007, Guimaraes *et al.*, 2010). The surface hydrophilicity and hydrophobicity of scaffolds play an important role in cell activity. The morphology study of fabricated scaffold was evaluated through scanning electron microscope (SEM). Furthermore, other characterization studies on water contact angle and attenuated Fourier transform – infrared spectroscopy (ATR-FTIR) were assessed as well. Finally, the cytotoxicity of PEDOT:PSS/nHA/CS/PCL scaffold was evaluated using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide).

EXPERIMENTAL

Materials

Hydroxyapatite nanoparticles (nHA) were prepared in-house using the nanoemulsion technique (Zhou *et al.*, 2008). PEDOT:PSS (1.1wt% dispersion in water, high-conductive grade), chitosan (medium molecular weight, degree of deacetylation = 90%), poly- ϵ -caprolactone (PCL), molecular mass (M_n) 70,000–80,000, Dulbecco's modified eagle medium (DMEM), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), phosphate buffered saline (PBS) and fetal bovine serum (FBS) were purchased from Sigma-Aldrich, USA. NaOH and other reagents were all of analytical grade.

Fabrication of biocomposite scaffold

To prepare a composite scaffold with 5% PCL (w/v), 1 g of PCL was weighed and dissolved in 20 ml glacial acetic acid under magnetic stirrer for 30 min at room temperature. Then, 2% (w/v) (0.4 g CS) chitosan was added to the solution and kept for stirring for hours, until it was completely dissolved and a clear, viscous solution was obtained. Then, 0.4 ml PEDOT:PSS was added into the polymer solution and magnetically stirred. After addition of 0.05g nHA, the solution was homogenized first prior to molding and lyophilization. For this purpose, the solution was transferred to glass molds and kept at -80°C for 24 hours. Solid-liquid or liquid-liquid phase separation was induced when the mixtures of solution were kept at -80°C . The frozen solutions were then lyophilized by a freeze-dryer (LABCONCO Freeze Dry System, USA) for 48 hours.

Characterization of biocomposite scaffold

Initially, the prepared scaffolds were cut with sharp razor blade into the cylinder shape specimens (1.2 cm diameter x 0.2 cm height of cylinder). Then, the prepared composite scaffolds were treated in NaOH (1N) for naturalization. Then, the scaffolds were washed thoroughly and dried. The prepared samples were then examined under scanning electron microscope (SEM, Hitachi TM3000, Japan) at an accelerating voltage of 15 kV. An energy dispersive X-ray (EDX) was used to confirm the constituent elements in the scaffold. The pore sizes of the scaffolds were measured using an image analysis software (ImageJ, NIH, USA). The porosity of the scaffolds was measured using a liquid-displacement method based on Archimedes' principle, as

described elsewhere (Sun *et al.*, 2014). The wettability of all three types of scaffolds was measured via the contact angle (VCA-Optima, AST Inc., USA) by dropping 2 μl of deionized water onto the scaffolds. Chemical bonding analysis of samples was performed using an ATR-FTIR (PerkinElmer 5 Series, USA Modal). All spectra were recorded in the range of 4400–600 cm^{-1} . This method was used for detecting the presence of functional groups in scaffold specimens. The scaffold conductivity was measured using a Digital Multimeter (VC830L, Victor Instruments, China). Furthermore, the scaffolds were evaluated for their cytotoxicity. The specimens in triplicate (diameter 1.2 cm and width 0.2 cm) were kept in a 24-well plate and sterilized. In this process, samples were washed three times using PBS with 1% penicillin and streptomycin, followed by exposure to UV light for 2 hours. A human fibroblast cell line (HSF 1184, ECACC, UK) was used to measure the cytotoxicity of composite scaffolds. These cells were cultured in DMEM and incubated at 37°C in an atmosphere of 5% CO_2 and 95% humidity. At 70–80% confluence, the cells were seeded on each scaffold in 24-well plates. The cell morphology of seeded cell was observed by using SEM. In this case, after a definite time interval, all specimens were moved to other well plate and washed with PBS. Then 4% glutaraldehyde was used to fix the cells for 1 hour at 4°C . After that, prepared specimens were underwent gradual dehydration in ethanol with different percentages (30%, 50%, 70%, 95%, and 100%). Samples were dried under biosafety cabinet (BSC) (1300 Series, Thermo Scientific). Finally, the samples were sputter-coated with gold for 20 second and observed with SEM.

RESULTS AND DISCUSSION

Physicochemical analysis of PCL-incorporated PEDOT:PSS/nHA/CS scaffold

The microstructure of fabricated biocomposite PEDOT:PSS/nHA/CS/PCL scaffolds was examined by SEM. The scaffolds were fabricated via freezing/lyophilization technique. Figure 1 shows the micrograph of fabricated scaffold. The average pore size was $59 \pm 82 \mu\text{m}$. The pore structure is an important factor for cell harboring, penetration and migration (Armentano *et al.*, 2010). The morphology study showed elongated and irregular pores, as well as connectivity between the pores.

The porosity of PEDOT:PSS/nHA/CS/PCL was measured by using ethanol through liquid displacement method. Table 1 illustrates the results of porosity measurement. The measured porosity in PEDOT:PSS/nHA/CS scaffold was 74% but, after addition of PCL, it was decreased to 66%. Blending of PCL with CS resulted in the increase of density, causing in the decrease of porosity. A report showed that the preferred porosity for cell penetration was 60-90 % (Chung *et al.*, 2008).

The ATR-FTIR analysis was carried out to identify the presence of functional groups within the composite scaffold. Fig.2 (a) illustrates the spectra of pure PCL that consisted of a strong peak at $\sim 1700 \text{cm}^{-1}$ due to the presence of carbonyl group. Fig.2 (b) indicates a broad peak at $3500\text{--}3200 \text{cm}^{-1}$ due to $-\text{NH}_2$ and $-\text{OH}$ groups in chitosan which were physically embedded within the scaffold (Cooper *et al.*, 2011). All of the PEDOT:PSS and nHA peaks were affected by the strong absorptions of the PCL peaks due to the higher PCL concentration (Wang *et al.*, 2011). These spectra indicated crosslinking of the chitosan chains.

The wettability of scaffold was evaluated through water contact angle measurement. Table 1 illustrates the results of two types of scaffolds. According to the results, the sample containing PCL indicated the higher water contact angle. By blending of PCL and CS formulation, the water contact angle was increased from $46.23^\circ \pm 2.34$ (PEDOT:PSS/nHA/CS) to $78.31^\circ \pm 3.22$ ((PEDOT:PSS/nHA/CS/PCL). CS has hydrophilic property because of the existence of three functional groups of amine, carboxyl and hydroxyl within its molecular structure (Chandrasekaran *et al.*, 2011) Meanwhile, PCL has hydrophobic characteristic. Blending of PCL with CS has resulted in moderate hydrophilicity and thus, rendering the suitability of it for biomedical application. Swelling behavior of scaffolds was presented in Figure 3, after soaking in PBS buffer. It showed that scaffolds containing PCL has lower water uptake compared to

PEDOT:PSS/nHA/CS. The water uptake for PEDOT:PSS/nHA/CS scaffold was 11%. After blending with PCL, water uptake was decreased to 6%. There was a direct relationship between water uptake and hydrophilicity (Roozbahani *et al.*, 2013). Due to hydrophilicity and diffusion process, highly porous material would show higher water uptake property.

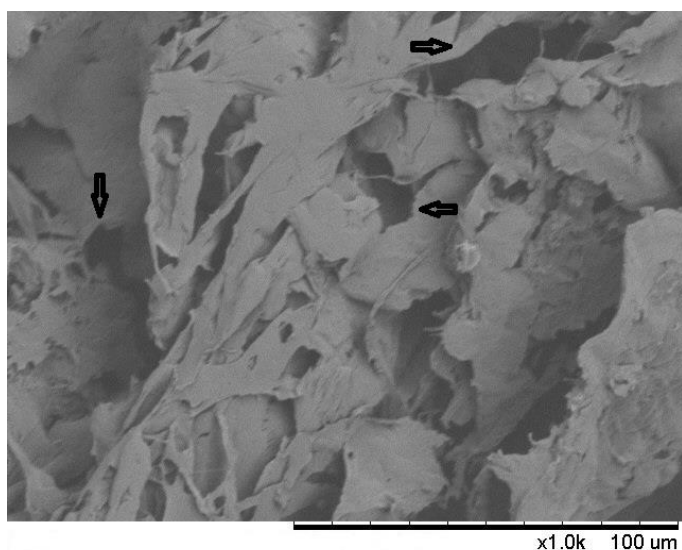


Fig. 1 SEM micrograph of PCL-incorporated PEDOT:PSS/nHA/CS biocomposite scaffold. The arrows show pores.

Table 1 Pore size, porosity and water contact angle measurements of biocomposite scaffold.

Scaffold	Pore size (μm)	Porosity (%)	Contact angle ($^\circ$)
PEDOT:PSS/nHA/CS	110 ± 72	74 ± 2	46.23 ± 2.34
PCL/PEDOT:PSS/nHA/CS	59 ± 82	66 ± 5	78.31 ± 3.22

Furthermore, porosity and pore sizes are also important factors that have effects on the swelling measurements (Min *et al.*, 2008). In addition, both samples were hydrophilic, mostly due to the hydrophilic property of chitosan, which has an important role on swelling rate. The suitable water uptake rate is important for tissue engineered scaffold for the healing of wound, in order to prevent dehydration and exudate built-up on the wound (Chandrasekaran *et al.*, 2011).

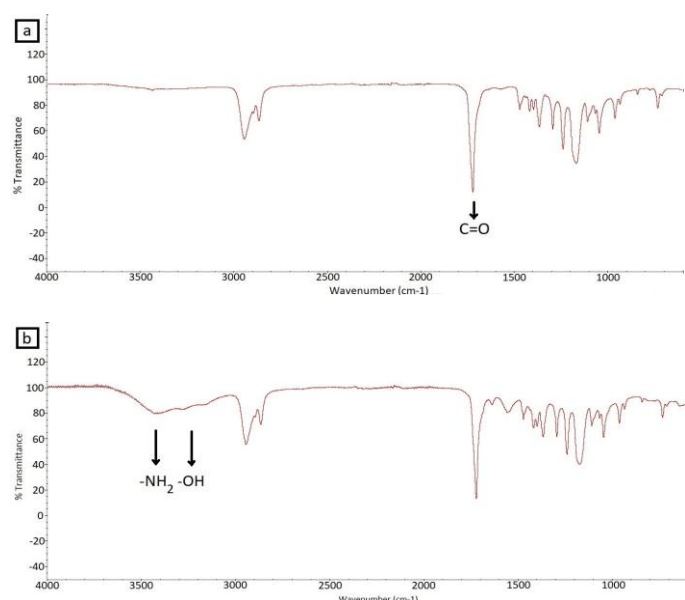


Fig. 2 ATR-FTIR graph (a) pure PCL (b) PCL-incorporated PEDOT:PSS/nHA/CS biocomposite scaffold.

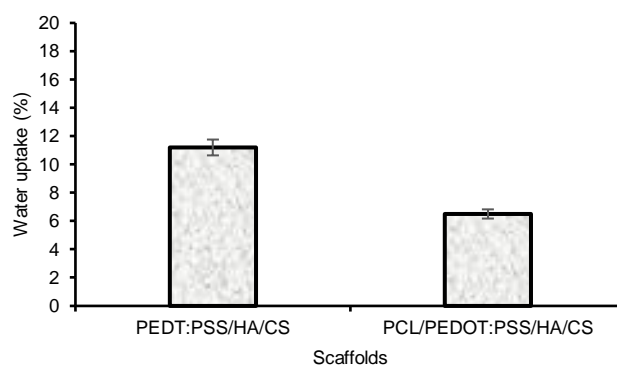


Fig. 3 The swelling behavior of biocomposite scaffolds.

The conductivity of fabricated scaffold was measured and calculated by using Pouillet's equation. The conductivity of PEDOT:PSS/nHA/CS/PCL scaffold was recorded at $3.55 \pm 0.64 \mu\text{S/m}$, which was decreased from $9.72 \pm 0.78 \mu\text{S/m}$ for PEDOT:PSS/nHA/CS scaffold. In this case, the conductivity was affected by addition of PCL. Although the PCL sample indicated low conductivity, it might still exhibit effect on better cell activity than the scaffolds without conductive property. Previous study reported that the electrical conductivity of scaffold could enhance the cell-scaffold interaction due to enhanced intracellular signalling process (Lari *et al.*, 2016).

Cytotoxicity of PCL-incorporated PEDOT:PSS/nHA/CS scaffold

The implant material for using scaffold structure has to be non-toxic to mammalian cells. The materials which are toxic or release waste or byproducts will damage the cells and give effect on environment of targeted implant. Therefore, in this study the cytotoxicity of prepared sample was tested by indirect MTT assays. The calorimetric method was used to measure the capacity of live cells to reduce the tetrazolium salt of MTT and change to formazan crystal with darker purple color. This test was carried out for seven days. MTT assay was performed to evaluate the cytotoxicity of scaffolds after 7 days and the results were shown in Fig. 4. The viability of HSF cells on biocomposite PEDOT:PSS/nHA/CS/PCL scaffold was 85% in comparison to the control (tissue culture plate without scaffold sample).

The morphology of HSF cells on scaffold was evaluated by SEM after 7 days of culture. The SEM micrograph in Fig. 5 shows the morphology of cells that was spread on the scaffold surface. The HSF cells were connected to each others and the cells were filopodia anchored on the surface of the scaffold. HSF cells were interacted and integrated well with the scaffolds while the ends of the dendritic extensions were observed to be penetrated into the walls of the scaffolds.

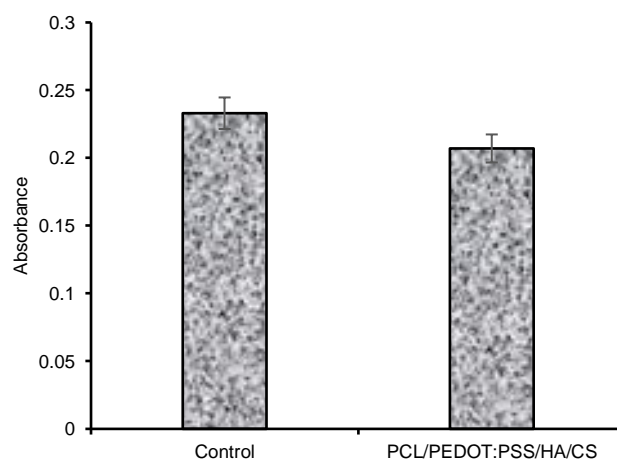


Fig. 4 MTT assay of HSF on PCL-incorporated PEDOT:PSS/nHA/CS biocomposite scaffold compared to control.

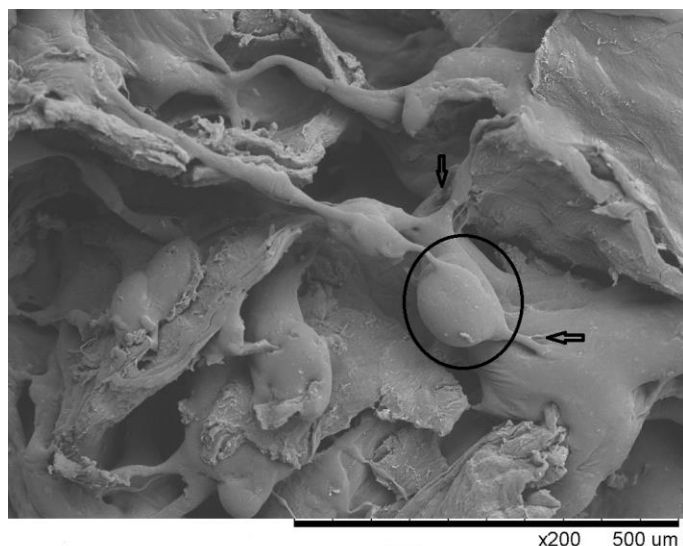


Fig. 5 SEM micrograph of HSF proliferation on PCL/PEDOT:PSS/HA/CS after 7 days culture. A cell was adhered to the surface of scaffold with filopodia (black arrows).

CONCLUSION

In conclusion, PEDOT:PSS/nHA/CS/PCL scaffolds were fabricated using freezing and lyophilization techniques. The morphology study showed elongated and irregular pores. The pores were well-connected. ATR-FTIR spectra showed the chemical bonding was caused by the presence of chitosan and PCL. The contact angle results indicated that the addition of PCL was moderated the hydrophilicity of composite scaffold and led to decrease in wettability. Swelling results were affected due to decrease in hydrophilicity of PEDOT:PSS/nHA/CS/PCL scaffold. The scaffold was non-toxic to the cells and the cells were well attached to the scaffold. PEDOT:PSS/nHA/CS/PCL scaffold has lower wettability. This change might lead to the increase in compressive mechanical properties of the scaffold.

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