Polymer 124 (2017) 95–100

Contents lists available at ScienceDirect

Polymer

journal homepage: www.elsevier.com/locate/polymer

In *vitro* biocompatibility of vapour phase polymerised conductive scaffolds for cell lines

Jong Seob Choi [a], Jin Seul Park [b], Boram Kim [c], Byong-Taek Lee [c], Jin-Heong Yim [b, *]

[a] Convergence Medical Device Research Center, Gumi Electronics and Information Technology Research Institute (GERI), 350-27, Gumidaero, Gumi, Gyeongbuk 39253, South Korea
[b] Division of Advanced Materials Engineering, Kongju National University, Budaedong 275, Seobuk-gu, Cheonan-si, Chungnam 31080, South Korea
[c] Institute of Tissue Regeneration, Department of Regenerative Medicine, Soonchunhyang University, Cheonan, Chungcheongnamdo, South Korea

ARTICLE INFO

Article history:
Received 18 May 2017
Received in revised form
14 July 2017
Accepted 18 July 2017
Available online 19 July 2017

Keywords:
Conductive 3D scaffolds
Conducting porous structure
Cell viability

ABSTRACT

Conductive polymers have been intensively studied for their potential applications in cell therapy, neural regeneration, and drug delivery. They can also be used as scaffolds for tissue engineering. Ideal scaffolds are porous, interconnected structures that allow cell entry and can thus mimic in *vivo* three-dimensional (3D) tissue regeneration. In the present study, poly(3,4-ethylenedioxythiophene)-silica and polypyrrole-silica composites were fabricated by a two-step procedure for use as 3D conductive porous scaffolds. A hybrid conductive composite layer was first formed by vapour phase polymerisation on a 3D microparticle assembly. Microparticles were then selectively removed, yielding a highly porous skeletal structure. The in *vitro* biocompatibility of the scaffolds was investigated by culturing with HepG2 and MC3T3-E1 cells, and evaluating cell viability with the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay and examining cell morphology by scanning electron and confocal microscopy. We found that the two scaffolds effectively promoted cell proliferation, indicating that conductive polymer-based scaffolds can be useful for investigating the behaviour of muscle and nerve cells under electrical stimulation.

1. Introduction

Current in *vitro* methods for screening drugs and toxic materials mainly rely on flask or plastic dish-based tissue culture systems owing to their low cost, rapidity, and ease of preparation. However, in *vitro* two-dimensional (2D) cell culture methods do not fully recapitulate the complexity and heterogeneity observed in *vivo*. Animal models are also limited by high cost, the unfeasibility of high-throughput screening, and ethical issues. To date, there are no appropriate alternatives to these approaches despite efforts made by many studies [1]. One possibility is to use 3D biomaterial-based scaffolds for tissue engineering and regenerative medicine that which are structurally similar to human organs and provide a suitable environment for cell-cell and cell-matrix interactions [2,3]. Most scaffolds in 3D cell-based culture systems are hydrogels obtained by 3D bioprinting [4], nano/microfiber meshes generated by electrospinning [5], or organic/inorganic composites formed by freeze drying [6]. These 3D scaffolds typically consist of either natural polymers [7] or proteins [7], or of biodegradable polymers [8] that are biocompatible and enhance the adhesion or the physical and mechanical properties of cells [9]. However, these materials are electrically non-conductive, which is a barrier for the regeneration of cardiac muscle and neural cells that require electrical stimulation. To overcome this drawback, it has been suggested that conductive polymers and carbon-based materials be combined with biocompatible materials to improve their electrical properties; such composites have been used to promote nerve and muscle cell growth and regeneration [10–12]. Conductive polymers as well as electrical composites have been investigated as drug delivery systems, for electrical stimulation therapy, and tissue regeneration owing to their stable/high conductivity and the possibility of controlling electrical stimulation [13]. However, in the case of electrical composites, conductivity is inversely proportional to biocompatibility according to the volume ratio of conductive additives to biomaterial, which is prohibitive for biomedical applications. To overcome this problem, we previously fabricated highly conductive scaffold: poly(3,4-ethylenedioxythiophene)-silica (PEDOT-SiO_2) composites [14]. We have described the protocol

* Corresponding author.
E-mail address: jhyim@kongju.ac.kr (J.-H. Yim).

http://dx.doi.org/10.1016/j.polymer.2017.07.047
0032-3861/

for constructing 3D conductive skeletal scaffold by vapour phase polymerisation (VPP) [14].

In the present work, we used a two-step procedure to generate two kinds of 3D conductive scaffolds (PEDOT-SiO_2 and Polypyrrole-silica (PPy-SiO_2)) with a high porosity. The conductive composites were first synthesised by VPP on a 3D microparticle assembly. Microparticles were then selectively removed to obtain a highly porous skeletal structure. The two resultant composites were compared in terms of biocompatibility and effect on cell viability. HepG2 hepatocellular carcinoma cells and MC3T3-E1 preosteoblasts were cultured on the scaffolds, and cell viability and behaviour were evaluated with the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and by scanning electron microscopy (SEM) and confocal microscopy. Our results demonstrate that these two highly conductive scaffolds can effectively promote cell proliferation as a preliminary study for electrical stimulation of electrically reactive cells such as muscle cells and neurons under 3D artificial scaffold.

2. Material and methods

2.1. Fabrication of conductive scaffolds

3, 4-EDOT (Sigma-Aldrich, St. Louis, MO, USA) as the monomer for PEDOT, Py (Acros Organics, Geel, Belgium) as the monomer for PPy, ferric p-toluenesulfonate (Sigma-Aldrich) as the oxidising agent, and TEOS (Samchun Pure Chemical Co., Seoul, Korea) as a precursor of the SiO_2 inorganic network were used without further purification. 1-Butanol (Junsei Chemical Co., Tokyo, Japan), which was used to dilute the oxidising agent, was used as received.

PS (MW = 350,000; Sigma-Aldrich) was used to fabricate the 3D assembly template; 5 wt% PS was dissolved in DCM (≥99%; Sigma-Aldrich) as an oil phase. The solution was poured into a 30 ml syringe and then injected into a beaker via a syringe pump at a constant rate of 15 ml/h along with an aqueous solution of 1 wt% poly(vinyl alcohol) (MW ≈ 9000–10,000; Sigma-Aldrich), with stirring at 150 rpm. After evaporating the DCM, PS particles were washed three times with distilled water and dried in an oven at 60 °C for 24 h; they were then dispersed in ethanol, placed in a mold, and heated at 80 °C using a hot press to form a 3D assembly. FTS was dissolved in 1-butanol at a concentration of 20 wt% by stirring the solution for approximately 30 min. The PS assembly template was immersed in the mixed oxidant solution for 5 min; it was spin-coated for 10 s at 300 rpm and then continuously coated for 30 s at 500 rpm. After drying the mixed oxidant solution at 60 °C for 5 min, the FTS-coated PS assembly template was transferred to a VPP chamber that was maintained as a closed reaction system, with the reaction proceeding at 60 °C and room temperature for PEDOT and PPy, respectively. The conductive monomer/TEOS mixture (volume ratio of 2:1) was placed at the bottom of the VPP reaction chamber, and FTS-coated PS particles were exposed to the mixed EDOT/TEOS or Py/TEOS vapour for 24 h. After polymerisation, PS particles were immersed in distilled water for 1 h to remove any unreacted oxidant and then dried at 60 °C. DCM was added dropwise to the conductively coated PS template to selectively remove PS microparticles, yielding the 3D conductive scaffolds.

2.2. Cell culture

The conductive scaffolds were sterilised under ultraviolet light followed by 70% ethanol, washed twice with phosphate-buffered saline (PBS), and coated with 10 μg/ml fibronectin at 37 °C for 1 h HepG2 and MC3T3-E1 cells were seeded onto scaffolds at a density of 10^{4-6}/scaffold in a 24-well plate with culture medium and cultured for 1, 3, and 7 days, with the medium changed every 2 days.

2.3. SEM analysis of HepG2 cells cultured on the conductive scaffold

HepG2 cells cultured on the 3D conductive scaffolds were washed twice with PBS and fixed with 6% glutaraldehyde for 2 h at room temperature. After thorough washing with distilled water, the cells were sequentially immersed in 30%, 40%, 50%, 60%, 70%, 80%, 90%, and 100% ethanol solution for 10 min. The remaining ethanol in the scaffold was evaporated by natural drying for 1 day. Samples were coated with palladium, and visualised by SEM and imaged.

2.4. MTT assay

The proliferation of cells grown on scaffolds was evaluated with the MTT assay (Sigma-Aldrich). After 1, 3, or 7 days of culture, 100 μl of MTT solution was added to each conductive scaffold for 4 h. After removing the medium, 1 ml dimethyl sulfoxide (Samchun Pure Chemical Co.) was added to each well under gentle shaking to dissolve the formazan crystals. The extract was transferred to a 96-well plate and the absorbance at 595 nm was measured on a microplate reader.

The growth of MC3T3-E1 cells (1×10^4/scaffold) seeded on conductive scaffolds was examined by immunofluorescence analysis. Briefly, after 1, 3, or 7 days of culture, the scaffolds were rinsed twice with PBS and fixed in 4% paraformaldehyde for 10 min at room temperature, permeabilised in 0.25% Triton X-100 for 10 min, and blocked in 1% bovine serum albumin for 1 h. Cells were labelled with FITC-conjugated phalloidin at 4 °C overnight. Nuclei were stained with Hoechst 33342. Cell morphology was examined under a confocal microscope (FV10i-W; Olympus, Tokyo, Japan) at 40× magnification.

2.5. Statistical analysis

Data were analysed using Graph Pad Prism v.5 software (GraphPad Inc., La Jolla, CA, USA). Differences between groups were evaluated by two-way analysis of variance followed by a Bonferroni post-hoc test. $P < 0.05$ was considered statistically significant.

3. Results and discussion

3.1. Fabrication of conductive scaffolds with high-porosity

The protocol for fabricating conductive scaffolds with high porosity is schematically illustrated in Fig. 1. Polystyrene (PS) particles with a diameter of 50–150 μm were assembled by colloidal sedimentation using a specific mold and were used as the template for 3D conductive porous scaffolds (Fig. 1a). The surface of the template was coated with ferric p-toluenesulfonate (FTS) solution added dropwise to initiate polymerisation of conductive monomers and tetraethyl orthosilicate (TEOS) (Fig. 1b). The FTS-coated, PS assembled template was transferred to a VPP chamber (Fig. 1c), which was maintained as a closed system during polymerisation. Assembled conductive composites were removed from the chamber (Fig. 1d) and immersed in dichloromethane (DCM) to remove the PS template, yielding 3D conductive scaffolds with macropores around 50–150 μm in diameter (Fig. 1e). VPP-based hybridisation with inorganic materials was adopted to enhance the electrical and mechanical properties. Our previous studies showed that VPP could confer higher conductivity to PEDOT than solution-based polymerisation [15], and that mechanical properties were also increased by adding Si-based inorganic materials during polymerisation [16]. VPP with monomers of conducting polymer and metal alkoxide produced mechanically robust conductive hybrid polymer–metal oxide composites [17–20]. The conductive monomer