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Effect of SWCNT introduction in random copolymers on material properties and fibroblast long term culture stability

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ABSTRACT

Polymeric nanostructured biomaterials can be used as synthetic cell interfaces with important applications in the study and control of cellular processes. Herein, we developed multifunctional nanocomposites based on synthesized biodegradable and biocompatible copolyesters of poly(butylene 1,4-*trans*-cyclohexanedicarboxylate) (PBCE) containing ether–linkages, and single walled carbon nanotubes (SWCNTs), employed as functional phase. Surface, thermal and mechanical characterization of the polymer and nanocomposite films were performed. The influences of AC conductivity and interfacial polarization on dielectric relaxation process, as well as the correlation between the dielectric behaviors and SWCNT content were investigated by measuring the dielectric properties. The effect of SWCNT incorporation, and amount of ether-oxygen atoms was also investigated in terms of fibroblast long-term culture stability, by performing adhesion and proliferation studies of cells seeded on the biomaterial surface, at different time points. Results showed that polymeric conductive nanocomposites were successfully developed with a low percolation threshold, and SWCNT presence maintained the polymer thermal degradation behavior. Moreover, the culture of primary fibroblasts indicated that these advanced functional materials are biocompatible and guarantee the cell adhesion and growth, being suitable substrates for regenerative medicine applications. Finally, their versatile structure and chemical properties may provide a robust platform to gain insight into cell–biomaterial interactions, being an important step towards the better understanding and control of cell interactions with nanomaterials.

1. Introduction

Electrically conductive polymeric materials have recently attracted considerable interest from academic and industrial researchers for exploring their potential biomedical application, such as in biosensors, drug delivery systems, biomedical implants and tissue engineering [1]. In this regard, nanocomposite technology permits to incorporate conductive nanostructures with unique features in a polymer matrix, in order to transfer and integrate specific properties into a single material, thereby enabling the development of new multifunctional materials [2]. Previous studies showed that the introduction of conducting fillers to such a well-studied tissue engineering matrix as polycaprolactone allows to obtain conducting biocompatible composites which can be used to proliferate cells under electrical stimulation [3,4].

One of the challenges of degradable and conducting polymers is the optimization of their conductivity. New polymer based materials that have a low content of the conducting species and still possess sufficient conductivity are still very desirable. Following the discovery of carbon nanotube (CNT) in 1991 [5], these nanosized materials have quickly become a technological platform for researchers in diverse fields of science including materials and biomedical engineering. CNT is one of the most useful nanoscale agents for material property modulation because of its extraordinary mechanical, electrical, and thermal properties [6–8]. The advantage of nanotubes lies mainly in increasing the surface area,

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improving electrical conductivity, and introducing improved properties of electron-transfer reactions [9,10].

Among other biodegradable and biocompatible polymer matrices, aliphatic polyesters (APs) are very interesting and well known materials thanks to their outstanding properties and versatility. Recently, our research group focused its attention on the design and development of heteroatom-containing APs whose properties have been tuned by properly varying the kind of heteroatom introduced along the polymer backbone [11,12], the chemical composition [13,14] and the molecular architecture [15,16].

In this work, we have explored the potentiality of novel polymeric nanostructured biomaterials developed combining newly synthesized biodegradable and biocompatible random copolymers based on 1,4-*trans*-cyclohexanedicarboxylic acid and diglycolic acid [17] with single walled carbon nanotubes (SWCNTs), employed as functional phase. In particular, poly(butylene 1,4-*trans*-cyclohexanedicarboxylate) homopolymer (PBCE) and two copolymers containing different amounts of ether–oxygen atoms, P(BCE90BDG10) and P(BCE70BDG30), have been considered as matrices of SWCNT based nanocomposites. The effect of incorporation of different amounts of SWCNTs (0.1–0.5–0.75–1%wt) on physico-chemical properties was deeply analyzed. The effect of both SWCNT incorporation, and copolymer composition was also investigated in terms of biocompatibility, adhesion and proliferation studies of human fibroblasts seeded on the biomaterials surface. We selected primary fibroblasts based on their own activity as supporting cells within the tissues. In fact, these cells are necessary for the tissue architectures by engaging direct interaction with neighboring cells through the production of extracellular matrix proteins [18]. Therefore, culture of fibroblasts on polymer surfaces, neat or SWCNT based nanocomposites, could be informative for tissue engineering application studies.

2. Material and methods

1,4-*trans*-cyclohexanedicarboxylic acid (CEDA) containing 99% of *trans* isomer, diglycolic acid (DGA), 1,4-butanediol (BD), and titanium tetrabutoxide ($Ti(OBu)_4$) (Aldrich) were reagent grade products; CEDA, DGA and BD were used as supplied, while $Ti(OBu)_4$ was distilled prior to use. All the reagents were purchased from Sigma Aldrich (Milan, Italy). Single walled carbon nanotubes (SWCNTs) were obtained from Thomas Swan & Co. Ltd (Elicarb™, Durham, UK).

2.1. Nanocomposite film development

Poly(butylene cyclohexanedicarboxylate/diglycolate) random copolymers (P(BCEmBDGn)) were synthesized in bulk by the usual two step melt polycondensation, as reported elsewhere [19]. Briefly, 1,4-butanediol (BD) and different molar ratios of CEDA and DGA, were employed in the syntheses, using 20% mol excess of glycol with respect to dicarboxylic acids and a concentration of titanium butoxide catalyst ($Ti(OBu)_4$) of about 150 ppm of Ti/g of polymer. Three different polyesters have been prepared: the homopolymer PBCE and two copolymers, namely P(BCE90BDG10) and P(BCE70BDG30) containing 10 and 30 mol% of BDG units, respectively.

The reactions were carried out in a 250-mL stirred glass reactor, with a thermostatted silicon oil bath; temperature and torque were continuously recorded during the polymerization. The first stage was run under pure nitrogen flow at a temperature of 190 °C until more than 90% of the theoretical amount of methanol was distilled (about 90 min). In the second stage, the pressure was reduced to about 0.1 mbar and the temperature was increased to 250 °C. The polymerizations were carried out until a constant torque value was measured.

Single walled carbon nanotube based nanocomposite films were prepared by means of solvent casting method in chloroform ($CHCl_3$), as previously reported and films of 90 μm thickness were obtained [20]. Composite samples containing 0.1, 0.5, 0.75 and 1% wt SWCNTs with respect to the polymer initial weight were prepared with the three different matrices. Neat PBCE, P(BCE90BDG10) (BDG10) and P(BCE70BDG30) (BDG30) polymeric films were also prepared by solvent casting for comparison.

2.2. Nanocomposite film characterization

2.2.1. Surface characterization

Field emission scanning electron microscope (FESEM, Zeiss Supra25) was used to analyze the surface morphology of the produced composite films, after gold sputtering.

Static contact angle measurements were performed on surface films by using a KSV CAM101 instrument (Helsinki, Finland) under ambient conditions by recording the side profiles of deionized water drops for image analysis. Five drops were observed on different areas for each film and contact angles were reported as the average value ± standard deviation.

2.2.2. Thermal and mechanical characterization

Thermogravimetric analysis (TGA) was performed by a Seiko Exstar 6300, Japan, microbalance under nitrogen flow (250 mL min^{-1}), a temperature range of 30–800 °C, and a heating rate of 10 °C min^{-1}.

Dynamic-mechanical analysis (DMA) was performed by an ARES N_2 instrument (Rheometric Scientific, USA) in a dynamic time sweep test, at a frequency of 1 Hz, and room temperature (RT). The strain 0.05% was chosen by means of an iso-frequency test in the elastic linear region. The reported storage elastic modulus (G') is the average of tests performed on five rectangular samples (10 mm × 40 mm, about 0.09 mm thick).

2.2.3. Dielectrical characterization

The real and imaginary parts of the complex impedance (Z^*) of the synthetized polymers and developed nanocomposite films were measured by Hewlett Packard 4284A Precision LCR Meter at RT, in the 20 Hz ÷ 1 MHz frequency range.

High frequency impedance measurements were made by a HP-4291A analyzer, with a 16453A text fixture in the frequency range of 10^6–10^9 Hz at RT.

2.3. Biological analysis

2.3.1. Culture of fibroblast on biopolymers

Rat Fibroblasts (rFFF; LONZA) were cultured in 25 cm^2 tissue-culture flasks (TCP) in DMEM (Dulbecco's Modified Eagle Medium, EuroClone) containing 10% of heat-inactivated fetal bovine serum (FBS), 2 mM L-glutamine, and 100 U/mL of penicillin-streptomycin (Euroclone) and incubated at 37 °C in a humidified atmosphere with 5% CO_2. The medium was refreshed every 3 days.

2.3.2. Long-time culture of rFFFs on biopolymers

rFFFs were seeded on the substrates selected in the study at a starting concentration of 500 cells/mL of control medium and maintained in culture for 27 days. As internal control rFFF were also cultured on TCP and glass coverslip. At defined time points, rFFF were harvested and evaluated for cytotoxicity, proliferation, and adhesion to polymer surfaces, according to methods described below.