Effect of multi-walled carbon nanotubes on the cross-linking density of the poly(glycerol sebacate) elastomeric nanocomposites

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GRAPHICAL ABSTRACT
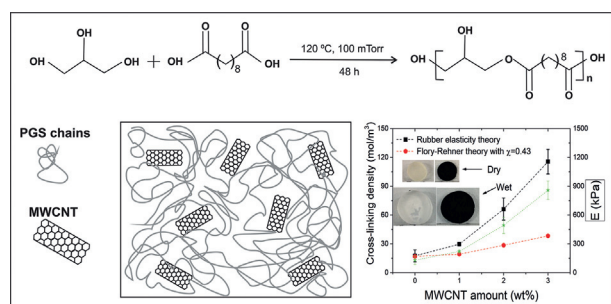
ARTICLE INFO

ABSTRACT

Processing conditions deeply affect the mechanical, chemical and biological properties of elastomeric based nanocomposites. In this work, multi-walled carbon nanotubes (MWCNTs) were dispersed in poly(glycerol sebacate) (PGS) prepolymer, followed by curing under vacuum at 120 °C. It was observed an increase of the water contact angle with the amount of MWCNTs added, as well as the tensile strength and Young modulus, without compromising the elastomeric behaviour of the pristine PGS matrix. The cross-linking degree was determined by the Flory-Rehner swelling method and through the mechanical rubber elasticity model, and an increase of more than six-fold was observed, which demonstrates the chemical conjugation between the MWCNTs and the PGS polymer chains, resulting in stiff and elastomeric nanocomposites. Finally, *in vitro* cell culture of adult mouse hypothalamus neurons A59 cells showed good support for cell viability and stimulation for axons and dendrites growth. The unique features of these nanocomposites make them promise for biomedical applications, as soft tissue substrates with tailored mechanical properties.

1. Introduction

Carbon nanotubes (CNTs) are widely used for different applications, such as supercapacitors [1], lithium batteries [2], wearable devices [3], electrochemical biosensors [4] and biomedical engineering [5], due to its extraordinary mechanical, electrical and thermal properties.

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CNTs are known to enhance the thermal and mechanical properties of polymer based composites [6]. Additionally, they are easily dispersed in liquid media by ultrasound techniques, and due to their intrinsic electrical properties, they are widely used induce electrical conductivity to insulator materials [6,7].

Owing to its unique features, the blending of CNTs with polymeric matrices allows the development of nanocomposites with enhanced properties when compared to the initial raw materials. For instance, the electrical conductivity can change dramatically, within several orders of magnitude, by adding a small amount of CNTs to the insulating polymer matrix [8].

Since the work of Ajayan et al. [9] in 1994, numerous polymers were reported for the manufacture of carbon nanotube-polymer based composites, including epoxy [9], polystyrene [10], polypropylene [11], poly(methyl methacrylate) [12], polyurethane, polyimide [13], among other soft matrix materials. With the introduction of carbon nanotubes into the polymer matrix, the physical properties (e.g. mechanical, and electrical) could be enhanced, especially when chemically functionalised fillers are used [6].

Electrically conductive composites have great potential as flexible electrodes in displays, electronic paper, motion-detected devices [14] or even for biomedical engineering purposes. Collagen [15], chitosan [5], poly(propylene fumarate) [16], among other biocompatible polymeric materials blended with MWCNTs, showed positive results as suitable substrates for tissue engineering applications. The diameters of carbon nanotubes are ranging from 1 nm (single-walled carbon nanotubes) to 10–100 nm (multi-walled carbon nanotubes), which are similar to those of small nerve fibers, synaptic contacts and growth cone filopodia [17], providing the morphological clue and support for neuron adhesion and long term survival [18]. Good electrical conductivity could lead to the formation of neuronal circuits and related stimulation [19,20], making carbon nanotubes excellent candidate for nerve tissue repair [18].

A homogeneous distribution of the MWCNTs among the polymer matrix is usually associated to mechanical [21] and electrical properties enhancement of the overall nanocomposites. Different methods have been reported in the preparation of the MWCNT-polymer composites, including solution mixing, in-situ polymerisation, melt blending and chemical treatment [7]. Solution mixing shows better efficiency in separating the carbon bundles, reducing cluster size, promoting a more homogeneous dispersion, which enhances the mechanical and electrical properties of the nanocomposites [14,22].

Poly(glycerol sebacate) (PGS) is a tough biodegradable elastomer, synthesised by poly-condensation of glycerol and sebacic acid [23]. The starting materials of PGS are safe for *in vivo* applications, as glycerol has been approved to be used in foods and sebacic acid is a natural human metabolic intermediate [24]. The mechanical properties and degradation kinetics of PGS could be tailored by controlling the curing time and temperature [25]. Moreover, PGS is a biocompatible and biodegradable elastomer used in soft tissue engineering applications, i.e. heart muscle [25], vascular tissue [26], cartilage [27], retina [28], nerve conduits [29].

Nanocomposites of multi-walled carbon nanotubes-poly (glycerol sebacate) (MWCNT-PGS) showed that the incorporation of the fillers increased the overall mechanical stiffness without compromising significantly the elasticity [30], which is an important feature for soft tissue applications [31]. In this work, MWCNT-PGS nanocomposites with different filler loadings were synthesised by solution mixing and two-step polycondensation synthesis of PGS. The chemical functional groups and molar mass of PGS prepolymer was studied by nuclear magnetic resonance (NMR). After curing, nanocomposites morphological, wettability, mechanical and chemical properties were systematically assessed. The influence of the filler in the polymer cross-linking density was studied by Flory-Rehner theory and correlated with the rubber elasticity theory. Finally, the suitability of the developed nanocomposites for biomedical applications was explored through *in vitro* biological assays of A59 neuron cell line.

2. Materials and methods

2.1. Synthesis of the nanocomposites

The MWCNT-PGS nanocomposites were prepared following the reported methods [23,30]. Briefly, PGS prepolymer was prepared by mixing equimolar of glycerol (≥99.5%, Sigma Aldrich) and sebacic acid (>95% GC, Sigma Aldrich) at 120 °C under argon for 24 h. Pristine PGS matrix was obtained by pouring PGS prepolymer into a poly(tetrafluoride) (PTFE) based mould and placed inside an oven at 120 °C under 100 mTorr vacuum (Vacuo-Temp, Selecta), during 48 h.

Nanocomposites of PGS with MWCNTs (Play with Carbon Pty) were prepared by dispersing the filler in tetrahydrofuran (THF, ≥99.9%, Sigma-Aldrich) with the help of an ultrasound bath (Bandelin, Sonorex Super RK106) during 6 h, followed by addition of the desired amount of PGS prepolymer. The nanocomposite solution was them placed on top of a magnetic stirrer (Velp) until complete dissolution. The amount of solvent related to the prepolymer was 1 g of PGS for 5.5 ml of solvent. The amount of MWCNTs on the elastomeric matrix ranged from 0 and 3 wt%. After complete dissolution of the PGS prepolymer, the solution was poured into a PTFE mould, and placed in a fume cupboard overnight to allow complete solvent evaporation, followed by the curing procedure described previously for PGS matrix.

2.2. Material characterisation

NMR (nuclear magnetic resonance) spectra, including 1D ^1H, 1D ^{13}C, 2D COSY (correlation spectroscopy), 2D TOCSY (total correlation spectroscopy), and 2D HSQC (heteronuclear single-quantum correlation spectroscopy), of the prepolymer in deuterated chloroform ($CDCl_3$, 99.8 atom %, with 0.1% v/v tetramethylsilane, Sigma-Aldrich) were collected with a Varian Inova 500-MHz NMR spectrometer at 25 °C. The molar weight of the prepolymer was measured by Shimadzu UFLC system equipped RID-10A and a Sedere ELSD detector, at room temperature using THF at a flow rate of 1 ml/min on Phenomenex Phenogel column (00k-0644-k0).

The nanocomposite samples were fractured after immersion in liquid nitrogen for 30 min. Platinum coating of around 10 nm was deposited on the cross section of each sample by sputtering (Dynavac Sputter Coater) and scanning electron microscope (SEM) images were taken by JEOL 7500 field emission scanning electron microscope.

Contact angle measurements (sessile drop in dynamic mode) were performed at room temperature in a Data Physics OCA20 device using ultrapure water as test liquid. A sessile drop (2 μL) of ultrapure water (Milli-Q, Millipore) was deposited on the surface of the samples and the contact angle was measured using a Data-Physics OCA 20 goniometer and SCA20 software. For each sample, contact angles were measured on 8 different spots and the result was expressed as their average and standard deviation. Infrared measurements (FTIR) were performed at room temperature (IRAffinity-1S, Shimadzu). Spectra were collected after 32 scans and with a resolution of 2 cm^{-1}, from 4000 to 650 cm^{-1}.

Mechanical measurements were performed in tensile mode on dog-bone shape samples with a gauge length of 18 mm, 2 mm width and 1 mm thick, in a universal testing machine (EX-L, Shimadzu) with a load cell of 10 N, at a testing rate of 1 mm/min. All experiments were performed at room temperature (∼22 °C). The mechanical parameters were obtained as the average of five individual measurements.