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Facile fabrication of poly(ε-caprolactone)/graphene oxide membranes for bioreactors in tissue engineering

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ABSTRACT

Promising polymer membranes of blended biocompatible poly(ε-caprolactone) and graphene oxide (PCL/GO) and PCL and partially reduced graphene oxide (PCL/rGO) with outstanding water and nutrient transport properties for cell culture bioreactors were prepared using phase inversion at mild temperatures. Some of the prepared PCL/GO membranes were subjected to a 'chemical-free' GO post-reductive process using UV (PCL/GO/UV) irradiation. The PCL/rGO membranes exhibited 2.5 times higher flux than previously reported biocompatible polymer membranes for cell culture bioreactors, which was attributed to the highly interconnected porosity. On the other hand, the formation of PCL-graphene oxide composites in the PCL/GO and PCL/GO/UV membranes was not conclusive according to spectroscopic analyses, thermal analyses and mechanical characterization, probably due to the low graphene oxide loading in the membranes (0.1%w/w). The presence of graphene oxide-based nanomaterials in the polymer matrix slightly reduced the mechanical properties of the PCL-graphene oxide membranes by limiting the polymer chain mobility in comparison to that of the plain PCL membranes. However, their mechanical stability was sufficient for the applications pursued. Finally, the biocompatibility assay indicated that the incorporation of GO and rGO into the PCL matrix enhanced the uniform distribution and morphology of the glioblastoma cells on the surface of the PCL-graphene oxide membranes.

1. Introduction

The medical field represents one of the most relevant markets for membranes when compared to other industrial applications, aside from the water industry [1]. Different relevant applications for membranes in medicine include drug delivery, haemodialysis, artificial organs and tissue engineering. Membranes for tissue engineering can be used as scaffolds for cells to be implanted *in vivo* to enhance cell differentiation in tissues and in bioreactors for *in vitro* cell culture proliferation and regeneration of *in vitro* 3D tissues. The 3D tissues regenerated by this technology can be further implanted *in vivo* or used as alternatives to animal models for drug screening or artificial organ supports. Particularly, perfusion bioreactors, using membranes as scaffolds, provide a series of benefits, such as reducing the internal and external diffusive limitations for nutrient transport. Furthermore, perfusion bioreactors enable the application of mechanical stimuli on cultured cells, in contrast to other bioreactor designs for tissue engineering [1,2].

The phase inversion casting technique is a versatile and facile method for producing highly porous scaffolds with nanofibrous structures and scalable, 3D, commercial membrane products. Phase inversion is the most important method employed for developing nanocomposite polymer membranes for water treatment applications [3]. For example, antifouling nanocomposite polyethersulfone (PES) membranes for ultrafiltration and nanofiltration have been produced by dispersing carbon nanotubes [4], TiO_2 particles [4] or graphene oxide (GO) [5] in the polymer solution prior to phase inversion. Similarly, to fabricate scaffolds for tissue engineering, the incorporation of nanomaterials, such as carbon nanotubes (CNT) [6], graphene [7], hydroxyapatite (HA) [8,9], and silver nanoparticles [10,11], in different polymer matrices to achieve mechanical reinforcement or to favour chemical or electrical cell stimuli or antibacterial properties has been investigated [12].

Due to the outstanding structural, optical, mechanical, thermal and electrical properties of graphene and its derivatives, these materials have been used in different application niches, such as energy, electronics, and biomedicine [13,14]. Particularly, the electroactivity of

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neural cells has promoted the use of graphene and its derivatives for neural tissue regeneration [15]. The biocompatibility and toxicity of graphene and graphene derivatives has been the source of controversial discussion among the research community. The thorough revision by Volkov et al. [16] showed the potential cytotoxicity of graphene and graphene derivatives and the potential risks under different types of exposures to these nanomaterials. However, they also found multitude of other interesting experimental works, where graphene and graphene derivatives demonstrated their improved biocompatibility for different biomedical applications, included implantable devices and regenerative medicine. Meanwhile long-term cytotoxic effects of graphene and its derivatives are elucidated, the potentiality of these nanomaterials should be explored. In our previous work [17], poly(ε-caprolactone) (PCL) membranes fabricated using phase inversion exhibited high porosity and a morphology that enhanced the adherence and proliferation of the neural type cells. We consider that the incorporation of small amounts of graphene oxide-based nanomaterials in our former PCL membranes may improve the intrinsic properties of the polymer matrix [18], i.e., mechanical reinforcement, electrical and/or thermal conductivity, nutrient flux and antifouling as well as ameliorating the intrinsic PCL-neural cell biocompatibility. The most common fabrication method used to produce PCL/GO composite scaffolds for tissue engineering is electrospinning [19–21]. In addition, electrospun composites of PCL with commercial graphene [22] and graphene produced using arc discharge methods [23] have been prepared. Ramzani and Karimi [24] compared the loading effects of graphene nanomaterials on the mechanical properties of electrospun composites of PCL with GO and rGO, respectively, and observed a critical graphene loading of 0.1 wt% in the PCL. While novel needleless electrospinning techniques have recently improved the production of electrospun fibres at large scale (up to 1.6 m) [25,26], the technique still does not reach the production scale (hundreds of meters) that can be achieved by means of phase inversion. Alternative methods to electrospinning for producing PCL/GO nanocomposites that have been reported in the literature involve solvent casting methods using complex and extreme temperature conditions and chemicals [21,27] or laborious and time consuming in situ polymerization techniques [28]. However, to our knowledge, the formation of PCL-graphene oxide composite membranes to be used as scaffolds in bioreactors for tissue engineering using the simple phase inversion technique under mild conditions has not been reported previously in the literature.

In this work, flat membranes of GO or partially reduced graphene oxide (rGO) with PCL were produced using phase inversion under mild temperature conditions and in the absence of toxic reductive chemicals. The effects of the oxidation state of the graphene oxide nanomaterials on the morphological, chemical and thermal characteristics and mechanical and nutrient transport properties of the PCL-graphene oxide membranes were assessed. The possible formation of PCL-graphene oxide nanomaterial composites was evaluated. Additionally, glioblastoma cell culture tests were conducted as preliminary tests for the biocompatibility of the membranes prepared in this study for use in bioreactors for neural tissue engineering.

2. Experimental

2.1. Materials

PCL pellets (Mw, 80 kDa), bovine serum albumin (BSA, A9647, Fraction V, p ≥ 96%) and dibasic sodium phosphate (Na_2HPO_4) were supplied by Sigma Aldrich, (Spain). Graphite powder (99%) and N-methyl pyrrolidone (NMP, 99%, extrapure) were purchased from Acros Organics. Sulfuric acid (95–98%) (H_2SO_4), hydrochloric acid (37%) (HCl), potassium permanganate ($KMnO_4$), sodium nitrate ($NaNO_3$), sodium chloride (NaCl), potassium chloride (KCl) and potassium dihydrogen phosphate (KH_2PO_4) were provided by Panreac. Hydrogen peroxide (H_2O_2, 30% v/v) was purchased from Scharlab (Spain), and 2-propanol (IPA, 99%) was obtained from Oppac (Spain). The aliphatic solvent, Shellshol D70, was supplied by Shell Chemicals (The Netherlands). All reagents were used as purchased.

2.2. Synthesis of the graphene oxide and reduced graphene oxide

GO was synthesized by chemical oxidation of graphite powder following a modified Hummer's method [29,30]. Briefly, 3 g of graphite powder and 1.5 g of $NaNO_3$ were added to 70 mL of H_2SO_4, and the mixture was stirred in an ice bath. Next, 9 g of $KMnO_4$ was slowly added to the solution at a constant temperature of 35 °C over 20 min. Afterwards, ultrapure water was added, and the temperature was raised to 98 °C for 15 min. The excess of $KMnO_4$ was removed with H_2O_2 and washed with ultrapure water to obtain graphite oxide. The graphite oxide was exfoliated using ultrasonication (VCX 500, Sonics & Materials, Inc., USA) for 30 min and centrifuged (Centrifuge 5810, Eppendorf, Spain) for 1 h. The GO powder was dried at 50 °C for 24 h.

Reduced graphene oxide (rGO) was synthesized using a hydrothermal method with the GO produced previously, according to an adapted method from Ribao et al. [30]. In summary, the GO was redispersed in ultrapure water (0.5 mg/mL) by sonication and heated at 200 °C during 3 h in a Teflon lined autoclave. The rGO precipitated during this process. The rGO was finally dried at 50 °C for 24 h.

2.3. Preparation of the PCL-graphene oxide flat membranes

The casting and phase inversion techniques described elsewhere for plain PCL scaffolds [17] were adapted here for preparing the PCL-graphene oxide flat membranes. First, a dispersion of GO or rGO in NMP was prepared using sonication for 30 min. After that, PCL was added in the GO/NMP or rGO/NMP dispersion and stirred (Roller Shaker 6 Basic, IKA, Spain) for 48 h at 37 °C until achieving a uniform PCL solution. The weight percentages of PCL and the GO (PCL/GO) or rGO (PCL/rGO) nanomaterials in the polymer solution were 15%w/w and 0.1%w/w, respectively. The nanomaterial loading was selected based on the critical loading of 0.1 wt% that was found by Ramzani and Karimi [24] during the preparation of PCL-graphene composites using electrospinning techniques. In addition, it was experimentally observed that using a 1%w/w graphene loading led to mechanically unstable membranes (see Fig. S1 of Appendix A. Supplementary material). Lower loading concentrations (0.25% and 0.5%w/w) were also tested unsuccessfully. The polymer solution was left to degasify overnight at room temperature and casted on a glass plate using a doctor blade casting knife through a 0.2 mm slit. The casted solution was immediately submerged into a 100%v/v IPA coagulation bath until the polymer film was completely precipitated. Then, the membrane was placed into a new IPA coagulation bath to complete the solvent exchange for 24 h. To completely remove the solvent traces, the PCL/GO and PCL/rGO films were subsequently immersed in ultrapure water that was changed periodically during 72 h. Furthermore, certain PCL/GO membranes were subjected to a UV post-treatment using a UV lamp (365 nm, 6 W, Model EA-160/FE, Spectroline, USA) for 48 h, for the purpose of reducing the graphene oxide present in the PCL/GO membrane. The membranes obtained from this procedure are referred to as PCL/GO/UV. Control membranes containing only PCL (15%w/w PCL in NMP) were also prepared for comparison.

2.4. Physical characterization

The structure and morphology of the surface and cross section of the PCL-graphene oxide membranes were determined using scanning electron microscopy (SEM, EVO MA 15, Carl Zeiss, Germany) at a voltage of 20 kV. For the cross-section images, the samples were frozen in liquid nitrogen and fractured. All the samples were kept overnight at 30 °C under vacuum and were gold sputtered before examination.

The thickness of the flat scaffolds, δ, was measured using an