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Effect of carboxylic acid functionalized graphene on physical-chemical and biological performances of polysulfone porous films

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ABSTRACT

This study highlights the preparation and characterization of new polysulfone/carboxylic acid functionalized graphene (PSF/G-COOH) porous composite films. Materials structure, morphology, topography, hydrophilic–hydrophobic character, as well as thermal, mechanical and biological behavior were thoroughly evaluated. Raman spectrometry, X-ray diffraction and transmission electron microscopy evidenced the formation of homogenous configurations, with well-dispersed G-COOH layers within the PSF matrix. Both film sides were found to develop larger pores in the presence of G-COOH, whereas changes of selective layer thickness and macrovoids shape were also observed. Profilometry emphasized a smoothing effect imposed by G-COOH on both surfaces, ~50% reduction of surface roughness was computed for 2 wt.% G-COOH. No significant vary was noticed for water contact angle, whereas biocomposites thermal and mechanical stability were found to be positively related to G-COOH concentrations. Biocompatibility and cell cytoskeleton assessments revealed good biocompatibility and emphasized cells affinity for G-COOH rich areas.

1. Introduction

Aimed to raise awareness of global current health status, numerous studies highlight the increase of both acute and chronic disorders occurrence over the past decades [1,2]. With general public relying on the eased medical act and enjoying noxious lifestyles, young people are frequently involved in accidents, while old ages are often associated with cardiovascular disease, liver failure, diabetes, or kidney failure [3,4]. Given this challenging situation, biomedical engineering has ascended with multiple and ingenious solutions in order to make human body reconstruction more accessible in nowadays. In this regard, biopolymers science has taken its well-deserved place within Research and Development. In contrast with the natural polymers, synthetic polymers bring out great benefits through their wide variety and optimal properties and thus found multiple clinical applications [5]. Such materials are used for the fabrication of disposable bags and flasks, suture wires and dental biomaterials, parts of implants and contact lens, catheters and stents, adhesives and meshes, as well as films and membranes for oxygenators, dialyzers, drug delivery systems and tissue engineering supports [6,7]. Consequently, synthetic polymers can be processed into a wide range of forms, amongst which porous films and membranes can be easily produced by common extrusion or solvent evaporation processes, through more ingenious techniques such as nanoarchitectonics, as well as by dry/wet phase inversion [8]. On the one side, the polymer melt can be extruded through cooled rolls to form flat cast films, or processed through blow extrusion when tubular films are desired [9]. On the other side, the bottom-up approaches are more often applied when envisaging precisely designed architectures at nanoscale. For instance, self-assembled monolayers and layer-by-layer strategies are reliable methods, which provide the advantage of process controllability in films assembly [8,10]. Not least among polymer film fabrication are the phase inversion techniques, either dry or wet, which imply the precipitation of polymers into thin films [11]. One of the most common polymers frequently processed to films and membranes for biomedical use is polysulfone (PSF), a thermoplastic polymer containing aryl-SO2-aryl repetitive units [12].

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When processed by wet phase inversion it forms asymmetric configurations, which are characterized by superior chemical and thermal stability and proper biocompatibility [7]. An important advantage from biomedical point of view is its high resistance against most types of sterilization, whereas it is disadvantaged by hydrophobic nature [7,13]. Thus overcoming its fouling phenomenon represents a debated subject for PSF membranes and films optimization. In order to impose a hydrophilic character or promote anti-fouling properties for PSF, numerous studies report the use of polymeric modifiers such as polyamide [13] and poly[2,2′-(m-phenylene)-5,5′-dibenzimidazole] [14], as well as $CaCO_3$ particles [15] or carbon based nanomaterials [16]. During the past decades, carbon allotropes and their conjugates tremendously ascended the scale of interest for the biomedical scientific community. Drug release and delivery, medical imaging, biosensoristics, nanobiotechnology, development of new biomaterials and coatings *etc*., are examples of research branches served by carbon nanomaterials within the biomedical sector [17,18]. Amongst all the above, carbon nanotubes (CNTs) were captured in the spotlight for a certain period of time, yet their use as biomaterials is deemed controversial. On the one hand, CNTs possess great properties for physics applications, and can be functionalized with various biomolecules, being advantageous for bioanalytics and medical imaging [17]. On the other hand, high length to width ratios result in cells penetration, tissue accumulation and consequently *in vivo* carcinogenesis [19]. Functionalized CNTs with low aspect ratio are therefore more recommended when envisaging potential use for medical applications [19]. Nowadays numerous research is focused on graphene (G). If carbon nanotubes are built up by tubular structures of carbon atoms arranged in hexagonal configurations [20], one may imagine graphene sheets as their planar version – opened carbon nanotubes. Similarly, G sheets are often covalently or non-covalently functionalized or conjugated with biomolecules, in order to achieve material candidates for biomedical applications. When used as modifying agents, G and its derivatives outrun common nanomaterials by meliorating the final material features at incomparable levels [21]. Graphene oxide (GO), a rather more hydrophilic version of G, is the most often used graphene derivative within biomaterials research [22]. Refocusing on biopolymer thin films, our previous works on PSF porous films loaded with GO emphasized on their potential to improve polymers thermal, mechanical and biocompatibility properties, as well as to act towards the establishment of a finer inner morphology [23,24]. Interestingly, similar studies report the best outcomes to occur at very low concentrations, for a wide range of polymers [25].

While G and GO are intensively studied, the effect of other G derivatives on PSF films and membranes formation and performance is currently insufficiently investigated [26]. It is the authors opinion that carboxylic acid functionalized graphene (G-COOH) might be a good option for adjusting the performances of such materials, by bringing together the strong and stable structure of G with the hydrophilic character of –COOH functional groups. Therefore, this study was aimed on evaluating the role of G-COOH concentration on PSF porous films characteristics. By ultrasonically mixing PSF with G-COOH, followed by wet phase-inversion method, PSF/G-COOH composite films were obtained. In contrast to other previous works [23,24], this study involves a more extensive analysis of nano-agents importance on materials structuration and physical and chemical, thermal and mechanical, as well as biological properties. Accordingly, structural analysis was brought out by Raman spectroscopy, X-ray diffraction (XRD) and transmission electron microscopy (TEM). In addition, both film surfaces were thoroughly investigated in terms of morphological features, surface profile and hydrophilic–hydrophobic character by scanning electron microscopy (SEM), profilometry and contact angle (CA), respectively. SEM studies were also performed for cross-sectional views. Eventually, materials stability against thermal and mechanical stress was assessed by dynamic mechanical analysis (DMA) and tensile tests, while biological investigation involved biocompatibility tests and monitoring cells cytoskeleton development and growth in contact with PSF/G-COOH membranes.

2. Materials and methods

2.1. Chemicals and reagents

Carboxyl Graphene (~99% purity, ~5% carboxyl ratio, 1–5 μm diameter, 0.8–1.2 nm thickness) was purchased from ACS Material, LLC (USA). Polysulfone ($[C_6H_4\text{-}4\text{-}C(CH_3)_2C_6H_4\text{-}4\text{-}OC_6H_4\text{-}4\text{-}SO_2C_6H_4\text{-}4\text{-}O]_n$, MW ~35.000 g mol^{-1} by LS), N,N-dimethyl-formamide DMF(99% purity, solvent) and absolute ethanol (99.8% purity) were supplied from Sigma Aldrich (USA). Distilled water was used during materials fabrication process.

Cell culture media, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reagent, Lactic Dehydrogenase (LDH) In Vitro Toxicology Assay Kit, Phalloidin-TRITC and 4′,6-diamidino-2-phenylindole (DAPI) were purchased from Sigma–Aldrich (Germany). LiveDead kit was purchased from ThermoFisher Scientific (USA). Murine mesenchymal stem cells (MSCs) were purchased from Gibco, Life technologies.

2.2. Samples preparation

PSF and PSF/G-COOH composite films with 0.25, 0.5, 1 and 2 wt.% G-COOH were prepared by a phase inversion method. Firstly, a 15 wt.% PSF solution was prepared by constantly stirring PSF pellets in DMF at 60 °C for 48 h. Subsequently, 5 equal volumes were extracted from the as obtained PSF solution, among which 4 volumes were mixed with G-COOH according to the aforementioned concentrations, for which dispersion was made by a 1.5 h ultrasound treatment. In order to avoid samples variances, pure PSF solution was treated in the same conditions. Sonicated solutions were alternatively poured onto a flat dish, leveled with a glass straightedge and rapidly thrown in 3:1 (v/v) ethanol-water coagulation baths. After phase inversion, PSF and PSF/G-COOH materials were purified by 24 h immersion in distilled water. Eventually, films were dried in the open air, at room temperature.

In order to perform biological assessments, PSF and PSF/G-COOH composite films surfaces were seeded with murine MSC. Resulted bidimensional cultures were maintained in corresponding complete cell culture media, and exposed to standard conditions of 37 °C, 5% CO_2 and humidity for a total of 7 days.

2.3. Ultrasound treatment

Ultrasound treatment involved the use of a VCX750 ultrasonic processor for small and medium volume applications (Sonics & Materials, Inc. Newton, CT USA), with a probe tip manufactured of Ti–6Al–4V and the source operating with 750 W and 20 kHz. Amplitude was set to 100% and a short-pulse sonication was done in 3 cycles of 30 min sonication and 15 min break. All samples were kept at low temperature in an ice bath container, in order to avoid materials alteration.

2.4. Raman spectrometry

Raman spectrometry was conducted (DXR Raman Microscope, Thermo Fischer Scientific, Waltham, MA, USA) with a laser line of 633 nm, focused by a 10× objective. Spectra were computed based on a number of 10 scans.