Carbon 129 (2018) 258–269

Contents lists available at ScienceDirect

Carbon

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

Liquid crystal behavior and cytocompatibility of graphene oxide dispersed in sodium alginate solutions

Xun Huang [a], Jiexiang He [b], Kai Sun [b], Yuanfeng Chen [a], Zhengang Zha [a, *], Changren Zhou [b, **]

[a] The First Affiliated Hospital of Jinan University, Guangzhou 510632, China
[b] Department of Materials Science and Engineering, Jinan University, Guangzhou 510632, China

ARTICLE INFO

Article history:
Received 5 August 2017
Received in revised form
28 November 2017
Accepted 2 December 2017
Available online 5 December 2017

ABSTRACT

The liquid crystal (LC) behavior and cytocompatibility of a biomimetic sodium alginate (SA)/graphene oxide (GO) LC system were proven in this work. A stable, nematic LC with a highly ordered microstructure forms at a GO concentration (C_{GO}) as low as 0.2 mg mL^{-1} in an alginate solution, but the C_{GO} in aqueous solutions has usually been higher than 10 mg mL^{-1} in previous reports. It is the lowest filler content ever reported for the formation of LC from any colloid, arising mainly from the strong interaction of hydrogen bonds between SA macromolecules and GO sheets. The LC SA/GO system can be used as a drug carrier and tissue engineering scaffold due to the formation of a well-organized, laminated structure with larger gap between its layers after lyophilization. Hence, this work has developed a new LC system with a high degree of orientation and good cytocompatibility that can be used to imitate the desirable features and structural properties of human tissues and microenvironments, and this new LC system will be helpful in biomedical research.

1. Introduction

The liquid crystal (LC) state is one of the most important factors in maintaining the structure and functions of humans [1]. The ideal biomaterial should imitate the desirable microenvironment and structural properties of human tissues. In bionics, research on LC-state biomaterials has received more attention since the 1990s, because of its unique structure and properties [2–5]. The existing studies have confirmed that graphene oxide (GO) can form lyotropic nematic LCs [6], and that the behavior of LCs is critical in their further processing into foams [7], films or fibers [8–11]. However, thus far, the cytocompatibility of GO-based LCs biomaterials has not been reported. Therefore, exploring the effect of the behavior of GO LCs on the structure, biocompatibility and biological function of GO-based biomaterials is needed, especially for the manufacture of highly bionic drug carriers and tissue engineering scaffolds with long-range, ordered microstructures and enhanced properties.

Sodium alginate (SA), a type of linear, anionic polysaccharide macromolecule, has been widely used in the biomedical field because of its excellent biocompatibility and biological function [12–15]. Several studies have demonstrated that the addition of GO to SA solution can improve thermal and mechanical properties and optimize the drug loading and release of the SA films and hydrogels [16–21]. The microstructures and properties of these GO-based composite materials strongly depend on the content and order degree of the GO sheets. The current GO-based LCs 1D fibers [22–24], 2D films and 3D foams with enhanced mechanical properties, but the GO concentrations (C_{GO}) is high [7,25,26]. In general, because of the nondegradation and nanotoxicity of GO, a higher GO content in composite materials results in less biocompatibility and biological function in the implant material. In other words, the GO content in biomaterial should be low. Therefore, if the low concentration of GO dispersed in SA solutions can form LC, the structure, properties and biocompatibility of the GO/SA composite will be optimized.

Here, we report on the LC behavior and cytocompatibility of GO dispersed in different concentration SA solutions, the interaction mechanism between GO and SA and the structural properties of a GO/SA LC system. This study was initiated to determine whether SA can promote the formation of a nematic LC with a highly ordered

* Corresponding author.
** Corresponding author.
E-mail addresses: zhzgg@vip.163.com (Z. Zha), tcrz9@jnu.edu.cn (C. Zhou).

https://doi.org/10.1016/j.carbon.2017.12.006
0008-6223/

microstructure at a lower C_{GO}, as low as 0.2 mg mL^{-1}. In addition, the structure and mechanical properties and the cytocompatibility of the GO/SA foams and films for biomedical applications are also discussed in detail. This work contributes to the research and development of new biomedical materials, such as implanted materials, tissue repair materials, medical instruments, drug carriers, and tissue engineering scaffolds.

2. Materials and methods

2.1. Materials

SA (medium viscosity: 2% solution, 25 °C, ≥2000 cP) was purchased from Sigma-Aldrich (Chemical Co, St. Louis, MO, U.S.A.). Graphite powder was obtained from Qingdao Henglide Graphite Co., Ltd. Other analytical grade reagents were used as received.

2.2. LC GO synthesis and LC GO/SA composite dispersion preparation

GO was prepared from natural graphite powder according to the modified Hummers' method [27]. Briefly, graphite powder (2.5 g) was vigorously stirred with a mixture of concentrated H_2SO_4 (200 mL) and $KMnO_4$ (15 g), and the temperature was maintained below 10 °C. The mixture was heated to 35 °C and stirred for 2.5 h. The mixture was then diluted with deionized water (0.2 L) and stirred for 2.0 h. Finally, additional deionized water (0.8 L) and 30% H_2O_2 (50 mL) were slowly added into the mixture. An aqueous LC GO dispersion was prepared by washing it with water and a 1 M HCl solution until the pH of the solution was approximately 5–6, and then, the dispersion was ultrasonicated for 60 min. The LC GO dispersions with different concentrations in aqueous solution were prepared via repeated centrifugation and dilution. The LC GO/SA composite dispersions were prepared by incorporating a series of SA concentrations (0.1–1 mg mL^{-1}) into different concentrations of GO aqueous solutions (0.1–1 mg mL^{-1}).

2.3. Fabrication of self-assembly LC GO and LC GO/SA biocomposite 3D foams and 2D films

The self-assembled LC GO and LC GO/SA biocomposite 3D foams were fabricated by casting 2.5 mL of the LC GO and LC GO/SA dispersions into culture dishes (diameter 3.5 cm) and 24-well culture plate, respectively, and freezing drying them. The SA foams were fabricated by the same method. The 3D foams with the desired densities and shapes, such as plates and cylinders, were readily accessible. The LC GO and LC GO/SA biocomposite films were made using a simple casting method followed by vacuum drying until the films could be peeled off for analysis. The SA films served as the control and were made using the same method.

2.4. Characterizations

AFM images of the GO and GO/SA sheets were taken in the tapping mode on a multimode 8 AFM system (Bruker, Germany), and the samples were prepared by first depositing the GO and GO/SA sheets onto freshly exfoliated mica substrates from diluted aqueous solutions. The AFM analysis of the LC GO and LC GO/SA films was conducted in the ScanAsyst mode (BioScope Catalyst Bruker Instruments, Germany). The morphology of the GO and GO/SA sheets was also assessed using a Philips Tecnai 10 transmission electron microscope (Philips, Netherlands) operating at an accelerating voltage of 120 kV. The SEM images of the LC GO and LC GO/SA foams were taken on a Zeiss Merlin high-resolution thermal field emission SEM system (Zeiss, Germany). The birefringence of the LC GO and LC GO/SA dispersions was examined via POM, (Zeiss SteREO Discovery V20, Germany) and the liquid samples were loaded into planar cells. Confocal observations were performed on a Carl Zeiss LCM 510 Meta Duo Scan laser scanning confocal microscope (LSCM) using 488 nm and 543 nm lasers for the excitation. The zeta potentials were measured using a Zetasizer Nano ZS instrument (Malvern Ltd., U.K.). The mechanical properties of the SA, LC GO and LC GO/SA foams were measured using a high-precision SHIMADZU AG-I electronic universal testing machine (Shimadzu, Japan). The compression testing specimens were cylindrical with diameters of 14 mm and an average thickness of 12 mm.

The chemical compositions of the prepared films were verified via FTIR (Bruker, Germany) over a range of 4000 to 400 cm^{-1}. Ultraviolet absorption spectra (UV) were recorded using a Shimadzu UV-2550 spectrometer from 200 to 600 nm. XRD studies were performed using a powder XRD system (MiniFlex 600 diffractometer, Rigaku, Japan) with Cu-Kα radiation (λ = 1.54 Å) operating at a generator voltage of 40 kV and a current of 20 mA. XPS was performed using an Axis Ultra DLD X-ray Photoelectron Spectroscopy/ESCA (Kratos Co., UK) with Al Ka (1486.6eV) as the X-ray source at 5 mA × 15 kV with a pass energy of 40 eV for the high-resolution scan.

Cell culture: MC3T3-E1 mouse preosteoblasts were maintained in MEM α at 37 °C in a 5% CO_2-humidified air environment. The medium was supplemented with 10% (v/v) fetal bovine serum (FBS) and 1% (v/v) penicillin-streptomycin.

2.5. Cell viability assay

The cell viability was assessed via an MTT assay. MC3T3-E1 cells were seeded on the SA, LC GO and LC GO/SA films for 1, 3 and 5 days. After discarding the medium, new medium mixed with 0.5 mg mL^{-1} MTT (Sigma, U.S.A.) was added to each well and incubated for 4 h at 37 °C. Subsequently, 150 mL of DMSO was added to each well to dissolve the formazan crystals. The dissolved formazan solution (100 mL) of each sample was moved to a 96-well plate, and the absorbance (OD) was measured at 490 nm using a microplate reader (Multiskan MK3, Thermo, U.S.A.) and a reference filter of 630 nm.

2.6. Live/dead staining and TUNEL assay

The cell viability and apoptosis on the SA, LC GO and LC GO/SA films cultured for 1 and 3 days were evaluated using a calcein AM/PI Double Stain Kit (Keygentec, China) and a TUNEL Assay Kit (Keygentec, China). For the calcein AM/PI assay, the cells were incubated with a calcein AM/PI (2 μM and 8 μM, respectively) solution for 30 min at room temperature and then washed three times with phosphate-buffered saline (PBS). The dead cells were stained red, and the viable cells were stained green. For the TUNEL assay, the cells were fixed in 4% paraformaldehyde and permeabilized by 0.1% Triton X-100 and were incubated in a DAPI-TUNEL (5 μg mL^{-1} and 50 μL, respectively) test solution for 60 min at 37 °C. All the cell nuclei were stained blue, and the apoptotic cells were stained red. All of the samples were viewed using a LSCM and were tested by flow cytometry.

2.7. Cell attachment and morphology study via LSCM observation

The attachment and morphology of the cells seeded on the SA, LC GO and LC GO/SA films were evaluated by labeling the actin cytoskeletons using phalloidin-rhodamine (Cytoskeleton, U.S.A.) and LSCM imaging. Briefly, the MC3T3-E1 cells were cultured on the SA, LC GO and LC GO/SA films for 24 h and then fixed in 4%