International Journal of Biological Macromolecules 114 (2018) 77–84

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: http://www.elsevier.com/locate/ijbiomac

Nanofibrous silk fibroin/reduced graphene oxide scaffolds for tissue engineering and cell culture applications

Hande Nalvuran [a], Ayşe Eser Elçin [a], Yaşar Murat Elçin [a,b,*]

[a] Tissue Engineering, Biomaterials and Nanobiotechnology Laboratory, Ankara University Faculty of Science, and Ankara University Stem Cell Institute, Ankara, Turkey
[b] Biovalda Health Technologies, Inc., Ankara, Turkey

ARTICLE INFO

Article history:
Received 20 October 2017
Received in revised form 17 December 2017
Accepted 14 March 2018
Available online 16 March 2018

Keywords:
Silk fibroin
Reduced graphene oxide
Nanofibrous composite biomaterials

ABSTRACT

Graphene and silk fibroin (SF) have been extensively investigated in the literature. Hybrid scaffolds of SF and graphene combine the properties of both of the materials and provide promising applications for tissue engineering purposes. In this study, reduced graphene oxide (RGO) (0.5%, 1.0% and 2.0% (w/v)) was incorporated into SF and fabricated into composite nanofibers through electrospinning. The fibers were characterized and analyzed by SEM, XRD, FTIR, TGA, circular dichroism analysis, contact angle measurements and tensile tests. Here, we document that the presence of RGO increases intermolecular forces between RGO and SF molecular chains in the SF matrix, which results in an increased silk II content. Upon the incorporation of RGO, thermal stability and mechanical properties of the fibers significantly improved. Furthermore, in-vitro findings showed that composite nanofibers supported cell viability and were hemocompatible. Finally, bone marrow mesenchymal stem cells were induced osteogenically on electrospun SF/RGO mats for 30 days, which showed that the substrate supported osteogenic differentiation. In this study, a feasible method is proposed to generate biocompatible and versatile SF/RGO-composite nanofibers that can influence biomedical applications.

© 2018 Elsevier B.V. All rights reserved.

1. Introduction

Incorporation of a small portion of inorganic/organic material into the organic structure may alter the properties of biomaterials. Natural, biodegradable and non-toxic biopolymers are among the preferred types of materials used in the manufacture of nano-biocomposites. Graphene and its chemical derivatives are a promising new class of nanomaterials, possessing a unique quantum behavior. This material is a two-dimensional sheet and consists of a single-layer honeycomb-shaped lattice configuration, composed of sp^2 hybridized carbon atoms [1]. Electrical and thermal conductivity, as well as the mechanical strength of graphene are high; in addition, graphene has excellent optical properties. Graphene composites are being developed and used in diverse range of applications such as the fabrication of optical and electronic products, energy storage, devices that are capable of biosensing [2], conversion [3,4], and also imaging [5]. Due to its versatility, graphene holds a great potential for use in various biomedical applications [6–8].

Upon the initial discovery of the potential applications of graphene [9], investigations have focused on the development of a chemical method for the production of reduced graphene oxide (RGO) and exfoliated graphene oxide (GO) [10]. Due to the presence of structural defects, the thermal and electrical properties of RGO are inferior when compared to pristine graphene [11,12]. However, RGO is valuable for practical applications. GO and RGO are suitable for chemical-based mass production processes. There are feasible methods enabling the high-yield synthesis of graphene variants with various chemical modifications that can be utilized as fillers in various hybrid systems and composites [13,14].

Fibroin [15] is a protein with a β-sheet secondary structure that produces hybrid films when combined with graphene [16,17], facilitating the growth of cells [18]. Silk fibroin (SF) is a high-potential biopolymer, which may be combined with graphene or its chemical derivatives. An example of such a configuration is the electrospun, non-woven, nanofiber-based mat. SF, derived from the cocoon of *Bombyx mori*, is a promising biomaterial, which offers some unique features including high biocompatibility, permeability, biodegradability, morphologic flexibility and a number of tangible mechanical properties. Electrospinning introduces some novel potential uses, which were previously unattainable. Production of silk nanofibers with a high surface-to-volume ratio and a highly porous three-dimensional structure is a major advantage of this technique. Nanofiber mats facilitate the growth of extracellular matrix (ECM), owing to their biomimetic structure, making them applicable for cell growth [18–20]. In particular, SF/RGO composites may have potential in tissue engineering [21], for the application of local electric fields or local

* Corresponding author at: Tissue Engineering, Biomaterials and Nanobiotechnology Laboratory, Ankara University Faculty of Science, Ankara, Turkey.
E-mail address: elcin@ankara.edu.tr (Y.M. Elçin).

https://doi.org/10.1016/j.ijbiomac.2018.03.072
0141-8130/© 2018 Elsevier B.V. All rights reserved.

ionic currents to biological interfaces [22], and for induced stem cell differentiation under electrical stimulation [23].

This study aims to use SF and RGO for the development of novel biocompatible nanofibrous composites for use in cell culture and tissue engineering applications.

2. Materials and methods

2.1. Silk fibroin processing

Silk, derived from *Bombyx mori* silkworm cocoons were used for the preparation of aqueous SF solutions by using procedures that have been published previously [24]. Silk cocoons were minced into 4–5 pieces, followed by 30 min boiling in 0.02 M Na_2CO_3 solution. Following rigorous washing in distilled water, the extracted SF was dissolved at 60 °C for 3 h in 9.3 M LiBr (Sigma, St. Louis, MO, USA) solution and dialyzed for 3 days against ultrapure water in cellulose membrane tubing (Sigma; 1 kDa MW cutoff) to obtain the regenerated SF (RSF). Filtrated SF solution was quickly frozen at −80 °C and the solvent was removed by lyophilization under vacuum at −56 °C (Christ Alpha 1–4 LD, Osterode am Harz, Germany) for 24 h until a foam-like dry substance was obtained [25,26].

2.2. Preparation of SF or SF/RGO nanocomposites by electrospinning

For the preparation of SF solutions, regenerated SF sponges were dissolved for 1 h in 98% formic acid. Concentration of SF solutions for electrospinning was 15% by weight. Chemically reduced graphene oxide was purchased from Grafen Inc. (Ankara, Turkey) and had the following characteristics; density: 1.91 g/cm^3; BET surface area: 422.69–499.85 m^2/g; composition: 77–87% C, 13–22% O; electrical conductivity: >600 S/m). RGO was incorporated into SF solutions to final concentrations of 0.5 mg/mL, 1 mg/mL and 2 mg/mL (w/v) (abbreviated as SF/RGO0.5, SF/RGO1, and SF/RGO2, respectively). RGO concentrations higher than 2 mg/mL were not preferred during electrospinning, since those compositions yielded micrometer-size fibers (which was not aimed in the study).

2.3. Electrospinning and post-treatment of SF or SF/RGO mats

Electrospinning was based on the design previously described by Jose et al. [27]. The system is comprised of an electrically-charged spinneret, a syringe pump and a grounded, metallic collector, inside an insulated cabinet housing. Electrospinning conditions were modified to produce a Taylor cone in a stable position. To the capillary tube, a voltage of +19 kV was applied. The polymer injection rate was 0.02 mL/min and the gap between the collector and tip of the tube was set to 13 cm. In all experiments, a total volume of 3 mL, containing 15 wt% SF mixture was electrospun onto a uniform surface. During the experiments, the environmental situations were documented and the ambient temperature was kept at 23–24 °C, and humidity at ~45–50%.

Electrospun scaffolds were fabricated and annealed for 45 min by immersion in absolute methanol to encourage a structural conversion to β-sheet. To prevent curling or folding, meshes were placed for 24 h between sheets of filter-paper to enable freeze-drying process.

2.4. Scanning electron microscopy (SEM)

We have used SEM to measure the diameter of the nanofibers formed by electrospinning. Electron micrographs demonstrated a

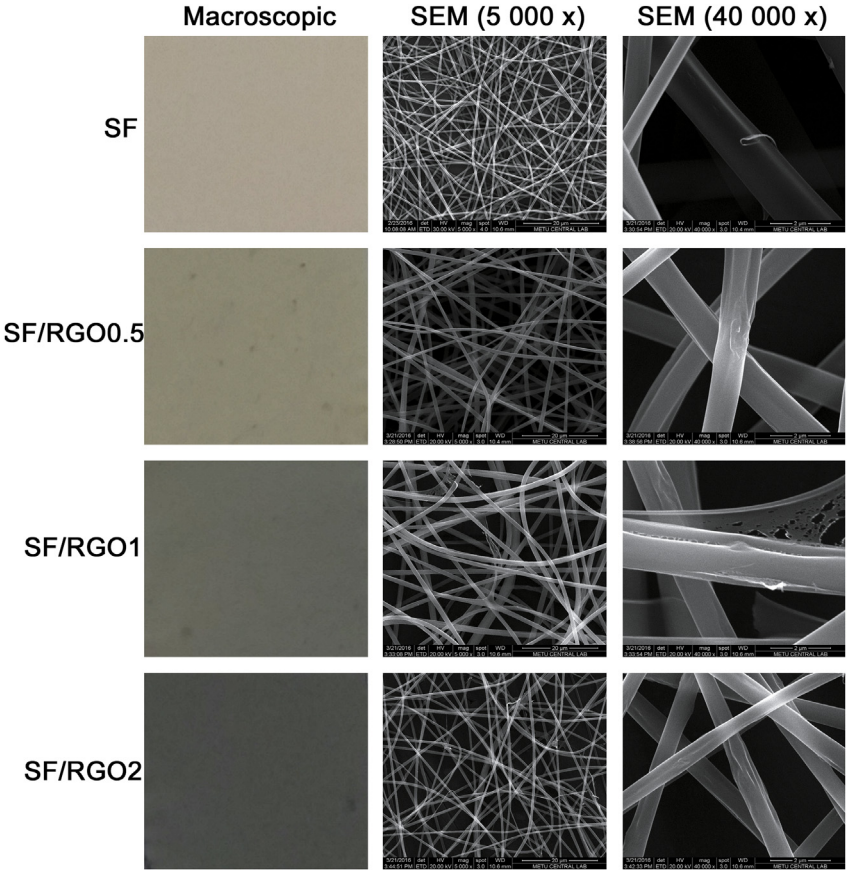

Fig. 1. SEM images of mats, post electrospinning generated at diverse amplifications and macroscopic presentations of similar materials, demonstrating a change in color stimulated by the incorporation of RGO into the white SF fibers. Groups indicated as "SF", contained no composites and represent negative controls, and the "SF/RGO0.5, SF/RGO1 and SF/RGO2", mats that have been incorporated with 0.5, 1, and 2 mg/ mL RGO suspensions, respectively.

Download English Version:

https://daneshyari.com/en/article/8327152

Download Persian Version:

https://daneshyari.com/article/8327152

Daneshyari.com