Journal of Non-Crystalline Solids 457 (2017) 145–151

Contents lists available at ScienceDirect

Journal of Non-Crystalline Solids

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

Morphology and cell responses of three-dimensional porous silica nanofibrous scaffold prepared by sacrificial template method

Honglin Luo [a,b], Zhongyuan Zhou [a], Gen Li [a], Wei Li [a], Zhiqing Li [c], Guangyao Xiong [a,*], Yong Zhu [d], Fanglian Yao [d], Ruisong Guo [b], Yizao Wan [a,b,*]

[a] School of Materials Science and Engineering, East China Jiaotong University, Nanchang 330013, China
[b] School of Materials Science and Engineering, Tianjin University, Tianjin 300072, China
[c] School of Optometry and Ophthalmology, Tianjin Medical University Eye Hospital, Tianjin 300384, China
[d] School of Chemical Engineering, Tianjin University, Tianjin 300072, China

ARTICLE INFO

Article history:
Received 30 August 2016
Received in revised form 22 November 2016
Accepted 29 November 2016
Available online xxxx

Keywords:
Silica
Nanofiber
Scaffold
Biomedical applications

ABSTRACT

Three-dimensional (3D) nanofibrous scaffolds morphologically mimicking natural extracellular matrix show great potential in the application of tissue repair. Herein, we report a novel 3D nanofibrous silica (SiO_2) scaffold fabricated via a sacrificial template method. The sacrificed template used was 3D bacterial cellulose and the resultant silica nanofiber has a ultrasmall diameter of 21 nm. SEM, TEM, and AFM evidence the 3D porous nanofibrous structure and pore structure measurement confirms the existence of macro- and meso-pores in the as-prepared silica scaffold. Cell studies using osteoblast cells demonstrate excellent biocompatibility and high ALP activity. These results suggest that the 3D silica nanofibrous scaffold is a promising candidate for hard tissue engineering.

1. Introduction

The fascinating properties of silica and other bioceramic nanomaterials have drawn much attention [1–6]. To date, various shapes have been fabricated such as nanoparticle [7], nanorod [8], nanotube [9], nanofiber [9], and nanoplate [10]. All these silica nanomaterials have been widely used in many fields ranging from gas sensing [11] and polymer nanocomposites [12] to biomedical [1,2,13,14] and food [15] applications. So far, many approaches have been employed to synthesize silica nanomaterials. A typical example is the bioinspired methodology that uses biological templates such as peptides [16,17] and proteins [18] under physiologically relevant conditions [19]. Up to now, various silica nanostructures have been created using different biotemplates [16,20–29]. For instance, in a previous report, Khripin et al. have demonstrated a protein-directed approach to synthesize silica with arbitrary three-dimensional (3D) architecture by employing cross-linked protein as the template [27]. It is believed that the protein can catalyze the formation of biotemplated silica [30,31]. Among various morphologies, silica nanofibers are considered to be one of the most significant morphologies due to its large specific surface area [32]. It has been demonstrated that nanofibrous silica is a fascinating material in biomedical applications as, in particular, scaffolds for tissue engineering and regeneration due to its resemblance to natural ECM (extracellular matrix) [33,34]. Obviously, the sacrificial biotemplates that determine the physical structure of the resultant silica products through replication play a decisive role in the production of nanofibrous silica. Therefore, seeking appropriate templates is of paramount importance to the production of silica nanofibers.

Recently, a natural 3D biopolymer, bacterial cellulose (BC), has shown good potential as a natural nano-scale template for the replication of ceramic nanomaterials [35–37]. BC shows many striking features as compared to other biological templates. Firstly, BC is much cheaper than other biological templates since it is commercially available and hundreds of tons of BC are produced each year in China alone. Secondly, BC has very high mechanical properties which enable it to bear the weight of ceramic deposits. Thirdly, the intrinsic BC fibers have a diameter in the range of dozens of nanometers which enable them to reproduce nano-sized ceramic via replication. Fourthly, intrinsic BC has 3D nanofibrous structure. Thus, the use of BC as the template makes it possible to fabricate 3D nanofibrous scaffolds that inherit the morphological features of pristine BC. Fifthly, the porous structure of BC pellicles allows for sufficient ion transport in the sol-gel solution, leading to uniform ceramic nanofibers in diameter and composition. Lastly, the large quantity of –OH groups on the surface of BC nanofibers allow versatile surface functionalization, which makes BC a versatile sacrificial template for various ceramic nanomaterials.

In a previous study, Sai et al. prepared a BC–silica composite aerogel with 3D porous network structure through a sol–gel process followed by freeze drying [38]. In our previous work, BC was used as a sacrificial template to synthesize a 3D network-structured silica nanotube scaffold

* Corresponding authors at: School of Materials Science and Engineering, East China Jiaotong University, Nanchang 330013, China.
E-mail addresses: xiongguangyao@163.com (G. Xiong), yzwantju@126.com (Y. Wan).

http://dx.doi.org/10.1016/j.jnoncrysol.2016.11.039
0022-3093/

[39]. However, literature survey indicates that the fabrication of 3D silica nanofibrous scaffolds using BC as the sacrificial template has not been reported yet.

In this work, we report, for the first time, the fabrication of a silica nanofibrous scaffold via a sacrificial template method in conjunction with the sol-gel route using a 3D porous BC nanofibrous scaffold as the sacrificial template. Unlike many previous reports in which acidic or alkalic catalysts were used to facilitate the formation of silica [40–42], the sol-gel process was carried out at mild conditions (near-neutral solution and room temperature). More importantly, unlike commonly used electrospinning method which is unable to produce fibers with diameters below 50 nm due to spinneret nozzle clogging [43], the silica nanofibers have a diameter as small as 21 nm. The main objective of this work was to provide a scalable method to prepare a silica nanofibrous scaffold with 3D structure. Moreover, the morphology, structure, and preliminary in vitro cell responses of the as-prepared silica nanofibrous scaffold are also investigated.

2. Experimental

2.1. Preparation of BC pellicles

The BC pellicles used in this work were prepared by conventional static culture using *Acetobacter xylinum* X-2 (a generous gift from Tianjin University of Science & Technology) as the bacteria strain. Similar to our previous work [44–47], the culture medium (pH = 4.5) used in this work was composed of 2.5% (w/v) glucose, 0.75% (w/v) yeast extract, 1% (w/v) tryptone, and 1% (w/v) disodium phosphate (Na_2HPO_4). After static culture for 3 days at a constant temperature of 30 °C, the BC pellicles were collected, rinsed with deionized water, purified in 0.5 M NaOH solution at 100 °C for 3 h, and washed again with deionized water to neutrality. The resultant BC pellicles were placed in tert-butyl alcohol (Guangfu, China) for freezing (− 10 °C) and drying to yield a BC scaffold.

2.2. Preparation of silica scaffolds

The BC scaffold was immersed in the solutions of tetraethyl orthosilicate (TEOS, Acros) and ethyl alcohol (EtOH, Guangfu, China) with varying concentrations (0.05, 0.1, and 0.2 M). After gentle stirring for 6 h at room temperature, the scaffolds with silica sol precursor were obtained. After washing, the scaffolds were immersed in another solution of 9 mL of EtOH and 1 mL of H_2O for 24 h at room temperature, yielding BC/silica hybrid scaffolds. The products were then washed with deionized water, dried, and calcined at 700 °C for 6 h, giving rise to silica nanofibrous scaffolds.

2.3. Characterization

The morphology and structure of the silica scaffolds were characterized by scanning electron microscopy (SEM, Nova Nanosem 430), transmission electron microscope (TEM, Tecnai G2F-20) operated at 200 kV, and atomic force microscope (AFM, Bruker Dimension Icon). The pore size, pore volume, porosity, pore size distribution, and Brunauer–Emett–Teller (BET) surface area were evaluated by nitrogen adsorption–desorption measurement using a surface area analyzer (NOVA 2200e) and mercury intrusion porosimeter (PoreMaster 60GT, Quantachrome Instruments).

2.4. Cell culture and seeding

The primary mouse osteoblast cells were used for cell studies. Cells were recovered and passaged to the fourth generation. The cells were cultured in L-DMEM medium supplemented with 10% fetal bovine serum (FBS) at 37 °C in a humidified incubator with 5% CO_2. The culture medium was renewed every 2 days.

The scaffold samples were sterilized with steam before they were incubated in L-DMEM medium for 12 h. The samples were then placed into 24-well culture plates. Cells seeded at a cell density of 1×10^5 cells/mL were incubated at 37 °C in a 5% CO_2 incubator. The cell-scaffold constructs were transferred to another 24-well plate, rinsed with PBS three times to remove non-adhering cells. The wells were then filled with culture medium and incubated for different lengths of time.

2.5. Cell morphology

Fluorescence microscope (Nikion, Eclipse Ti-U) was used to observe cell attachment and proliferation. After 1, 3, and 5 days' culture, the cell-scaffold constructs were rinsed with PBS and then stained with live/dead staining reagent which identifies metabolically active cells

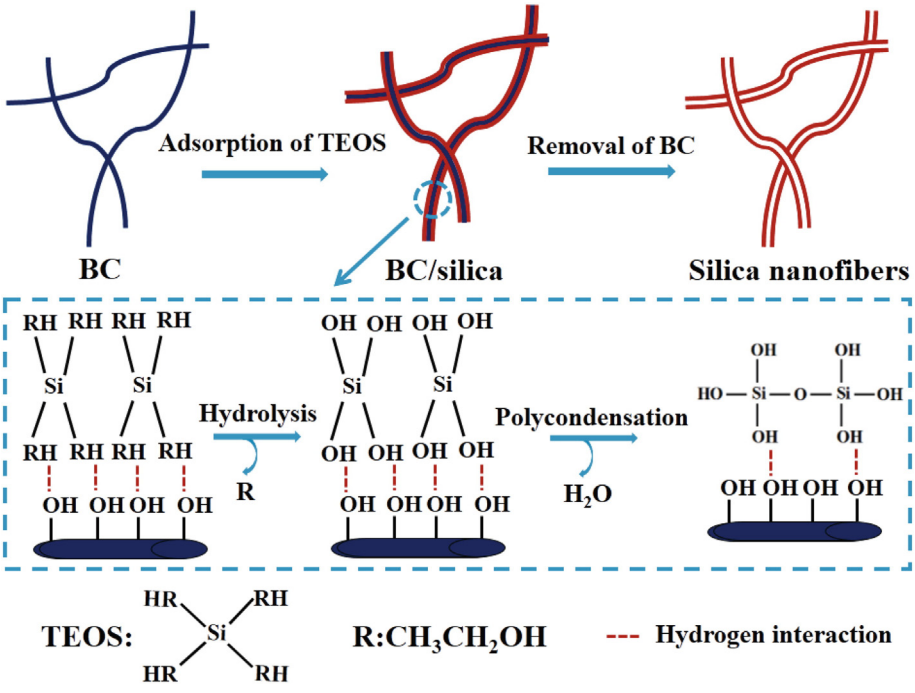

Fig. 1. The typical fabrication process of 3D porous silica nanofibrous scaffold.