Materials Science and Engineering C 62 (2016) 678–685

Contents lists available at ScienceDirect

Materials Science and Engineering C

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

# Synthesis of nanofibrous gelatin/silica bioglass composite microspheres using emulsion coupled with thermally induced phase separation

Da-Young Noh [a,1], Young-Hyeon An [a,1], In-Hwan Jo [a,b], Young-Hag Koh [a,b,*], Hyoun-Ee Kim [c,d]

[a] School of Biomedical Engineering, Korea University, Seoul 136-701, South Korea
[b] Department of Bio-convergence Engineering, Korea University, Seoul 136-701, South Korea
[c] Department of Materials Science and Engineering, Seoul National University, Seoul 151-742, South Korea
[d] Biomedical Implant Convergence Research Center, Advanced Institutes of Convergence Technology, Suwon-si, Gyeonggi-do 443-270, South Korea

ARTICLE INFO

*Article history:*
Received 18 November 2015
Received in revised form 26 January 2016
Accepted 3 February 2016
Available online 4 February 2016

*Keywords:*
Microspheres
Nanofibrous
Hybrid
Bioactivity
Bone tissue engineering

ABSTRACT

This study proposes an innovative way of synthesizing porous gelatin/silica bioglass composite microspheres with a nanofibrous structure using emulsion coupled with thermally induced phase separation (TIPS). In particular, a mixture of the solvent (water) and non-solvent (ethanol) was used to induce a unique phase separation of gelatin/silica mixtures (*i.e.* gelatin/silica hybrid-rich and liquid-rich phases) at −70 °C for the creation of a nanofibrous structure. All the composite microspheres synthesized with silica contents of 10 wt.%, 15 wt.%, and 20 wt.% had well-defined spherical shapes between 124 and 136 μm in size. In addition, they were comprised of nanofibrous gelatin/silica composite walls (several tens of nanometers in thickness), where the sol–gel derived silica bioglass phase was uniformly distributed throughout the gelatin matrix. The *in vitro* apatite-forming ability and biocompatibility of the nanofibrous gelatin/silica bioglass composite microspheres was significantly enhanced with an increase in silica content, demonstrating their great potential for the promotion of bone tissue regeneration.



## 1. Introduction

For the last few decades, biodegradable microspheres have been widely used for tissue regeneration, for example, as key components of injectable fillers and 3-D porous scaffolds, as well as carriers for the delivery of bioactive molecules (*e.g.*, drugs, growth factors, and cells) [1]. More recently, special attention has been given to the synthesis of porous microspheres due to their much larger surface area and longer paths for mass transport, allowing for the high loading efficacy of bioactive molecules and their controlled release kinetics [1–3]. Thermally induced phase separation (TIPS)-based techniques are one of the most useful techniques for producing porous microspheres with a controlled open porous structure [4–6]. These techniques can readily create 3-dimensionally interconnected pores by the removal of a solvent crystal network formed by the phase separation of a polymer solution into polymer-rich and solvent-rich phases below a critical temperature threshold.

As a biodegradable polymer, gelatin, a derivative of collagen, has attracted special attention, since it can induce excellent tissue regeneration *in vivo* [7] and exhibit a high loading efficacy for bioactive molecules through the formation of polyion complexes [8,9], and their sustained release over an extended period [10–14]. However, as is often the case with biodegradable polymers, gelatin by itself has relatively poor bioactivity, and thus is of limited clinical uses in bone tissue engineering. To overcome this limitation, bioactive calcium phosphate (CaP) particles with a chemical composition similar to the inorganic phase of native bone tissue have often been incorporated into gelatin matrixes [15,16]. More recently, sol–gel derived silica, which can possess excellent bioactivity, reasonable biodegradation nature, and mesoporous structure [17], has gained increasing interest as a bioactive inorganic phase, particularly since it can be directly hybridized with biocompatible polymers at the molecular level to provide significantly enhanced biological functions and mechanical properties [18–20]. However, despite the potential, only a few attempts have been made to formulate gelatin/silica composites into microspheres, particularly those with a porous structure [6,21], which can be very usefully applied to bone regeneration.

This study proposes an innovative way of synthesizing porous gelatin/silica bioglass composite microspheres with a nanofibrous structure using a combination of emulsification and TIPS processes, as shown in Fig. 1. In particular, a mixture of solvent (water) and non-solvent (ethanol) was used to induce the unique phase separation of gelatin/silica mixtures (*i.e.* gelatin/silica hybrid-rich and liquid-rich phases) at −70 °C for the creation of a nanofibrous structure [19]. Gelatin/silica hybrid mixtures with various silica contents (0 wt.%, 10 wt.%, 15 wt.%, and 20 wt.%) were emulsified in oil and then frozen at −70°C to induce TIPS, followed by solvent exchange and freeze drying in sequence. The resulting morphology

* Corresponding author at: School of Biomedical Engineering, Korea University, Seoul 136-701, South Korea.
*E-mail address:* kohyh@korea.ac.kr (Y.-H. Koh).
[1] These authors contributed equally.

http://dx.doi.org/10.1016/j.msec.2016.02.017
0928-4931/

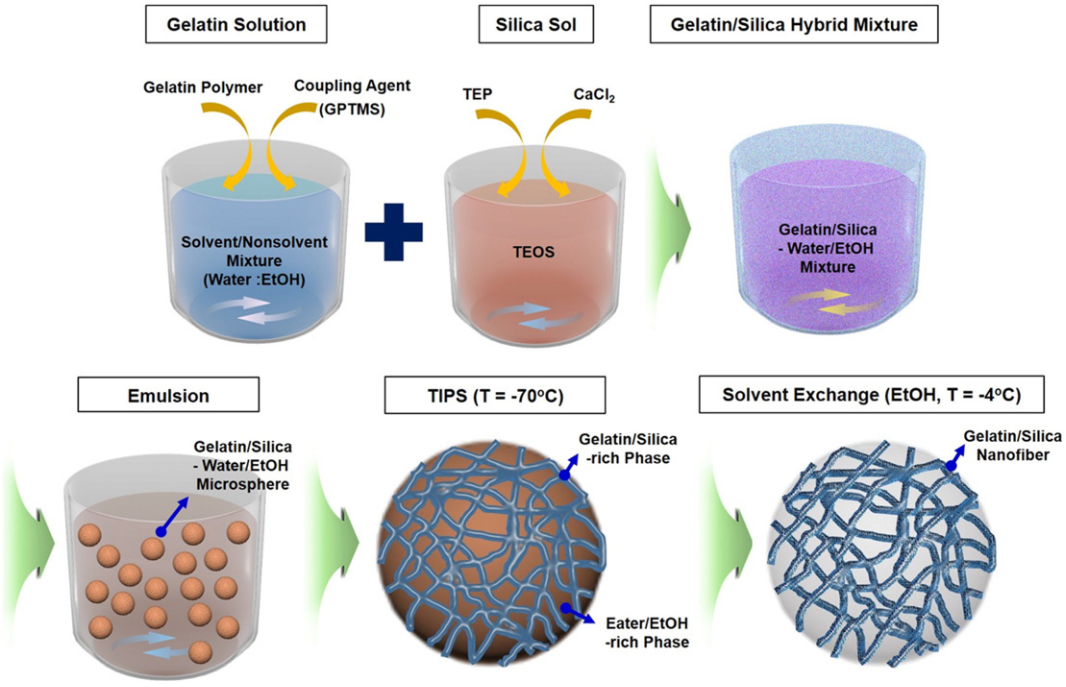


**Fig. 1.** Schematic diagram showing an experimental procedure for producing nanofibrous gelatin/silica bioglass composite microspheres by emulsification coupled with thermally induced phase separation (TIPS) technique.

and porous structure of the nanofibrous gelatin/silica bioglass composite microspheres were characterized by scanning electron microscopy. Furthermore, to evaluate their potential applications for bone tissue regeneration, the *in vitro* apatite-forming bioactivity and biocompatibility of the nanofibrous composite microspheres was also examined.

## 2. Materials and method

### 2.1. Gelatin/silica mixtures preparation

Unless specified otherwise, all reagents were purchased from Sigma-Aldrich (Sigma Aldrich, St. Louis, MO, USA). The preparation of the gelatin/silica hybrid mixtures was detailed in our previous study [19]. In brief, a gelatin solution with a concentration of 8 *w*/*v* was prepared by dissolving gelatin particles (Type B, bovine) in a mixture of ethanol (nonsolvent) and water (solvent) (1:1 EtOH/$H_2O$) using magnetic stirring at 40 °C for 1 h. In addition, a coupling agent to, 3-glycidoxyproyltrimethoxysilane (GPTMS), was added to the gelatin solution at a concentration of 1 vol.% to covalently link the gelatin to silica phases [18]. Separately, a silica sol with a molar composition of 60% $SiO_2$, 36% CaO and 4% $P_2O_5$ was prepared by mixing tetraethyl orthosilicate (TEOS), triethylphosphate (TEP), and calcium chloride in water containing hydrochloric acid (HCl, 1 N) using magnetic stirring for 2 h, which would result in sol–gel derived silica bioglass. To prepare the gelatin/silica hybrid mixtures, gelatin solutions were mixed with predetermined amounts of the silica sol (0 wt.%, 10 wt.%, 15 wt.%, and 20 wt.%) using magnetic stirring at 40 °C for 1 h.

### 2.2. Gelatin/silica microsphere synthesis

The prepared gelatin/silica mixtures were poured into vegetable oil and magnetically stirred at 200 rpm for 30 min for emulsification. After which, the composite microspheres dispersed in the oil were frozen at −70 °C for 4 h to induce TIPS. The synthesized composite microspheres were immersed in ethanol at −4 °C for 24 h and then in acetone for 24 h for solvent exchange to take place and finally freeze dried for 24 h. To improve stability in physiological environments, the nanofibrous gelatin/silica bioglass composite microspheres were treated with a 1% glutaraldehyde solution for 24 h and then rinsed with ethanol, followed by drying at 37 °C for 12 h.

### 2.3. Morphology, porous structure and microstructure evaluation

The morphology, porous structure, and microstructure of the nanofibrous gelatin/silica bioglass composite microspheres synthesized with various silica contents (0 wt.%, 10 wt.%, 15 wt.%, and 20 wt.%) were characterized by field emission scanning electron microscopy (FE-SEM) (JSM6701F, JEOL, Japan). The size distributions of the nanofibrous composite microspheres were roughly evaluated by measuring the diameter of individual microspheres based on the SEM images. Approximately 50–60 microspheres were measured for each test to obtain average and standard deviation.

### 2.4. Chemical composition and structure analysis

The chemical compositions of the nanofibrous gelatin/silica bioglass composite microspheres synthesized with various silica contents (0 wt.%, 10 wt.%, 15 wt.%, and 20 wt.%) were characterized by energy dispersive spectroscopy (EDS) attached to the SEM, while their chemical structures were investigated by attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR, Nicolet 6700, Thermo Scientific, USA). The final content of the sol–gel derived silica bioglass in the composite microspheres was determined by thermogravimetric analysis (TGA; TA Instruments, New Castle, DE, USA). The composite microspheres were heated up to 1000 °C at a heating rate of 10 °C/min in a flowing nitrogen atmosphere. The weight loss of the samples during the tests was monitored and the silica bioglass content was calculated by considering the residue of gelatin in $N_2$.

### 2.5. In vitro apatite-forming ability evaluation

The *in vitro* apatite-forming bioactivity of the nanofibrous gelatin/silica bioglass composite microspheres synthesized with various silica contents (0 wt.%, 10 wt.%, 15 wt.%, and 20 wt.%) was characterized using simulated body fluid (SBF) solution [22,23]. The composite microspheres were immersed in SBF and then placed inside an incubator at a