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Highly porous gelatin–silica hybrid scaffolds with textured surfaces using new direct foaming/freezing technique

Bo Lei [a], Kwan-Ha Shin [a], In-Hwan Jo [a], Young-Hag Koh [a,b,*], Hyoun-Ee Kim [c]

[a] Department of Dental Laboratory Science and Engineering, Korea University, Seoul 136-703, Republic of Korea
[b] Department of Orthopaedics, Korea University Medical Center, Guro Hospital, Seoul 152-703, Republic of Korea
[c] Department of Materials Science and Engineering, Seoul National University, Seoul 151-742, Republic of Korea

HIGHLIGHTS

- Highly porous gelatin–silica hybrid scaffolds were produced using direct foaming/freezing.
- Hybrid scaffolds had high porosity, large pores and large interconnections.
- Tailored surface textures were created after immersion in ethanol at −20 °C for 24 h.
- Hybrid scaffolds showed much significantly enhanced mechanical properties.

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ABSTRACT

Highly porous gelatin–silica hybrid scaffolds with high porosity, large pores and large interconnections, as well as tailored surface textures were produced using a newly developed direct foaming/freezing. Two different types of precursors as the silica source, 3-glycidoxyproyltrimethoxysilane (denoted as "GS") and sol–gel derived silica (denoted as "SS"), were used for producing the porous GLA–GS and GLA–GS–SS hybrid scaffolds. In this method, air bubbles could be vigorously incorporated into the GLA–GS and GLA–GS–SS mixtures and then stabilized by rapid freezing of the foamed mixtures at −70 °C. Both the porous GLA–GS and GLA–GS–SS hybrid scaffolds produced herein had a highly porous structure (porosity > 90 vol%, pore size = 200–500 μm, interconnection size = 100–200 μm) with a uniform distribution of the silica phase in the gelatin matrix. In addition, surface textures with a rugged morphology could be created after immersion of the porous GLA–GS and GLA–GS–SS hybrid scaffolds in ethanol at −20 °C for 24 h. The porous GLA–GS and GLA–GS–SS hybrid scaffolds showed much higher mechanical properties than the porous GLA scaffold, while preserving excellent *in vitro* biocompatibility, demonstrating potential application as the bone scaffold.

1. Introduction

The fabrication of 3-dimensional porous scaffolds with a tightly controlled porous structure is one of the most important requirements for successful tissue regeneration, which primarily determines not only cell seeding efficiency but also cells attachment, migration, proliferation differentiation and new tissue formation [1–4]. As a scaffolding material, gelatin is considered as one of the most promising biodegradable organic phases since it can resemble the chemical structure and biological functions of collagen in the native ECM without immunogenicity concerns [5]. In addition, gelatin can be hybridized with bioactive sol–gel derived silica at the molecular level [6], which can more closely mimic the chemical composition and nanostructure of the native bone ECM [7], leading to significantly improved mechanical properties and biological performances.

Thus far, a range of manufacturing methods have been developed to produce highly porous polymer-based scaffolds with porosity >90 vol% [8], including freeze-drying [9], solvent casting/salt-leaching [10] and gas-based foaming techniques [11]. Freeze drying can create pores in pure gelatin, gelatin-calcium phosphate composites and gelatin-siloxane hybrids by removing frozen solvent used to dissolve gelatin polymer [12,13]. However, this technique is inapplicable to a mixture of gelatin and silica sol because of

* Corresponding author. Department of Dental Laboratory Science and Engineering, Korea University, Seoul 136-703, Republic of Korea. Tel.: +82 2 940 2844; fax: +82 2 909 3502.
E-mail address: kohyh@korea.ac.kr (Y.-H. Koh).

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a considerable amount of alcohol associated with the condensation of the silica sol. On the other hand, gas-based foaming technique can achieve very high porosity, large pores and large interconnections between the pores via either direct incorporation of gases or chemical reaction [14–17]. However, this technique often suffers from severe floatation of light gas bubbles, resulting in a nonuniform porous structure.

We herein propose the direct foaming/freezing technique as a novel way of producing highly porous gelatin–silica hybrid scaffolds with high porosity (>90 vol%), large pores (>200 μm) and large interconnections between the pores (100–200 μm), as well as tailored surface textures. This technique can directly incorporate air bubbles into the gelatin–silica mixtures and stabilize them without any noticeable flotation via fast freezing of the foamed mixtures at −70 °C. Furthermore, surface textures with a rugged morphology could be successfully created by treating the hybrid scaffolds in ethanol at −20 °C. The porous structure (e.g. porosity, pore size, interconnections between the pores), surface morphology, chemical compositions and chemical structures of the porous gelatin–silica hybrid scaffolds were characterized using a range of analysis tools. The mechanical properties and *in vitro* biocompatibility of the porous hybrid scaffolds were examined for evaluating their potential applications for bone regeneration.

2. Materials and method

2.1. Materials

Unless specified otherwise, all regents were purchased from Sigma–Aldrich (Sigma Aldrich, St. Louis, MO, USA). Two different types of precursors as the silica source, 3-glycidoxyproyltrimethoxysilane (denoted as "GS") and sol–gel derived silica (denoted as "SS"), were used to achieve homogenous GLA–GS and GLA–GS–SS hybrids at the molecular level. The components of the porous pure gelatin (GLA) and gelatin-silica hybrid scaffolds (GLA–GS and GLA–GS–SS) are summarized in Table 1.

2.2. Gelatin–silica hybrid mixtures preparation

First, a 10 wt% gelatin solution was prepared by dissolving gelatin (type A, from porcine skin) in distilled water or 0.01 N hydrochloric acid (HCl) at 40 °C for 2 h by magnetic stirring for the production of pure porous GLA and GLA–GS hybrid scaffolds, respectively. Sodium dodecyl sulfate (SDS) at a concentration of 0.1 wt% used as the surfactant and stabilizer was also added to the gelatin solutions. Subsequently, predetermined amounts of GS (Table 1) were added to the gelatin solutions and hybridized at 35 °C for 6 h by magnetic stirring for preparing homogenous GLA–GS mixtures. Similarly, GLA–GS–SS mixtures were also prepared using a sol–gel derided silica sol that had been synthesized by dissolving tetraethyl orthosilicate (TEOS) in 0.01 N HCl using magnetic stirring for 3 h according to a method reported in the literature [18]. The prepared silica sol was hybridized with the GLA–GS mixture by magnetic stirring for 30 min.

Table 1
Compositions of the porous pure gelatin scaffold (GLA) and the porous gelatin–silica hybrid scaffolds (GLA–GS and GLA–GS–SS).

Hybrid scaffolds		GLA [g]	GS [ml]	SS [g]
GLA		1	0	0
GLA–GS	GLA–GS1	1	1	0
	GLA–GS2	1	2	0
	GLA–GS3	1	3	0
GLA–GS–SS		1	1	0.67

2.3. Porous gelatin–silica hybrid scaffolds production

The foaming process was carried out at 35 °C by repeatedly pressing commercially available polyurethane sponges (30 ppi, Jeil Urethane Co., Korea), which had been immersed in the GLA, GLA–GS and GLA–GS–SS mixtures. The foamed mixtures were then immediately transferred to plastic containers and rapidly frozen at −70 °C for 2 h for gelation of the gelatin phase, followed by freeze drying for 48 h. In addition, in order to create surface textures with a rugged surface, the frozen GLA, GLA–GS and GLA–GS–SS hybrid scaffolds were immersed in ethanol at −20 °C for 24 h.

2.4. Porous structure characterization

The porous structure and microstructure of the porous pure gelatin (GLA) and gelatin–silica hybrid scaffolds (GLA–GS and GLA–GS–SS) were examined by field emission scanning electron microscopy (FE-SEM) (JSM6701F, JEOL, Japan). The pore size ranges of the porous hybrid scaffolds were calculated by counting the numbers of pores >50 from the SEM images. The porosity of the porous hybrid scaffolds was calculated by considering their apparent density and skeletal density by assuming the theoretical density of the gelatin (ρ_G = 1.35 g cm^{-3}) and silica (ρ_S = 2.65 g cm^{-3}).

2.5. Chemical composition and structure characterization

The chemical compositions and elemental distribution of the porous gelatin–silica hybrid scaffolds (GLA–GS and GLA–GS–SS) were characterized by energy dispersive spectroscopy (EDS) attached to the FE-SEM. In addition, their chemical structures were evaluated by attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR, Nicolet 6700, Thermo Scientific., USA).

2.6. Mechanical properties test

The mechanical properties of the porous pure gelatin (GLA) and gelatin–silica hybrid scaffolds (GLA–GS and GLA–GS–SS) were evaluated using the compressive strength tests. The samples with a diameter of 8 mm and a height of 8 mm were compressed at a cross-head speed of 0.5 mm min^{-1} using a screw-driven load frame (Oriental Testing Machine Co, Korea). The stress versus strain responses of the samples during the compressive strength tests were recorded. The compressive modulus was determined by considering the initial linear region of the stress–strain curves. At least three specimens were tested to obtain the mean and standard deviation for each scaffold.

2.7. In vitro biocompatibility evaluation

The *in vitro* biocompatibility of the porous gelatin–silica hybrid scaffolds (GLA–GS and GLA–GS–SS) was evaluated using a pre-osteoblast cell line (MC3T3-E1; ATCC, CRL-2593, Rockville, MD, USA) according to a method reported the literature [7]. Briefly, the MC3T3-E1 cells with a density of 2×10^4 cells mL^{-1} were seeded on the hybrid scaffolds and cultured in a humidified incubator in an atmosphere containing 5% CO_2 at 37 °C for 1 and 5 days. After 1 and 5 days of culturing, the morphologies of the attached cells on the porous hybrid scaffolds were examined by FE-SEM. Prior to these observations, the samples were washed three times by phosphate buffer solution (PBS), fixed with 2.5% glutaraldehyde for 30 min and then dehydrated in graded ethanol (75, 85, 95 and 100% ethanol in sequence), followed by drying at ambient condition for 1 day.

The cell viability and growth of the porous GLA–GS and GLA–GS–SS hybrid scaffolds was examined using an MTS