Materials Science and Engineering C 70 (2017) 969–975

Contents lists available at ScienceDirect

Materials Science and Engineering C

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

Effect of inorganic/organic ratio and chemical coupling on the performance of porous silica/chitosan hybrid scaffolds

Daming Wang [a], Wei Liu [a], Qian Feng [b], Chaoqun Dong [b], Qisong Liu [a], Li Duan [a], Jianghong Huang [a], Weimin Zhu [a,c], Zemeng Li [a], Jianyi Xiong [a], Yujie Liang [d], Jielin Chen [a], Rong Sun [e], Liming Bian [b], Daping Wang [a,*]

[a] Shenzhen Key Laboratory of Tissue Engineering, Shenzhen Second People's Hospital, Shenzhen 518035, China
[b] Division of Biomedical Engineering, The Chinese University of Hong Kong, Shatin, New Territories 999077, Hong Kong
[c] Shenzhen Centre of Sports Medicine & Engineering Technology, Shenzhen 518035, China
[d] Laboratory of Chemical Genomics, School of Chemical Biology and Biotechnology, Shenzhen Graduate School of Peking University, Shenzhen 518055, China
[e] Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences, Shenzhen 518055, China

ARTICLE INFO

Article history:
Received 30 January 2016
Received in revised form 19 March 2016
Accepted 4 April 2016
Available online 9 April 2016

Keywords:
Hybrid
Chitosan
Cytocompatibility
Sol-gel
Tissue regeneration

ABSTRACT

Inorganic/organic hybrid scaffolds have great potential for tissue engineering applications due to controllable mechanical properties and tailorable biodegradation. Here, silica/chitosan hybrid scaffolds were fabricated through the sol-gel method with a freeze drying process. 3-Glycidoxypropyl trimethoxysilane (GPTMS) and tetraethylorthosilicate (TEOS) were used as the covalent inorganic/organic coupling agent and the separate inorganic source, respectively. Hybrid scaffolds with various inorganic/organic weight ratios (I/Os) and molar ratios of chitosan and GPTMS (GCs) were examined and compared in this study. FTIR showed that higher GPTMS content resulted in the increased covalent cross-linking of the chitosan and the silica network in hybrids. Compression testing indicated that increasing the GPTMS content greatly improved the compressive strength of scaffold. LIVE/DEAD assay showed that enhanced cytocompatibility was obtained as the silica content increased. Therefore, the results confirmed that the two parameters I/O and GC can largely influence the scaffold performance, which can be used to tailor the hybrid properties.

1. Introduction

The development of tissue engineering provides a strategy to create a scaffold to stimulate tissue regeneration [1]. There are two mainstreams of tissue engineering strategies associated with tissue-regenerating scaffolds: scaffolds themselves that directly enable the stimulation of in situ tissue regeneration; and scaffolds pre-processed with seeded cells and the subsequent pre-grown tissue in vitro before implantation [2,3]. The scaffolds have the ability to restore, maintain, or improve the tissue function [4]. An ideal tissue-engineered scaffold should mimic both the structure and mechanical properties of the targeted tissue [5]. Generally, the scaffold should be:

a. Biodegradable and its degradation products must be non-toxic and biocompatible, which is one of the most important principles of material selection for tissue implants. The degradation rate should be controlled to match new tissue ingrowth.
b. The scaffold should have the ability to bond to the targeted tissue and induce the tissue regeneration. It requires the surface chemistry of the scaffold to enable the stimulation of cell attachment, migration, proliferation and differentiation. Ideally, the scaffold surface should also contain some active functional group for biomolecule attachment.
c. The scaffold should act as a template to direct cell ingrowth in three dimensions for the formation of effective construction. An interconnected pore network with appropriate porosity is required to encourage the transport of nutrients, oxygen and metabolic wastes.
d. Mechanical properties similar to the host tissue are required, especially for resisting load bearing in specific tissue such as articular cartilage.
e. The scaffold should meet international regulations for clinical use and should be easy to produce commercially and to be sterilized.

Bioactive glasses fulfil almost all of the strict requirements simultaneously; however, they are brittle in nature and thus are not suitable for the defect sites that are under cyclic loading [6]. As a consequence, a tougher material is needed but one that maintains all the beneficial properties of bioactive glass scaffolds. Introducing a biodegradable polymer into the glass to fabricate hybrid scaffolds will be a promising strategy [6]. In class II hybrids, the inorganic and the organic components are

* Corresponding author.
E-mail address: dapingwang1963@foxmail.com (D. Wang).

http://dx.doi.org/10.1016/j.msec.2016.04.010
0928-4931/

covalently cross-linked at the molecular level, and thus they cannot be distinguished at micro-scale [6]. These novel hybrid materials can inherit bioactivity from bioactive glasses due to the exposure of both inorganic and organic components to host tissue/cells as if it is one material [7]. In addition, both inorganic and organic components have a congruent degradation behaviour and hybrids have the potential for tailoring the material properties such as mechanical properties, degradation rate, scaffold morphology and et al. [6–10].

Chitosan, a common natural polysaccharide, has been widely used in biomedical applications [11,12]. So far several studies have been reported on the synthesis of inorganic/organic hybrids using chitosan as the organic source. In these hybrid materials GPTMS was used to covalently cross-link to chitosan [13–20]. Connell et al. [17], Wang et al. [16] confirmed that the hybrid reaction occurred between the epoxide ring of the GPTMS by the primary amine group (—NH_2) of the chitosan (Fig. 1). Also, inorganic/organic weight ratios (I/Os) and molar ratios of chitosan and GPTMS (GCs) showed great influence on the inorganic/organic coupling, the mechanical properties, the degradation behaviour and the scaffold morphology [16,17]. However, biological properties of these hybrids were not examined. Shirosaki et al. [13–15] focused on the in vitro study of silica/chitosan hybrids using MG63 osteoblastic cells. However, in these work GPTMS was the only inorganic component, which means the inorganic/organic coupling degree cannot be adjusted independently of the inorganic/organic ratios. How I/Os would affect the cytocompatibility of silica/chitosan hybrids still remains unknown.

Therefore, the aim of this work was to determine the influence of I/Os and GCs on the chemical, physical and cytocompatibility of silica/chitosan hybrid. Scaffolds with various compositions were fabricated through the sol-gel method and the freeze drying process. The results may be applied to many other types of inorganic/organic hybrid and will be of help to the researchers in relevant filed for the optimization of the hybrid synthesis and the design of a satisfactory tissue-regenerating hybrid material.

2. Materials and methods

2.1. Fabrication of silica/chitosan hybrid scaffold

All reagents were purchased from Sigma Aldrich China unless otherwise indicated. Silica/chitosan hybrid scaffolds fabricated with the following compositions were investigated in this work: hybrids containing 60 wt% organic with different molar ratios of chitosan and GPTMS (4060 GC1 and 4060 GC4) and hybrid with 40 wt% organic component (6040 GC4), as shown in Table 1. This was achieved by altering the GPTMS and hydrolyzed TEOS content while keeping the chitosan concentration unchanged.

The fabrication of porous silica/chitosan hybrid scaffold involved two main steps: the preparation of class II silica/chitosan hybrid sol and the subsequent freeze drying process to form a solid scaffold. Here, the silica/chitosan hybrid sol was prepared as described previously [16]. Briefly, chitosan powder (M_w: 50 – 150 kDa, degree of deacetylation of 75%) was dissolved in hydrochloric acid (HCl) of pH 4. The final chitosan solution concentration reached ~17 mg/mL. An appropriate amount of GPTMS was added subsequently for the functionalization of chitosan and GPTMS. The reaction continued for 3 days at 40 °C. TEOS was used as the separate silica source in this study. For obtaining the silica sol TEOS was hydrolysed with a vigorous agitation for 1 h, using an R ratio of 4 (the molar ratio of water to TEOS) and a water/HCl (2 N) volume ratio of 3. The fully hydrolyzed TEOS was then added to the functionalized chitosan sol, which was mixed for further 1 h to produce a class II silica/chitosan hybrid sol.

The aforementioned sol was cast into 6-well cell culture plates, sealed, aged at 40 °C for 3 days, frozen at −20 °C for 24 h and finally transferred to a LGJ-10D freeze-drier (Four-ring Science Instrument Plant Beijing Co., LTD) for a three-day drying process. After the sublimation of the ice crystals inside the sample, porous silica/chitosan hybrid scaffolds were obtained.

2.2. Hybrid cross-linking and chemical structure

A Nexus470 FT-IR spectrometer (Thermo Nicolet) was used to obtain Fourier transform infrared spectroscopy (FTIR) spectra of silica/chitosan hybrid scaffolds. Scaffolds were ground into fine powder and mixed with potassium bromide (KBr) in the same proportion to form the pellets. Spectra of the pellets were obtained in the range of 500 – 4000 cm^{-1} at a resolution of 8 cm^{-1} and averaged over 10 scans.

2.3. Dissolution study

A Thermo Scientific iCAP 6300 inductive coupled plasma-optical emission spectroscopy (ICP-OES) was used to obtain the soluble silica release profiles in TRIS buffer solution, for examining the dissolution behaviour of silica/chitosan hybrid scaffolds. 80 mg of hybrid scaffold was immersed into 120 mL 0.05 M TRIS buffer solution (pH 7.3) at 37 °C, with the subsequent orbital shaking at 120 rpm. 1 mL sample solution was extracted at 1, 2, 4, 8, 24, 72, 168 h and replaced by 1 mL fresh

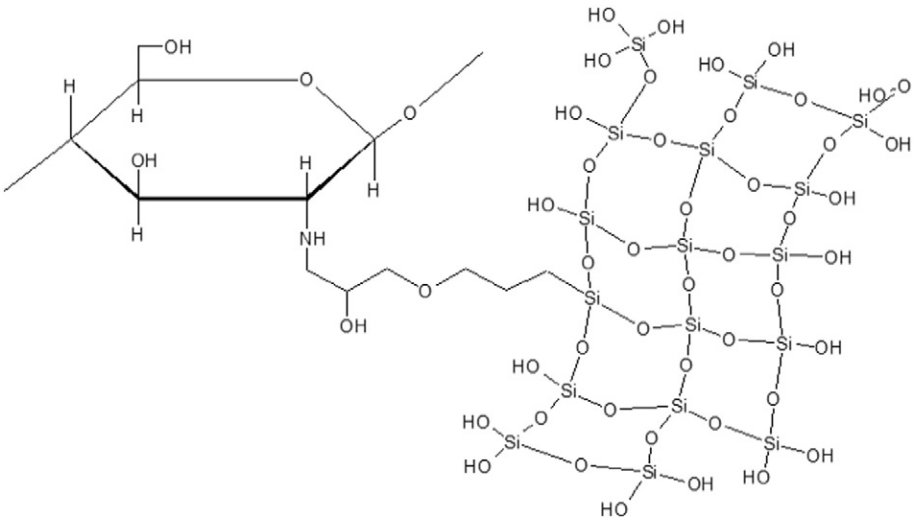

Fig. 1. Schematic for the chemical structure of silica/chitosan hybrid scaffolds.