Materials Science and Engineering C 70 (2017) 736–744

Contents lists available at ScienceDirect

Materials Science and Engineering C

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

Super-paramagnetic responsive silk fibroin/chitosan/magnetite scaffolds with tunable pore structures for bone tissue engineering applications

Shamsa Aliramaji, Ali Zamanian *, Masoud Mozafari

Biomaterials Research Group, Nanotechnology and Advanced Materials Department, Materials and Energy Research Center (MERC), Tehran, Iran

ARTICLE INFO

Article history:
Received 28 May 2016
Received in revised form 25 August 2016
Accepted 20 September 2016
Available online 24 September 2016

Keywords:
Silk fibroin
Chitosan
Responsive scaffolds
Magnetite nanoparticles
Bone tissue engineering
Freeze-casting

ABSTRACT

Tissue engineering is a promising approach in repairing damaged tissues. During the last few years, magnetic nanoparticles have been of great interest in this field of study due to their controlled responsive characteristics in specific external magnetic fields. In this study, after synthesizing iron oxide (magnetite) nanoparticles through a reverse coprecipitation method, silk fibroin/chitosan-based magnetic scaffolds were prepared using different amounts of magnetite nanoparticles (0, 0.5, 1 and 2%) by freeze-casting method. The physicochemical activity of the scaffolds was monitored in phosphate-buffered saline (PBS) solution to determine the biodegradation and swelling behaviors. The stability of the magnetite nanoparticles in the fabricated scaffolds was determined by atomic absorption spectroscopy (AAS). Moreover, the cellular activity of the magnetic scaffolds was examined under a static magnetic field. The results showed that the lamellar structured scaffolds having MNPs in the walls could not affect the final structure and deteriorate the biological characteristics of the scaffolds, while the ability of magnetic responsivity was added to the scaffolds. This study warrants further pre-clinical and clinical evaluations.

1. Introduction

Tissue engineering is a promising approach to repairing or substituting damaged tissues. In this approach three-dimensional scaffolds play a very important role, in which their main function is to direct the growth of cells within the porous structure of the scaffolds [1]. Every day new scaffolds with different biomaterials, structures and properties are fabricated in order to develop advanced tissue engineering constructs. Among different biomaterials, silk fibroin is a biocompatible, oxygen and water permeable, stable, low immunogenic and non-cytotoxic, versatile in processing with adequate tensile strength. These properties make silk fibroin a great biomaterial to be used in tissue engineering [2,3]. However, silk fibroin is relatively brittle and difficult to handle as a scaffold biomaterial, especially when implanted in load-bearing sites [4,5]. It is also difficult to achieve a porous structure using silk fibroin alone, and therefore imbedding bioactive agents in these scaffolds is hard to be obtained [6]. To overcome the mentioned limitations, silk fibroin can be combined with natural polymers such as polysaccharides, among which one of the most popular polysaccharides that can be combined with silk fibroin is chitosan [5,7].

Chitosan, an abundant natural polymer produced by the hydrolysis of chitin has glycosaminoglycans (GAG) in its structure which is one of the components of Extracellular Matrix that interacts with collagen fibers and is very important in cell-cell adhesion. Depolymerisation of chitosan yields bioactive anti-microbial products which are metabolized from the body easily. Therefore, chitosan is biodegradable and has excellent biocompatibility with almost all kinds of tissues. Chitosan is obtained by removing enough acetyl groups for the molecule. This process, which is called deacetylation, releases amine groups (—NH) and gives the chitosan a cationic characteristic [8,9]. Due to its cationic nature, it can easily form complexes with anionic materials such as silk fibroin [10].

It can be used in bone tissue engineering because of its biocompatibility and non-toxicity, porous structure, suitability for cell adhesion and proliferation, antibacterial nature, and biodegradable properties [11–13]. Chitosan is osteoconductive and induces proliferation of osteoblast [14]. However, it is difficult to control its degradation and swelling properties [15].

To overcome the shortcomings of silk fibroin and chitosan and optimize their properties as scaffold materials, these two can be combined with each other. Furthermore, addition of chitosan to silk fibroin enhances the roughness of the surface of these kinds of scaffolds and therefore increases cellular attachment [7,16].

There are different methods to fabricate porous scaffolds such as freeze drying, salt leaching, gas foaming, injection molding, electrospinning, rapid prototyping and freeze-casting [17–19]. Freeze-casting is a versatile process and the scaffolds fabricated with this method have a combination of interconnected porous structure and adequate mechanical properties. It also provides control over pore size and architecture, and therefore it can be used for reengineering of different types of tissues and organs [20–24].

* Corresponding author.
E-mail address: a-zamanian@merc.ac.ir (A. Zamanian).

http://dx.doi.org/10.1016/j.msec.2016.09.039
0928-4931/

Table 1
The components of the prepared scaffolds.

Sample name	Chitosan to silk fibroin ratio	Magnetite content (wt%)
SF/C	1:3	0
$M_{0.5}$SF/C	1:3	0.5
M_1SF/C	1:3	1
M_2SF/C	1:3	2

One of the most recent strategies in tissue engineering is the use of magnetic nanoparticles for the preparation of magnetic responsive scaffolds. The most important aspect of these nanoparticles is their capability to be controlled from a distance by the use of an external magnetic field. This leads to a great deal of potentials in tissue engineering such as magnetic cell patterning, cell seeding and three-dimensional tissue-like structures [25–27]. These features make magnetic scaffolds promising candidate and new generation of scaffolds used in tissue engineering. An example of the use of these kinds of magnetic scaffolds is distribution of magnetic nanoparticles in macroporous ferrogel scaffolds embedded in iron oxide nanoparticles in order to optimize porous structure of the scaffolds for cell delivery [28]. In another study, Gloria et al. [29] have reported on magnetic poly (1-caprolactone)/iron-doped hydroxyapatite nanocomposites for the simultaneous use of repairing damaged tissues and further hyperthermia treatment. Most recently, Panseri et al. [27] have reported hydroxyapatite/collagen magnetic scaffolds to attract growth factors and cells attached to other MNPs.

It has been also reported that the presence of moderate external static magnetic field may have positive effects on osteoblast cells even without the presence of magnetic nanoparticles and it is being used for pain release and wound healing [30–32]. The presence of magnetic nanoparticles itself in scaffolds impacts biological properties and cellular behavior. In some cases, it has been reported that the incorporation of magnetic nanoparticle could even improve the bioactivity of the scaffolds [33–35]. Magnetic nanoparticles are super-paramagnetic with a magnetic moment like any other magnetic material which can induce a magnetic field [36,37]. When an external static magnetic field is applied to a magnetic scaffold, a synergistic effect is made which can alter cell behavior such as proliferation and differentiation [38–40].

In this study, different percentages of magnetite nanoparticles were incorporated into the structure of silk fibroin/chitosan scaffolds. The scaffolds were fabricated by freeze-casting method to obtain highly interconnected porous structures suitable for bone tissue engineering the presence of the magnetite nanoparticles on the physicochemical and biological properties of the scaffolds were investigated in detail.

2. Materials and methods

2.1. Synthesis of magnetite nanoparticles

In this study, magnetite (Fe_2O_3) nanoparticles were synthesized by coprecipitation method under ultrasound condition, as previously described in detail in a report by Aliramaji et al. [41]. Briefly, $FeCl_2 \cdot 4H_2O$

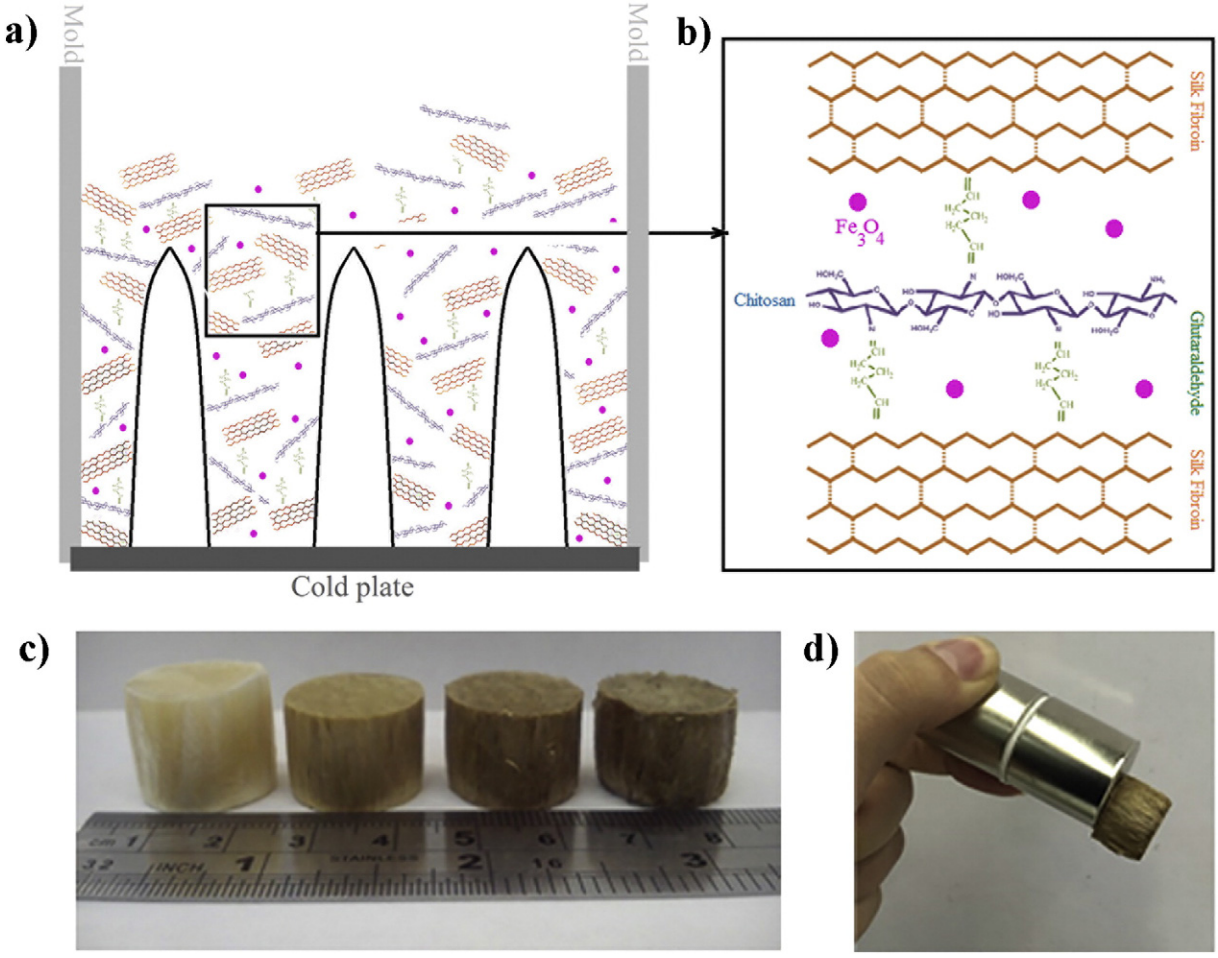

Fig. 1. (a) The schematic illustrates the freeze-casting technique showing the sublimation of the solidified solvent, and then densification of the walls, which result in a porous structure with unidirectional channels, where pores are the ice crystals. (b) Magnified illustration of the chemical structures occurs in the scaffold solution. (c) The fabricated scaffolds; as can be seen a color variation is clearly shown, due to the different amounts of nanoparticles used in the scaffolds. (d) Attraction of the magnetic scaffold to a permanent magnet.