Materials Science and Engineering C 33 (2013) 3958–3967

Contents lists available at ScienceDirect

Materials Science and Engineering C

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

Microstructure and characteristic properties of gelatin/chitosan scaffold prepared by a combined freeze-drying/leaching method

M. Alizadeh [a,b], F. Abbasi [a,b,*], A.B. Khoshfetrat [c], H. Ghaleh [a,b]

[a] Institute of Polymeric Materials, Sahand University of Technology, Tabriz, Iran
[b] Faculty of Polymer Engineering, Sahand University of Technology, Tabriz, Iran
[c] Faculty of Chemical Engineering, Sahand University of Technology, Tabriz, Iran

ARTICLE INFO

Article history:
Received 3 January 2013
Received in revised form 4 May 2013
Accepted 16 May 2013
Available online 24 May 2013

Keywords:
Gelatin
Chitosan
Scaffold
Porosity
Cross-linking
Degradation

ABSTRACT

A combined freeze-drying and particulate leaching method for scaffold synthesis showed an improvement in the horizontal microstructure of the gelatin/chitosan scaffolds. Type and concentration of the cross-linking agent, freezing temperature, concentration of the polymeric solution and gelatin/chitosan weight ratio were the variables affecting the scaffold properties. Assessment of the tensile properties of the scaffolds revealed that for a scaffold with 50% chitosan, glutaraldehyde, as a cross-linking agent, created much tighter polymeric network compared to N,N-(3-dimethylaminopropyl)-N′-ethyl carbodiimide (EDC). However, in the case of gelatin scaffolds, EDC was identified as the stronger cross-linker. Compressive behavior of the scaffolds satisfied formulations obtained from the theoretical modeling of the low-density, elastomeric foams. The investigation of the scaffold degradation indicated that the increase in the mechanical strength of the scaffolds would not always reduce their degradation rate.

© 2013 Elsevier B.V. All rights reserved.

1. Introduction

At a simplistic level, biological tissues consist of cells, signaling mechanisms and extracellular matrix (ECM). To replace or repair tissues in the human body being damaged through a disease or trauma, tissue engineering has to synthesize substitutes or scaffolds. The scaffolds should mimic the natural ECM to induce the growth of new functional tissue in vitro or in vivo [1]. Yannas used collagen-glycosaminoglycan (GAG) scaffold as an ECM analog, for the first time, to induce the dermis layer of skin regeneration. This collagen-GAG scaffold was later commercialized under the name of Integra® [2–6]. At present, there are many other commercial skin substitutes, extensively reviewed in the literature [7–10].

Skin is composed of the epidermis and dermis with a complex nerve and blood supply. The epidermis is composed primarily of keratinocytes, and fibroblasts are the major cell type of the dermis [11,12]. Collagen is the most abundant protein present in the human body, and it is the major component of skin and other musculoskeletal tissues. The major sources of collagen used currently for biomedical applications are bovine or porcine skin or equine Achilles tendons. One disadvantage of these collagen-based biomaterials is their mild immunogenicity. Other concerns include the high cost of pure collagen, variable physicochemical and degradation properties and the risk of infectious diseases transmission due to the allogenic or xenogenic origin of the material [13]. The denatured type collagen, gelatin, is practically more convenient than collagen. While its chemical composition is very similar to collagen, gelatin is known to have no antigenicity and it is far more economical than collagen. Furthermore, a concentrated solution is extremely difficult to prepare from the native collagen. Gelatin is a biodegradable and biocompatible polymer and is able to make polyion complexes. Due to these properties, gelatin is commonly used in drug and cell delivery for tissue engineering applications targeting several tissues such as bone, cartilage and skin [14–19]. Gelatin is also used in medicine as wound dressing [20–22], plasma expander, adhesive, and absorbent pad for surgical use. It activates the macrophages and shows high hemostatic effect [23].

Chitin, poly(β-(1 → 4)-N-acetyl-D-glucosamine), is a natural polysaccharide of major importance, found particularly in the shell of crustacean, cuticles of insects and cell walls of fungi. Chitosan is obtained by partial deacetylation of chitin (at least about 50%) in the solid state under alkaline conditions or by enzymatic hydrolysis in the presence of a chitin deacetylase. Chitosan is analogous to GAGs in structure, but it is very cost effective, in comparison [24]. It has been proved to be biologically renewable, biocompatible, biodegradable, non-antigenic, nontoxic and biofunctional. It exhibits antivirus and antiphage activities [25]. Chitosan can also enhance blood coagulation and accelerate the wound healing. The direct application of chitin and chitosan to the wounds stimulates several different physiological activities such as collagen deposition. Due to these properties, chitosan can act as an ideal wound dressing [26–28]. Gelatin/chitosan composites have a wide spectrum of

* Corresponding author at: Institute of Polymeric Materials, Sahand University of Technology, Tabriz, Iran. Tel./fax: +98 412 3459090.
E-mail address: f.abbasi@sut.ac.ir (F. Abbasi).

0928-4931/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.msec.2013.05.039

tissue engineering applications, such as drug carriers, hepatocyte culture, skin, bone, cartilage, and nerve repair [29–34].

It is required for an ideal skin substitute to have well-defined porous microstructure, specific surface area [3], chemical composition and appropriate degradation rate [5], mechanical and handling properties [12,35]. Different techniques used to fabricate gelatin containing scaffolds with defined shapes and a complex porous internal architecture include particulate leaching [36], emulsion templating [37,38], electrospinning [34], rapid prototyping [39], and mostly freeze-drying [3,40–45]. Despite some deficiencies of quench freeze-drying technique [3], this method is commonly applied for the scaffold fabrication, even in current researches [41,44,45]. In this work, we reassessed freeze-drying method, in terms of the created microstructure, mainly, to investigate the potency of the method for use in skin tissue engineering. Some parameters such as surface tension, chitosan content and combination of freeze-drying with the particulate leaching method were studied how to influence the scaffold microstructure and its uniformity. Rather than aldehyde, prevalent cross-linker of gelatin/chitosan scaffolds [31,39,44,46,47], EDC was employed as the main cross-linking agent. EDC is known to be non-toxic and biocompatible as it is not incorporated into the cross-linked sponge structure [23]. Subsequently, we assessed degradation and mechanical properties of the scaffolds (1) to compare aldehyde and EDC's capability in the crosslinking of gelatin, chitosan and gelatin/chitosan scaffolds (2) to confirm that our scaffolds can be modeled as the low-density, elastomeric foams, and (3) to assess degradation behavior of the scaffolds versus their mechanical properties.

2. Materials and methods

2.1. Materials

Gelatin, N-hydroxysuccinimide (NHS), acetic acid and acetone were obtained from Merck, Darmstadt, Germany. N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrocholoride (EDC) and glutaraldehyde (GA), 25% solution in water, were obtained from Merck, Schuchardt, Germany. Chitosan (percentage deacetylation > 90%, mol. weight $10^5 - 3 \times 10^5$) was purchased from Acros Organics (Geel, Belgium) and lysozyme, chicken egg white, was obtained from Sigma-Aldrich, Steinheim, Germany. All the materials were used without any further purification.

2.2. Scaffold fabrication

Aqueous solution of gelatin was prepared by mixing gelatin in the distilled water with a magnetic stirrer at 50 °C for 30 min. Chitosan was dissolved in acetic acid 1 vol.% at room temperature. The solutions were mixed to have a desired weight ratio of gelatin to chitosan and stirred at 50 °C for 30 min. NaCl powder was added to the solution and dissolved, and then the solution was degassed by centrifugation at 5000 rpm for 10 min. Next, 8 g of this solution was poured into the Wilton® molds (57 mm in diameter), quickly frozen at −20 or −80 °C and lyophilized for 24 h. The obtained sponge was cross-linked by immersing directly in 20 ml of acetone:water mixture (19:1 v:v) containing EDC/NHS or glutaraldehyde, at room temperature for 24 h. The cross-linked scaffolds were finally washed in the distilled water to remove the salt particles and residual cross-linker, and then lyophilized at the same conditions. NHS was used by 25 wt.% of EDC in the cross-linking solution [48].

Eqs. (1) and (2) were applied to prepare gelatin/chitosan solutions with the desired concentrations and gelatin to chitosan weight ratios.

$$m_c = x_c \times \frac{C_t}{C_c} \tag{1}$$

$$C_g = C_t \times \frac{1-x_c}{1-m_c} \tag{2}$$

where m_c is the weight fraction of chitosan solution in the final solution, x_c is the chitosan weight fraction in the scaffold, C_c, C_g and C_t (g/g) are mass concentrations of chitosan, gelatin and final solution, respectively. Table 1 presents the operational conditions applied in the preparation of different gelatin/chitosan scaffolds.

2.3. Porosity

The porosity of the porous gelatin/chitosan scaffolds (P) or the pore volume fraction of the scaffolds was obtained by the following equation [49]:

$$P = \frac{V_p}{V_f} = 1 - \frac{V_s}{V_f} = 1 - \frac{\rho_f}{\rho_s} \tag{3}$$

where V_p and V_s are the volume occupied by the pores and polymeric materials, respectively. V_f is the total volume of the scaffold. ρ_f is the scaffold density and ρ_s is the density of the solid from which it is made. ρ_f was obtained by measuring the scaffold mass and dimensions. To measure ρ_s for each sample, the polymeric solution was dried by exposing it to the air and the film density was then calculated using the film mass and dimensions.

2.4. Microstructure

To characterize the scaffold microstructure, the surfaces and cross-sections of the samples were viewed by scanning electron microscopy (SEM, Tescan MV2300). At least 200 pores were selected from each sample. To measure a pore size, the area of its cross-section was assessed by BEL View image analysis software, then assuming the pore like a sphere, equivalent diameter was reported as the pore size. The distribution of the pore sizes in the scaffolds was assumed to be Gaussian and the following equation was used to determine the pore size polydispersity index (PSPI) [50]:

$$\mathrm{PSPI} = \frac{\sum n_i d_i^2 / \sum n_i}{\left(\sum n_i d_i / \sum n_i\right)^2} \tag{4}$$

PSPI = 1 would be satisfied if all pores in a sample had equal sizes.

2.5. Water uptake

Water uptake ability of the scaffolds (with the dimensions of 2 cm × 1.5 cm × T, T is the thickness of the scaffold) was determined by swelling them in the distilled water for 1 h, enough time for the scaffolds to be saturated. Once the samples were taken out of the water, they were kept hung for 20 s and weighed (W_w). Equilibrium swelling of the scaffolds (E_w) was calculated by Eq. (5).

$$E_w = \frac{W_w - W_d}{W_d} \tag{5}$$

where W_d is the weight of dry samples before swelling.

2.6. In vitro biodegradation

Pieces of sponges (3.5 cm × 3.5 cm × T) were immersed into about 35 ml of phosphate-buffered saline (PBS) solution (pH = 7.4) containing 10,000 U/ml (0.1 mg/ml) lysozyme. After incubation at 37 °C, every two days, the sponges were taken from the medium, washed with the distilled water and freeze-dried. The total duration of the incubation was 8 days. The extent of the in vitro degradation was calculated as the percentage of weight difference of the dry sponge

Download English Version:

https://daneshyari.com/en/article/10614575

Download Persian Version:

https://daneshyari.com/article/10614575

Daneshyari.com