Materials Science and Engineering C 42 (2014) 553–561

Contents lists available at ScienceDirect

Materials Science and Engineering C

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

Chitosan–silica hybrid porous membranes

Christos Pandis [a,b,*], Sara Madeira [c], Joana Matos [c], Apostolos Kyritsis [a], João F. Mano [c,d], José Luis Gómez Ribelles [b,e]

[a] *Physics Department, National Technical University of Athens, Zografou Campus, 15780 Athens, Greece*
[b] *Centro de Biomateriales e Ingeniería Tisular, Universitat Politècnica de València, Camino de Vera s/n, 46022 Valencia, Spain*
[c] *3B's Research Group—Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, 4806-909, Taipas, Guimarães, Portugal*
[d] *ICVS/3B's, PT Government Associate Laboratory, Braga, Guimarães, Portugal*
[e] *Ciber en Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN), Spain*

ARTICLE INFO

Article history:
Received 19 February 2014
Received in revised form 3 May 2014
Accepted 30 May 2014
Available online 7 June 2014

Keywords:
Chitosan
Silica
Genipin
Scaffolds
Hybrid
Mechanical properties

ABSTRACT

Chitosan–silica porous hybrids were prepared by a novel strategy in order to improve the mechanical properties of chitosan (CHT) in the hydrogel state. The inorganic silica phase was introduced by sol–gel reactions in acidic medium inside the pores of already prepared porous scaffolds. In order to make the scaffolds insoluble in acidic media chitosan was cross-linked by genipin (GEN) with an optimum GEN concentration of 3.2 wt.%. Sol–gel reactions took place with Tetraethylorthosilicate (TEOS) and 3-glycidoxypropyltrimethoxysilane (GPTMS) acting as silica precursors. GPTMS served also as a coupling agent between the free amino groups of chitosan and the silica network. The morphology study of the composite revealed that the silica phase appears as a layer covering the chitosan membrane pore walls. The mechanical properties of the hybrids were characterized by means of compressive stress–strain measurements. By immersion in water the hybrids exhibit an increase in elastic modulus up to two orders of magnitude.

1. Introduction

Chitosan (CHT) is a linear polysaccharide derived from chitin, a naturally abundant organic material obtained mainly from the exoskeleton of the crustacean, for example lobsters, shrimps and insects as well as from the cell walls of some bacteria and fungi [1,2]. The deacetylation of chitin yields a copolymer composed of glucosamine and N-acetylglucosamine units linked by (1–4) glycosidic bonds. The degree of deacetylation (DD), defined as the ratio of glucosamine to the sum of glucosamine and N-acetylglucosamine, has influence in molecular weight, crystallinity, solubility, mechanical strength and biological properties of CHT and CHT-based biomaterials [3]. CHT does not dissolve in either organic solvents or in water at neutral or basic conditions but dissolves in acidic medium ($pH < 6$), for example in acetic and formic acids, due to protonation of free amino groups.

The biocompatibility, biodegrability [4], anti-bacterial activity, non-toxicity [4] and cellular compatibility of chitosan have boost the scientific research interest in the latest two decades to explore its use in biomedical applications [3]. Furthermore, its ability to be shaped in various forms such as films, microparticles [5], fibers [6] and porous scaffolds [7], its naturally hydrophilic character [8], the pH-dependent cationic nature and its tendency to interact with anionic glycosaminoglycans (GAGs), heparin, proteoglycans, and nucleotides like DNA or siRNA make CHT particularly suitable as a biomaterial for tissue repair and regeneration [9–11]. Due to its biological properties CHT is considered one of the most valuable polymer for biomedical and pharmaceutical applications such as drug delivery systems [5], surgical suture, dental implants, artificial skin, rebuilding of bone and cartilage, corneal contact lenses and encapsulating material, wound healing accelerator [12], weight loss effect, blood cholesterol control, artificial blood vessels and gene therapy. CHT has been shown to be osteoconductive in vitro and in vivo [13]. However, the mechanical properties of CHT are not adequate for bone regeneration because CHT has low Young's modulus especially in wet porous scaffolds.

The chemical modification of CHT appears as a promising method for the preparation of new materials based on chitosan with advanced physicochemical properties [14]. Furthermore, covalent and ionic cross-linking of chitosan is a very efficient approach to improve the properties of chitosan gels [15]. Tripolyphosphate (TPP) [16] is widely

---

[*] Corresponding author at: Physics Department, National Technical University of Athens, Zografou Campus, 15780 Athens, Greece.
E-mail address: pandis@mail.ntua.gr (C. Pandis).

http://dx.doi.org/10.1016/j.msec.2014.05.073
0928-4931/© 2014 Elsevier B.V. All rights reserved.

used for physical cross-linking while the most common chemical cross-linkers used are dialdehydes such as glyoxal and in particular glutaraldehyde (GTA) [15]. However, in the field of biomedical technology genipin (GEN) [17,18], a cross-linker of natural origin, is often favored over GTA for chitosan cross-linking due to its advantages in terms of cytotoxicity [19].

Another very interesting approach for the preparation of new materials is the combination of chitosan with a second component to fabricate polymer blends and composites. Polymers, like gelatin [20], poly(vinyl alcohol) [21], poly(lactic acid) [22] and poly(ε-caprolactone) [23,24], to name a few, have been utilized for preparing blends with chitosan while inorganic materials, for example hydroxyapatite (HAp) [25], layered silicates [26], carbon nanotubes (CNTs) [27] and glass/ceramic particles [28–31], have been used to form chitosan composites. Especially for applications that mechanical reinforcement is required the incorporation of an inorganic phase into the chitosan matrix to form hybrid materials appears as a very effective strategy. Chitosan/silica hybrids have been prepared by mixing chitosan in the silane solution [32]. Reactions with 3-glycidoxypropyltrimethoxysilane (GPTMS) can link polymer chains to silica network through covalent bonds [33–35]. Furthermore, porous structures of chitosan/TEOS have been fabricated as described in references [36,37].

In this work silica was added in a previously formed chitosan scaffold in the form of a coating layer of the pore walls within the porous structure. The initial highly porous scaffolds were prepared by the freeze gelation technique [38]. CHT was cross-linked by GEN following the optimal procedure combined with the optimal mixture composition. The hybrids were produced by the in-situ synthesis of the silica inorganic part inside the pores of the scaffolds by sol–gel reactions [39] in acidic medium. Extending the successful procedure reported in previous publications of our group [40,41], in this work the coupling agent GPTMS was used in order to facilitate the wetting of the pores and to introduce chemical bonding between the organic and inorganic phases of the hybrid. The influence of silica incorporation in morphology, thermal and mechanical properties of chitosan scaffolds is assessed and discussed.

2. Experimental

2.1. Materials

Chitosan (CHT) with low molecular weight and deacetylation degree (DD) of 75–85% was purchased from Sigma–Aldrich. Acetic acid, hydrochloric acid and sodium hydroxide (NaOH) were supplied from Panreac. Genipin (GEN) was purchased from Wako Chemicals. 3-glycidoxypropyltrimethoxysilane (GPTMS) and tetraethyl orthosilicate (TEOS) were obtained from Sigma–Aldrich. Ultrapure (MilliQ) water was used in all preparation procedures.

2.2. Preparation of hybrids

2.2.1. Preparation of cross-linked films and scaffolds

A starting CHT solution obtained by dissolving 1.5 wt.% of CHT in 1% wt acetic acid was used in all the procedures for preparing films and scaffolds. The solution was left stirring for 24 h and then filtered using a 70 μm millipore membrane.

In order to study the effect of cross-linking and to decide on the optimal GEN concentration to be used later, chitosan films with varying cross-linking degree were prepared. To that end the appropriate amount of GEN was mixed with 30 mL of the above CHT solution to obtain 0.66, 3.2, 6.2, 14.2 and 25 wt.% of GEN in the final CHT films. The mixtures were placed into Petri dishes to react for 24 h at room temperature and the films were obtained by solvent casting. The films were washed repeatedly with distilled water in order to remove residues of GEN and immersed in a 0.5 M solution of NaOH to neutralize them. Finally, were dried at 40 ºC in vacuum for 24 h.

Highly porous scaffolds were prepared using the freeze-gelation method [38,42]. According to this method the chitosan solution being transferred in Teflon crystallizers was frozen at −80 °C using liquid nitrogen. Then the frozen chitosan was fully immersed in the gelification environment of NaOH 0.5 M in ethanol aqueous solution, precooled at −20 °C. The frozen chitosan was left immersed for 72 h at −20 °C, a temperature lower than its freezing point. In that way chitosan was gelled and the acetic acid solution was removed leaving a porous structure. Finally, the obtained scaffolds were cut into disks of 8 mm diameter and washed thoroughly with distilled water up to neutral pH.

The final cross-linked CHT scaffolds were prepared by the two methods, namely method I (metI) and method II (metII) presented schematically in Fig. 1 and described as follows:

2.3. Method I

The CHT solution was mixed directly with GEN under vigorous stirring for 1 h to make a mixture containing 3.2% of GEN referring to the final CHT cross-linked samples. The blend was placed into Teflon crystallizer to react for 24 h at room temperature. Scaffolds were obtained by the freeze-gelation method and finally lyophilized using a Telstar Lyoquest apparatus in order to be left dried and with the pores fully opened for the subsequent steps.

2.4. Method II

The already prepared scaffolds or films of pure CHT were immersed into aqueous GEN solution (0.0065% w/v) at room temperature for 48 h and protected from light. To remove the excess of GEN the cross-linked films and scaffolds were washed with distilled water repeatedly. Then, the scaffolds were freeze-dried at −80 ºC for 24 h and the films were dried under vacuum.

2.4.1. Introduction of silica by sol–gel

In a second step the silica precursors were introduced inside the pores of the lyophilized scaffolds. For this purpose, the silica network was synthesized in situ by the sol–gel method [39] using TEOS and GPTMS as precursors. The molar ratio of TEOS with respect to GPTMS in the sol–gel solution was either 1:0.1, 1:0.5 or 1:1 and the molar ratios of the rest of the components (water, ethanol and chloride acid) of the starting solution were kept constant (1, 2 and 0.0185, respectively) with respect to TEOS. After 1 h of stirring, the above solution was transferred to a glass tube fully covering the CHT scaffolds. In order to assure that the pores of the polymer will be filled by the precursor solution, a vacuum pump attached to the reaction tube was pumping for 5 min prior to the introduction of the solution. After being immersed for 15 min in the sol–gel solution the samples were superficially rinsed with water/ethanol. Then, the scaffolds with silica layers on the pore walls, were left at room temperature for 24 h and subsequently dried for 24 h. Finally, the samples were washed with distilled water for 4 h and then dried again at 50 °C in vacuum for 24 h. The weight content of the silica phase (including organic side chains of GPTMS) was calculated by the increase of weight of the membrane after sol–gel reaction (Table 3) ranging between 74 and 89 wt.% of the obtained hybrid. The content of SiO_2 can be estimated from the residual weight after pyrolysis which ranges between 26 and 43 wt.% (Table 3).

2.5. Material characterization

2.5.1. Morphology

The morphology of the CHT, CHT–GEN and CHT–GEN/silica scaffolds was studied by means of Scanning Electron Microscopy (SEM) using a JSM-6300 (JEOL) microscope, with the samples previously sputter coated with gold. Porosity was measured by a gravimetric method. The samples were weighed dried and then immersed into octane in