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Fabrication of chitosan based hybrid porous scaffolds by salt leaching for soft tissue engineering

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1. Introduction

Tissue engineering research is an effective and confident method to design scaffolds with the ability of biomimicking the natural extracellular matrix (ECM), which guides, cellular migration, provides mechanical support and regulates cellular activities [1,2]. Hybrid porous three-dimensional polymeric scaffolds are of critical importance for their application in tissue engineering. [3]. The scaffolds need to be biocompatible, biodegradable, high porosity, high pore interconnectivity, large specific surface, uniform pore distribution with a degradation or resorption rate that the tissue replacement and possess good mechanical properties [4]. Scaffolds are desired to minimize the amount of implanted material and to increase the specific surface area for cell attachment, a vibrant environment for regeneration of damaged tissue and tissue in growth [5–7]. Cells are developed on a biodegradable porous scaffold in which the tissue is forming and growing, the scaffold gradually degrades. [8] Due to high permeability, ease of preparation and suitable elasticity, scaffolds are widely used as a 3D scaffold [9]. These scaffolds for tissue engineering have been achieved by various methods, such as the phase-separation process, solvent casting, lyophilization and salt leaching technique [10]. Recently, salt leaching technique has attracted much attention for the fabrication of polymeric porous scaffolds. Moreover the salt-leaching procedure proposed in the present contribution does not affect the properties of the scaffold [11].

Chitosan is a biodegradable, nontoxic, natural polymer has been widely investigated for biomedical applications and tissue engineering. Chitosan is known to have various biological activities including immune enhancing effects, antitumoral, antifungal and antimicrobial activities. Chitosan is degraded by enzymatic hydrolysis however, its tensile strength and elasticity is not suitable for some biomedical applications such as wound dressing and skin tissue replacement. Chitosan joined to other polymers opened a window of research for altering or tailoring the property of interest [12].

Poly(vinyl alcohol) (PVA) is a water soluble synthetic polymer of great interest because of its many desirable characteristics [13,14]. PVA possesses desirable properties such as non-toxicity, biocompatibility, high hydrophilicity, easier film forming ability, chemical resistance, and mechanical resistance [15]. Methylcellulose is a water soluble cellulose with excellent film-forming properties. Methylcellulose (MC) has specific advantages compared to other conventional cellulose since it is readily dissolved in water and can be easily prepared in the form of large area thin films with excellent mechanical strength and moderate oxygen permeability [16].

Previously we have reported a novel fabrication method of chitosan based hybrid porous scaffolds by lyophilisation technique. The resultant scaffolds had a highly porous and well interconnected pore structure with mechanical properties, and good

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biocompatibility [17]. In this regard, to improve the properties of chitosan and PVA polymers, the salt-leaching method has been used to prepare porous hybrid scaffolds employing excess NaCl crystals as a porogen. Nevertheless, two main drawbacks to producing these scaffolds need to be overcome: (i) Lyophilisation method is very cost effective and (ii) Hybrid scaffolds have small interconnected pores. However, in the present study, rather than processing by lyophilisation technique, salt leaching method was used and methylcellulose, a common plasticizer for chitosan and PVA was introduced.

2. Materials and methods

2.1. Materials

Chitosan, Poly vinyl(alcohol) and methylcellulose (analytically pure) were purchased from S&D fine Chemicals, Mumbai, India. Table salt (NaCl) was used as the particulate porogen. The salt was dried under vacuum at 100°C prior to blending. All other chemicals used were of analytical grade.

2.2. Fabrication of CS/PVA/MC scaffolds through the salt-leaching technique

Chitosan and PVA were blended by mixing a 1% solution of chitosan in 1% aqueous acetic acid and 1% solution of PVA in warm water. 1% Chitosan and PVA solutions were slowly added to 0.1%, 0.3% and 0.9% MC solutions to obtain blends of 25%, 50% and 75% MC, respectively. The mixtures were prepared by 6 h magnetic stirring at room temperature to obtain homogeneous solutions. Then, 4 g of granular sodium chloride (NaCl, 200–600 μm) was homogeneously added into the polymer solutions in disk-shaped Teflon coated container. The solution was left at room temperature for 24 h to allow gelation, prior to washing with deionised water in order to leach out the salt crystals. Next, the scaffolds were left to dry for 24 h and the salt-leached porous CPM scaffolds were obtained.

2.3. Attenuated total reflectance-fourier transform infrared spectroscopy (ATR-FTIR)

The chemical structure of the prepared porous scaffold was characterized using an attenuated total reflectance Fourier transform (ATR-FTIR) spectrophotometer (Shimadzu IR affinity – 1S). Each spectrum was acquired in transmittance mode on a Quest ATR ZnSe crystal cell by accumulation of 250 scans with a resolution of 4 cm^{-1} and a wavenumber range of 4000 – 500 cm^{-1}.

2.4. X – ray diffraction (XRD)

X-ray diffraction patterns of CS/PVA/MC/NaCl scaffolds were recorded with an X-ray diffractometer (XRD; Rigaku Co, model; DMAX – 2200, Japan). The voltage and current used were 40 Kv, and 36 mA, respectively. X-rays of 1.5406 Å wavelengths were generated by a CuK source and the 2Θ angle was varied from 5° to 80°.

2.5. Swelling behavior

The dried scaffolds were immersed in an excess of deionized water at room temperature. The swollen samples were weighed at definite time intervals after wiping off the excessive surface water with wet filter paper. The swelling ratio (SR) of the scaffold was determined using the following equations: where W_t was the weight of the swollen scaffold at time t and W_d was initial weight of the scaffold.

$$SR = \frac{\mathrm{Wt} - \mathrm{Wd}}{Wd} \times 100 \quad (1)$$

The swelling kinetics in 4wt%NaCl solution were tested in the same way. The equilibrium water content (EWC) was calculated as follows: where weight of the We was weight of the swollen scaffold at equilibrium and Wo was intial weight of the scaffold.

$$EWC(\%) = \frac{\mathrm{We} - \mathrm{Wo}}{We} \times 100 \quad (2)$$

2.6. Water retention test

To measure the water retention ability, the wet scaffolds were transferred to centrifuge tubes with filter paper at the bottom, centrifuged at 500 rpm for 5 min and weighed immediately (W_{wet}). The percentage of water retention (WR) of the scaffolds at equilibrium was calculated using following (2):

$$WR\% = \frac{Wwet(R) - Wdry}{Wdry} \times 100, \quad (3)$$

where $W_{wet(R)}$ is the wet weight after a predetermined time and W_{dry} is the original weight of the sample .

2.7. Porosity

Scaffold samples were immersed in ethanol for 2 h. The volume of a scaffold immersed in the fluid is equal to the volume of the displaced fluid, and we can calculate the porosity from the following equation.

$$P\% = \frac{W1 - W3}{W2 - W3} \times 100 \quad (4)$$

Where W_1: weight of the scaffold before immersion, W_2: weight of the scaffold after immersion and W_3: weight after drying by this method we can just get the porosity percentage (P %).

2.8. Scanning electron microscopy (SEM)

A scanning electron microscope (SEM), Hitachi SR-4700, operated at 1 Kv, was used to examine the scaffold morphology. The CPM/NaCl scaffold were fractured in liquid nitrogen and etched with deionized water for 48 h at ambient temperature to selectively extract the water soluble components (MC/PVA and NaCl salt particles). The cross-sections were coated with platinum using a sputter coater (Emitech K575 X) operating at 15 mA for about 25 s under argon atmosphere.

2.9. In vitro degradation test

In vitro degradation of the samples was studied in PBS under pH 7.4 and 37°C. Film specimens (10 mm × 10 mm) were incubated in test tubes containing 15 ml PBS. The tubes were stored in a water bath at 37° C for 20 weeks. The pH value of PBS was measured weekly and then the percentages of weight loss (%Wt loss) of the samples were calculated by Eq. (3)

$$\%Wt\ loss = \frac{W0 - Wt}{W0} \times 100, \quad (5)$$

where W_0 is original dry weight, and Wt is the dry weight at t [18].

2.10. Statistical analysis

All data were presented as means ± standard deviations. Comparisons among the three groups were made with one-way analysis of variance.