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Processing and characterization of chitosan/PVA and methylcellulose porous scaffolds for tissue engineering

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ABSTRACT

Biomimetic porous scaffold chitosan/poly(vinyl alcohol) CS/PVA containing various amounts of methylcellulose (MC) (25%, 50% and 75%) incorporated in CS/PVA blend was successfully produced by a freeze drying method in the present study. The composite porous scaffold membranes were characterized by infrared spectroscopy (FTIR), X-ray diffraction (XRD), thermogravimetric analysis (TGA), differential scanning calorimetry (DSC), scanning electron microscopy (SEM), swelling degree, porosity, degradation of films in Hank's solution and the mechanical properties. Besides these characterizations, the antibacterial activity of the prepared scaffolds was tested, toward the bacterial species *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*). FTIR, XRD and DSC demonstrated that there was strong intermolecular hydrogen bonding between the molecules of CS/PVA and MC. The crystalline microstructure of the scaffold membranes was not well developed. SEM images showed that the morphology and diameter of the scaffolds were mainly affected by the weight ratio of MC. By increasing the MC content in the hybrid scaffolds, their swelling capacity and porosity increased.
The mechanical properties of these scaffolds in dry and swollen state were greatly improved with high swelling ratio. The elasticity of films was also significantly improved by the incorporation of MC, and the scaffolds could also bear a relative high tensile strength. These findings suggested that the developed scaffold possess the prerequisites and can be used as a scaffold for tissue engineering.

1. Introduction

The structure and properties of three-dimensional porous polymeric scaffolds fabricated from biodegradable polymers have been widely used as temporary extracellular matrices (ECM) and play critical importance in tissue engineering and in situ tissue reconstruction [1,2]. The scaffolds need to be cell/tissue biocompatibility, biodegradable high porosity, large specific surface, uniform pore distribution, and pore interconnectedness to allow sufficient transport of nutrients and possess good mechanical properties to match with a degradation or resorption rate that matches the tissue replacement [3,4].

A variety of biodegradable polymer scaffolds have been extensively used for the regeneration of various soft and hard tissues. Use of biodegradable polymers, with numerous biomedical applications, has been progressively increased during the past year. Composite scaffolds may prove necessary for reconstruction of multi-tissue organs, tissue interfaces, and structural tissue including bone, cartilage, tendons, ligaments and muscles [5]. A scaffold, if prepare from a single polymer, cannot impart all these desired properties, but by taking two or more polymers in combination, it is possible to tailor a scaffold with the desired characteristics [6,7]. Various polymers have been employed for scaffold fabrication. Among the tissue scaffolds, hydrogel-based scaffold materials based on biopolymers such as PVA, methylcellulose, and chitosan, are appealing due to their structural similarities to macromolecular based human tissues, and their biocompatibility, low toxicity, relative low cost and availability [8,9].

Chitosan (CS) as a natural biomaterial, partially deacetylated derivative of chitin and a component of the native extracellular matrix (ECM) is extensively investigated for preparation of porous scaffolds for cartilage tissue engineering [10]. However the weak mechanical strength of the scaffolds prepared from chitosan remains a major hurdle behind the clinical application. An effective method to overcome chitosan drawbacks is to blend it with a synthesized polymer to develop a composite. Chitosan with other polymers to form polymer blends, which is one of the most versatile ways of producing new scaffolds for tissue engineering technology [11–14]. Many studies are now attempting to approach physicochemical attributes of hybrid polymers of natural and synthetic [15].

This is mainly due to its simplicity, availability of a wide range of synthetic and natural polymers for blending and effectiveness for practical utilization. PVA is a water-soluble, biodegradable and non-toxic polymer which is gaining growing interest for biomedical applications, both as a structural component, e.g., for artificial cartilage or tissue

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engineering scaffolds as a functional component, e.g., for controlled drug delivery systems [16]. Blending of PVA and chitosan is favourable because both of them are polar substances which have many hydroxyl groups in their chemical structure. These highly polar hydroxyl groups tend to form intermolecular and intramolecular hydrogen bonds. The formation of hydrogen bonds between the hydroxyl groups of chitosan and PVA tends to promote the localized stability and subsequently improve the miscibility of chitosan and PVA [17] have studied the effects of various plasticizers on the different biopolymers [18].

Methylcellulose (MC) is biodegradable cellulose ether that presents good solubility in water at low temperature. The interest in the use of MC arises not only from its biodegradable character, but also from the fact that it can be used to produce gels and fine chemicals in pharmaceuticals, foods, paints, ceramics, detergents, agriculture, polymerization, adhesives and cosmetics for many years [19,20].

The plasticizing efficiency and compatibility are the two main criteria for selecting the plasticizer. Usually the plasticizing efficiency of the plasticizers could be enhanced with the content. But the phase separation between the plasticizer and polymer would occur at high plasticizer content. Our previous work has proved that MC has a high plasticizing efficiency for CS and PVA compatibility between these polymers was very good [21]. Based on the common ground between the molecular structure of CS and PVA, here we would have used different weight ratios of MC as a high plasticizing efficiency for porous scaffold system.

The composition of this ternary blend of chitosan/PVA/MC was based on the fact that MC can form miscible blends with chitosan and that these are compatible with CS/PVA. Although a few researchers have studied CS/PVA blend films by solution casting method, all of these studies were used in other fields such as food packaging [22] instead of tissue scaffolds. The main objective of this study was to enhance the physical, chemical and mechanical properties of hybrid polymer of porous scaffolds based on CS/PVA/MC where MC in different ratios has been used to satisfy the requirements of the tissue engineering. To our knowledge, this is the first report, where such a hybrid ternary system of porous scaffolds of CS/PVA/MC was fabricated and extensively characterized by morphological, spectroscopy and mechanical aspects with different polymer contents, regarding to the hybrid network formation (polymer–polymer), swelling performance and also biocompatibility behaviour.

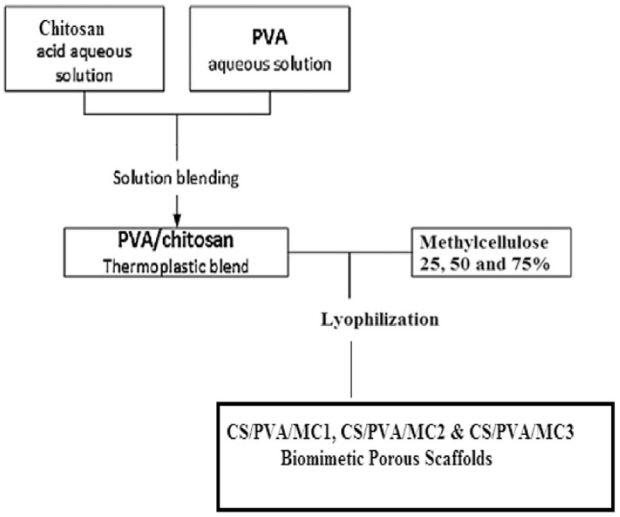
2. Experimental

2.1. Materials

Chitosan (92% deacetylated) was the kind gift of India seafoods Cochin, Kerala, India. PVA and methylcellulose (analytically pure) were purchased from S&D fine Chemicals, Mumbai, India. All other chemicals used were of analytical grade.

2.2. Preparation of porous hybrid chitosan-poly(vinyl alcohol)-methylcellulose scaffolds (CS/PVA/MC)

The preparation of the porous CS/PVAMC blend scaffolds is systematically shown in Scheme 1. The porous chitosan based hybrid ternary blend scaffolds, were prepared by a combination of film casting and lyophilization methods. One gram chitosan powder was added into a 100 ml of 0.1 M acetic acid and the mixture was stirred to form a 1 wt.% clear chitosan solution. Meanwhile, 1 g PVA powder was charged into 100 ml at 80 °C purified water while stirring to form a 1% clear PVA solution and methylcellulose powder was charged into 100 ml purified water and stirred to form 1 wt.% methylcellulose solutions, respectively. In the remaining of the manuscript, MC1, MC2 and MC3 means CS/PVA/MC scaffold membranes, which have 25%, 50% and 75% of methylcellulose content, respectively.



Scheme 1. Flow diagram of the fabrication process for porous scaffold.

2.3. Scaffold formation — lyophilization method

To form a porous scaffold each of the degassed mixture (10 ml) was poured into Teflon petri dishes, refrigerated at 4 °C, frozen at −20 °C in a deep freezer for 24 h. The frozen dishes were placed in a lyophilizer at −40 °C until dried porous scaffolds were obtained. The resulting scaffolds (area 2 cm^2, thickness 3.0 mm) were neutralized to remove acetate by immersing them in 10% NaOH followed by washing with water until neutralized, followed by lyophilization.

2.4. Characterisation of the prepared scaffolds

2.4.1. Attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) spectra

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 4000–400 cm^{-1}.

2.4.2. X-ray diffraction (XRD)

The XRD experiment was performed using an X-ray powder diffractometer (XRD-SHIMADZU XD-D1) using a Ni- filtered Cu Kα X-ray radiation source.

2.4.3. Thermogravimetric analysis (TGA)

The thermal behaviour of the samples was performed with a SDT Q600 V8.0 build 95 thermal gravimetric analyzer under a nitrogen atmosphere and at a heating rate of 10 °C/min in the temperature range of 25–600 °C.

2.4.4. Differential scanning calorimetry (DSC)

The thermal behaviour of the porous scaffold was studied with a DSC technique. DSC was performed with a Q500 (TA instruments, New Castle, USA) under atmosphere. About 5 mg of the sample was heat-treated from 50 °C to 200 °C at a heating rate of 10 °C/min.

2.4.5. Swelling degree

The swelling behaviour of scaffolds was investigated at room temperature by exposing them to phosphate buffer solution (PBS). A known weight of scaffold material was placed in PBS solution for 30 days. The wet weight of the scaffold was determined by first blotting the scaffold surface with filter paper, to remove excess surface water