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Regenerated cellulose/β-cyclodextrin scaffold prepared using ionic liquid

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ABSTRACT

Regenerated cellulose/β-cyclodextrin (RC/β-CD) tissue engineering scaffolds were fabricated by two particulate leaching techniques using environmental-friendly 1-butyl-3-methylimidazolium chloride (BMIMCl) ionic liquid. In one case, the pore inducing particles were water-soluble (edible salt) and in the other, they were water-insoluble poly (methyl methacrylate) (PMMA). The water-soluble particulate leaching and water-insoluble methods resulted in scaffolds with 70–75% and 91–93% porosity, respectively. X-ray diffraction revealed lower crystallinity of the RC/β-CD scaffolds as compared to pure RC. The RC/β-CD scaffolds prepared by water-insoluble leaching method showed the highest water uptake properties. In vitro cytotoxicity test demonstrated that RC/β-CD scaffolds are cytocompatible.

1. Introduction

Tissue engineering has attracted enormous attention over the past two decades. It aims at restoring, maintaining, or improving tissue function [1,2]. One of the key factors for a successful tissue engineering approach is the fabrication of a 3-D biodegradable scaffold. A scaffold must have certain properties such as controlled porosity and pore size distribution, biodegradation and biocompatibility [3]. Different natural and synthetic biodegradable polymers have been proposed for tissue engineering applications [4]. One major category of natural polymers is polysaccharides. Cellulose, as the most abundant polysaccharide found on earth, is the best example [5]. Cellulose, which is a linear polysaccharide composed of β-1–4-linked D-glucopyranose repeating units, has drawn much attention due to its unique properties such as high mechanical strength, chemical stability, biodegradability and biocompatibility [6]. In order to increase the functionality of cellulose, modification with β-CD is implemented. β-CD are cyclic oligosaccharides consisting of seven (β) units linked by 1,4-α-glucosidic bonds from the enzymatic degradation of starch [7]. β-CDs can form inclusion complexes with a wide variety of molecules. Therefore, these compounds have been used in various areas such as drug delivery, biochemistry, organic synthesis, and catalysis [8,9].

However, on account of the extensive and large network connecting hydrogen bonds and partially crystal structures, cellulose cannot be dissolved in water or most conventional organic solvents [10]. Recently, ionic liquids (ILs) have received great attention because of their low toxicity [11–13], chemical and thermal stability, relative non-volatility and recyclability [14]. Despite the wide range of ILs available, among the most promising ILs for wood dissolution are the imidazolium-based ILs with short side chains. 1-butyl-3-methylimidazolium chloride (BMIMCl) is the most frequently used hydrophilic IL which can dissolve cellulose to produce a cellulose solution without any derivative [15,16].

In the present study, regenerated cellulose/β-cyclodextrin scaffolds have been successfully prepared from BMIMCL solution and in vitro cytotoxicity test has been demonstrated for potential applications as tissue engineering scaffolds.

2. Experimental

2.1. Materials

Microcrystalline cellulose, a commercial reagent from Sigma was used. β-CD was purchased from Merck. The (BMIMCl) with ≥95% was supplied by Sigma. The PMMA granules with a size distribution between 120 to 250 μm and the KI crystals with a size distribution between 250 to 350 μm were supplied by Sigma. All other chemicals were purchased from Aldrich, were of analytical quality and were used as obtained with no further purification.

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2.2. Preparation of compact and porous RC/β-CD samples

2.2.1. Preparation of porous RC/β-CD scaffold by leaching water-soluble particulates

Cellulose was initially dissolved in BMIMCL by heating at 90 °C for 24 h and constant stirring. The ratio of BMIMCL/cellulose was 94/6 (wt.%). RC/β-CD solutions were mixed with potassium iodide (KI) particles in a Petri dish and allowed to stand at 5 °C for 24 h. The mass ratio of cellulose solution to KI was 1:3 (KI Volume fraction, $V_{f\ (KI)}=0.210$). Within this period, gelation occurred and synthesis started. The sol was removed from the Petri dish and the gel was washed with double distilled water several times in order to ensure complete removal of the solvent and the salt particles. The products of this process were swollen porous scaffolds.

2.2.2. Preparation of porous RC/β-CD scaffold by leaching water-insoluble particulates

Solution of RC/β-CD in BMIMCL was mixed with PMMA particles in a mass proportion of 1:2 (PMMA Volume fraction, $V_{f\ (PMMA)}=0.198$). After standing for 24 h, they were washed with double distilled water several times for 48 h in order to ensure complete removal of the solvent system. The sample was immersed in and washed with methanol several times and then allowed to dry for 24 h at 40 °C. Following this procedure, the macroscopic shapes of the gels were retained and no shrinking occurred. After drying, the samples were immersed in dichloromethane to leach PMMA particles and, finally, were dried in vacuum at 40 °C for 24 h.

2.2.3. Preparation of compact RC/β-CD samples

A compact RC/β-CD sample was prepared by a procedure similar to the one used for porous samples but without the KI salt or PMMA particles. The scaffolds prepared with different methods were coded as listed in Table 1.

2.3. Characterization

X-ray diffraction (XRD) patterns were obtained using a XRD diffractometer (Rigaku Miniflex II). Patterns with Cu Kα radiation (λ= 0.15406 nm) at 40 kV and 30 mA were recorded in the region of 2θ from 5 to 30. The morphology of samples was examined using a JEOLJSM-6701 F field emission scanning electron microscope (FESEM). The average pore diameter of the samples was calculated from the SEM pictures using the appropriate software (ImageJ 1.32j). The pore size distributions were obtained by image analysis of the FESEM micrographs by the appropriate software (ImageJ 1.32j). For every sample, at least two different FESEM pictures were used and for each picture at least 30 pores were measured. The water uptake (WU) ability of the scaffolds was analyzed for 80 h. Small pieces of the scaffolds with equal weights (W_0) were immersed in deionized water at room temperature. The wet weight of the samples (W_t) was determined after several times, by gently blotting them on filter paper. The water uptake ability was calculated according to Eq. (1).

$$WU(\%) = \frac{W_t - W_0}{W_0} \times 100 \quad (1)$$

Table 1
Samples identifications.

Samples	Sample identification
Regenerated cellulose/β-cyclodextrin	RC/β-CD
RC/β-CD scaffold prepared with KI salt	RC/β-CD-S
RC/β-CD scaffold prepared with PMMA particles	RC/β-CD-P

where W_t and W_0 are the wet and initial dry sample weight, respectively.

Cell proliferation of the samples were evaluated on human skin fibroblasts (HSF 1184) using dimethylthiazol diphenyl tetrazolium bromide (MTT) assay. The absorbance was measured at 570 nm using the spectrophotometric microplate reader (ELISA, ELx808). Experiments were run in triplicate per each sample. Statistical comparisons were performed using one-way analysis of variance ANOVA with the "Turkey's Post Hoc test". P values <0.05 were considered statistically significant.

3. Results and discussion

Fig. 1 shows the XRD patterns of β-CD powder, RC, compact and porous RC/β-CD scaffolds. The crystalline form of RC with typical diffraction peaks at $2\theta=12.24^\circ$ and $2\theta=21.6^\circ$ are assigned to $(1\bar{1}0)$ and (1 1 0)/(0 2 0) lattice planes of cellulose II crystalline structure [17]. β-CD exhibited many crystalline peaks, indicating that β-CD mainly existed in a crystalline form. However, the diffraction peaks corresponding to β-CD were not observed in the diffractograms of the developed nonporous and scaffold RC/β-CD samples, indicating that β-CDs were in the amorphous phase (Fig. 1). In addition, the weaker diffraction intensity of the RC/β-CD samples at $2\theta=12.24^\circ$ implied that they had lower crystallinity compared to the pure RC film [18]. This indicated that the hydrogen bond formation between RC and β-CD considerably slows down the re-crystallization of RC.

FFESEM micrographs of compact and porous RC/β-CD samples are shown in Figs. 2a–d. It is clearly observed from Fig. 2a–b that the compact RC/β-CD shows nonporous structure. The RC/β-CD scaffold was prepared with KI particles having a size distribution between 23 ± 3 and 153 ± 41 μm. The RC/β-CD-S exhibited an average pore size of 91.80 ± 6 μm (around 80% of pores have pore size between 85 ± 7 and 98 ± 5 μm) with 70–75% porosity (Fig. 2c–d). The pores of the RC/β-CD-S scaffold exhibited a low interconnection and surface porosity. As shown in Fig. 2e–f, the RC/β-CD-P scaffold revealed the well interconnected pores and surface porosity. The RC/β-CD scaffold was prepared with PMMA particles having a size distribution between 0.358 ± 0.1 and 4.9 ± 0.8 μm. The RC/β-CD-P scaffold has an average size distribution of 3.40 μm (around 89% of were found between 3.1 ± 0.2 and 3.6 ± 0.1 μm) and high 93–95% porosity. It is worth pointing out that interconnectivity is much higher in the case of PMMA leaching, although the proportion of porogen (PMMA) to polymer (RC/β-CD) was lower than that in the case of salt leaching.

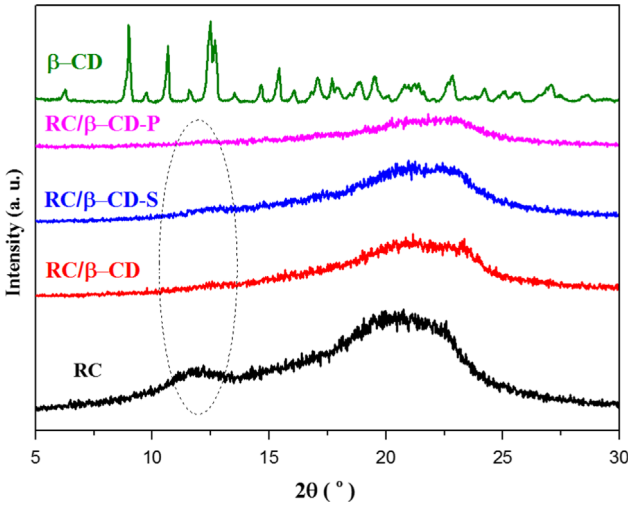

Fig. 1. XRD patterns of β-CD, RC, compact and porous RC/β-CD.