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Fabrication and degradation of poly(L-lactic acid) scaffolds with wool keratin

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ABSTRACT

As a natural protein, wool keratin was used to improve the cell affinity of poly(L-lactic acid) (PLLA). Small keratin particles were prepared from keratin solution by the spray-drying process. Keratin particles were blended with PLLA/1,4-dioxane solution and paraffin micro-spheres which were used as progens. After the mixture was molded and dried, the paraffin micro-spheres were removed by cyclohexane. PLLA/keratin scaffolds with controlled pore size and well interconnectivity were fabricated. Keratin releasing rate was detected by Fourier transform infrared (FTIR) after the scaffold was immersed into PBS up to 4 weeks. The surface chemical structure was examined by X-ray photoelectron spectroscope (XPS). The results suggested that the keratin could be held into the scaffold which was expected to improve the interactions between osteoblasts and the polymeric scaffolds.

1. Introduction

Tissue engineering is based on synthetic or natural degradable materials as scaffolds, which support cells and guide tissues and organs regeneration [1,2]. Because the biomechanical properties and biodegradabilities of poly(α-hydroxy acids) could be adjusted chemically for various applications, they have been extensively investigated [3–6]. In spite of poly(α-hydroxy acids), including polylactic acid (PLA), polyglycolic acid (PGA), and their copolymers poly(lactide-co-glycolide) (PLGA), are biodegradable and biocompatible, they lack cell-recognizable signals to attract cell seeding and migration [7,8]. Many cell affinity materials, such as collagen and hydroxyapatite, have been used to improve their capacity to support cell adhesion and proliferation [9–13].

Keratins are the major structural fibrous proteins constructing hair, wool, nail and so on [14], which are characteristically abundant in cysteine residues (7–20 number% of the total amino acid residues) [15]. As alternative natural proteinous biomaterials for collagen [16], wool keratins have been demonstrated to be useful for fibroblasts [17] and osteoblasts [18], owing to their cell adhesion sequences, arginine–glycine–aspartic acid (RGD) and leucine–aspartic acid-valine (LDV), biocompatibility for modification targets. Moreover, they are biodegradable in vitro (by trypsin) an in vivo (by subcutaneous embedding in mice) [19]. Keratin sponges with controlled pore size and porosity was fabricated by a compression-modeling/particulate-leaching method [20].

2. Materials and methods

2.1. Materials

Poly(L-lactic acid) (PLLA) with an inherent viscosity of 7.0 dl/g was purchased from PURAC (Netherlands) and used as received. Poly(vinyl alcohol) (PVA) (88% hydrolyzed, average molecular weight 25,000 g/mol), paraffin (melting point 53–57 °C), 1,4-dioxane, and cyclohexane were purchased from Acros (Belgium). The wool keratin particles used in this study was prepared from keratin solution by a spray-drying process.

2.2. Preparation of PLLA/keratin scaffolds

Paraffin micro-spheres were prepared by solidifying tiny paraffin drops in PVA solution at 60 °C [9,10,21,22]. PLLA and keratin powder was dissolved in 1,4-dioxane with a desired concentration and ratios. The PLLA/keratin (1:1 in weight) solution was then mixed with paraffin micro-spheres. A plastic syringe tube without end was used as the mold for preparing PLLA/keratin scaffold. Some of PLLA/keratin/paraffin micro-spheres suspension was poured into the mold. Caution was taken to ensure that there was no air bubble trapped inside the mold. The solution and paraffin spheres were compressed until no extra PLLA/keratin solution were found out of the mold. At this point, the paraffin spheres contacted each other and the inter space among paraffin spheres was fully filled with PLLA/keratin solution. The final samples were about 3 mm in thickness. After the scaffolds were dried in an incubator at 37 °C for 12 h, they were immersed in 40 mL of cyclohex-

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ane at room temperature for 12 h to dissolve and remove the paraffin. The morphology of scaffold was observed by SEM (LEICA, Stereoscan 440).

2.3. Biodegradation properties

PLLA/keratin scaffolds were immersed in 50 ml phosphate buffer saline (PBS, pH 7.4) at 37 °C for various periods up to 4 weeks. The degradation medium was changed daily for the first week, once at day 10 and day 14, and then weekly for the rest of the remaining period. Scaffolds were taken out at 1, 3, 7, 14, and 28 days. The samples were examined by FTIR and XPS (Perkin-Elmer, PHI 1600ESCA), respectively.

2.4. Keratin releasing rate

PLLA/keratin samples before and after degradation were examined by Fourier transform infrared (FTIR). The characteristic peaks of PLLA and keratin were used to calculate their ratios after different degradation periods. Samples with different ratios of PLLA and keratin were tested by FTIR to determine the ratio of absorption coefficients (k). Then, the percentage of PLLA (C_P) and keratin (C_k) were calculated using the following equations: $C_P = \frac{R}{k+R}, C_k = \frac{k}{k+R}$, where R is the ratio of absorbance of PLLA (A_p) and keratin (A_k) : $R = \frac{A_P}{A_k}$.

2.5. X-ray photoelectron spectroscope

The samples were characterized with an X-ray photoelectron spectroscope (XPS) (Perkin-Elmer, PHI 1600ESCA). Analyses were performed on a PHI 1600 model surface analysis system with a 250 W MgK X-ray (1253.6 eV) source at a base pressure ranging from 10^{-8} to 10^{-9} Torr. Samples were attached to the aluminum sample platform with double-sided tapes. All XPS spectra were average results from a surface area of 0.8 mm^2. Quantitative analyses were performed using peak areas and elemental sensitivity factors. Elemental surface composition was expressed in atomic percent (at %). Nitrogen (N) was used to characterize the amount of entrapped keratin on the surface of PLLA scaffolds. Mean values were from three replicate tests.

3. Results and discussion

Fig. 1 shows the morphology of wool keratin particles which has an average diameter of about 8 μm. The PLLA/keratin scaffold was highly porous (Fig. 2). The pores were well interconnected and reflected a negative replica of the paraffin micro-spheres. After the paraffin micro-spheres were dissolved and removed, the space occupied by them was emptied as pores which corresponded in

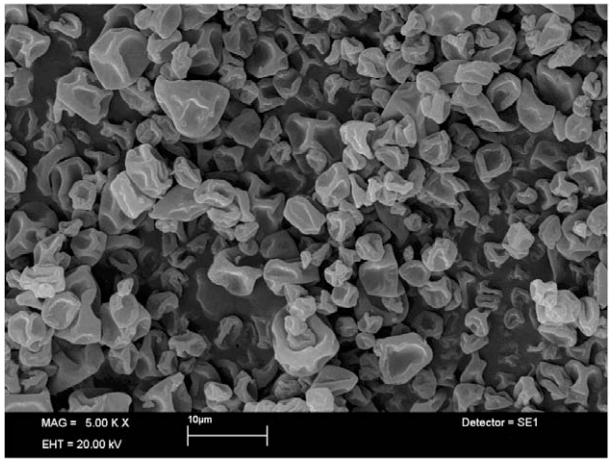

Fig. 1. SEM micrograph of wool keratin particles.

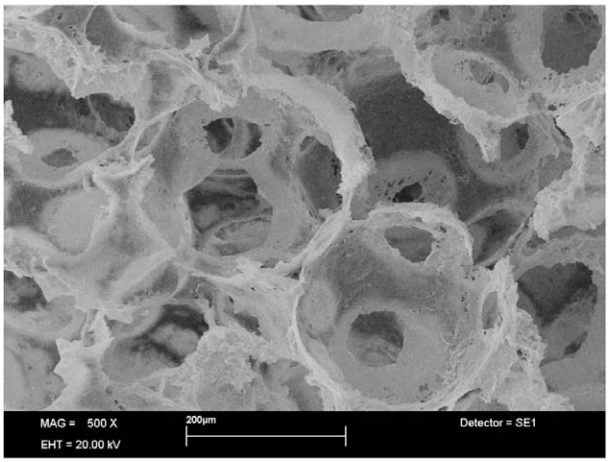

Fig. 2. SEM micrographs of PLLA/keratin scaffold. The size range of the paraffin spheres was 180–250 μm.

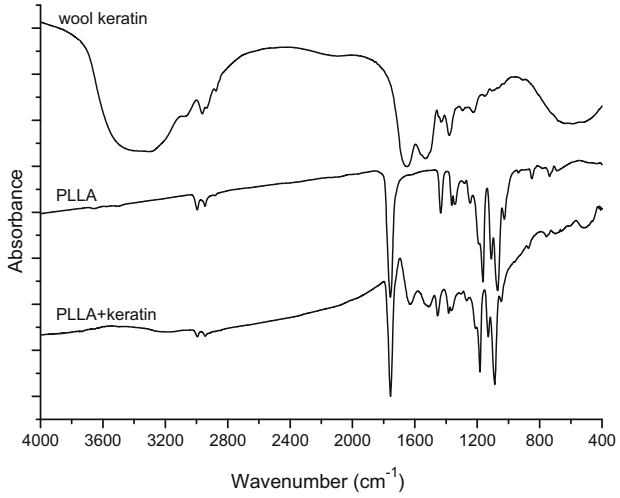

Fig. 3. FTIR of PLLA, keratin and PLLA/keratin membrane. For PLLA/keratin composite membrane, two peaks appeared at 1600—1700 cm^{-1} and 1550 cm^{-1} which belong to keratin.

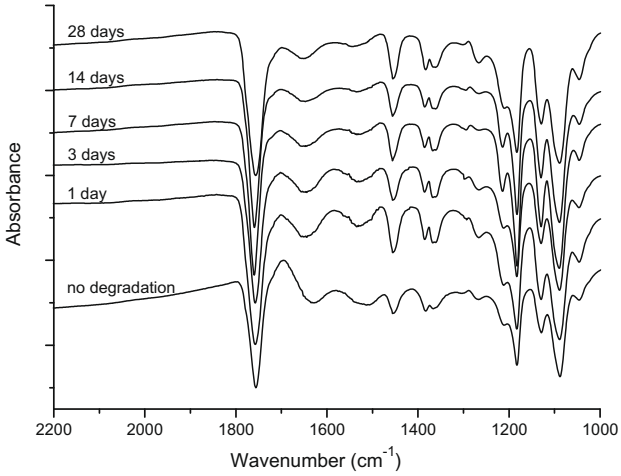

Fig. 4. FTIR spectra of PLLA/keratin scaffolds as a function of degradation periods.