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Isolation and characterization of biofunctional keratin particles extracted from wool wastes

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ABSTRACT

In the present study, wool fibers were hydrolyzed in the acidic environment. The pH value of wool hydrolyzed solution was adjusted based on the principle of isoelectric precipitation. Two kinds of keratin polypeptide precipitates were collected at pH 3.22 and pH 5.55, respectively. The keratin suspensions were spray-dried and denoted as KP3 and KP5. Both of KP3 and KP5 particles possessed smooth, round and uniform surfaces by scanning electron microscope (SEM). Compared with pristine wool fibers, KP3 and KP5 had higher glass transition temperature (100.3 °C, 78.2 °C) and lower decomposition temperature (179.3 °C, 187.0 °C) examined by thermogravimetry and differential scanning calorimetry (TG/DSC). Fourier transform infrared spectroscopy (FTIR) revealed that the disulphide bond (S–S) of the wool keratin was broken down thoroughly in the process of acidic hydrolysis. X-ray diffraction (XRD) analysis identified an amorphous form of these two kinds keratin polypeptides. The nontoxicity and biocompatibility of KP3 and KP5 were examined by the cell morphology and cell viability test. The Alamar Blue assay illustrated that 0.1 mg/ml KP3-PBS medium and 1 mg/ml KP5-PBS medium possessed beneficial impacts on cell viability. All these results demonstrated that both KP3 and KP5 with different amino acids compositions could be tailored from wool hydrolyzed solution based on isoelectric-point precipitation and have potential application future as biomaterials for wound healing, drug delivery, and so on.

1. Introduction

Recent years, many natural proteins have been introduced for tissue engineering to modify the synthetic polymers in order to improve the biocompatibility and surface modification for implantation [1]. The similarity between the natural proteins and the bio-molecules in host tissues suggested a possibility of higher biocompatibility and lower immunological reactions [2–4]. Keratin, known as a kind of protein in all mammals with a highly conserved amino acid sequence [5,6], was expected to enhance interaction responses between implantations and cells. In the area of tissue engineering, keratin could play a role of protein scaffolds with structural and regulatory functions in a cell-type-specific manner [7–9]. Based on the analysis of these model systems so far, keratin was reported for preferable impact on cell architecture, cell size and proliferation. The bio-functions of the keratin-based scaffolds for tissue engineering could be achieved due to the presence of a LDV cell binding domain in keratin amino acid sequence [10]. Keratin is insoluble structural protein in wool and feathers, which can be applied in many biofunctional applications.

Keratin was mainly composed of 19 amino acids constituted by five elements: carbon, hydrogen, oxygen, nitrogen, and sulphur, linked together as ladder-like polypeptide chains by peptide bonds with abundant natural sources like wool fibers [11,12]. Danalev et al reported in their study that the hydrolysis treatment of wool could lead to many modified amino acid products and they also synthesized markers to prove the dipeptide mimetics in wool keratin [13]. As a matter of fact, cysteine and glutamic acid were two major amino acids in wool keratin. Meanwhile glycine, leucine, proline as well as tyrosine were also components of keratin [14]. Since the bio-functions varied among different amino acids, amino acid groups could be selected based on the specific application purposes. For example, glutamatic acid was a key amino acid in cellular metabolism in human body, which increased brain function and mental activity [15,16]. Cysteine provided resistance to the body against harmful effects by enhancing the white blood–cell activity. It was essential for the proper functioning of the skin and could help in recovery from surgery [17].

To further investigate wool keratin, many studies have explored the keratin hydrolysis procedures [18,19]. There were mainly three methods to regenerate keratin from the original sources: enzymatic hydrolysis, alkaline hydrolysis and acidic hydrolysis. Enzymatic hydrolysis method offered advantages such as less species alteration, safer laboratory

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conditions and less contaminant waste [20]. However, enzymatic hydrolysis also had its limitations. Enzymes were expensive and the efficiency of hydrolysis was low and a long elapsed time was required for completion of hydrolysis. In the process of alkaline hydrolysis, some important amino acids, such as asparagines, arginine, serine, and glutamine, could be destroyed while others were racemized [21]. Because of high hydrolysis speed, simplicity, racemization and destruction of amino acids, acidic hydrolysis has been commonly used in wool hydrolysis.

The aim of this study is to explore the feasibility of transforming wool wastes into the functional protein biomaterials. In the present study, two kinds of bio-functional keratin polypeptide were collected through isoelectric-point precipitation process. Wool fibers were hydrolyzed in hydrochloric acid (HCl) solution. Then, by adjusting the pH value of the hydrolyzed solution, two kinds of keratin polypeptides were collected and characterized. The results indicated that it was viable to extract keratins with different compositions of polypeptides for future application. The keratin polypeptides could be developed as the tailored biofunctional raw materials for specific purposes in tissue engineering.

2. Methods and experiments

2.1. Materials

Wool was obtained from Australia. Hydrochloric acid 37% GR for analysis ACS (IL, USA, analytical purity) and sodium hydroxide powder (IL, USA, analytical purity) were employed to hydrolyze wool fibers.

2.2. Fabrication and characterization of polypeptide particles

2.2.1. Acidic hydrolysis of wool

60 g of wool was immersed into 4 mol/L hydrochloric acid (400 ml) solution, and then incubated in 95 °C water bath to hydrolyze the wool fibers until the wool fibers were dissolved thoroughly. Then the prepared hydrolysis solution was filtered twice for further process.

2.2.2. Measurement of hydrolysis degree of wool keratin

The amount of amino acid (W_{AA}) was measured by ninhydrin reaction [22]. The amount of the component which contained peptide bond (W_P) was measured by biuret reaction [23]. The hydrolysis degree was defined as in Eq. (1).

$$\text{The hydrolysis degree} = \left(1 - W_p/W_{AA}\right) \times 100\% \quad [1]$$

2.2.3. Isoelectric-point precipitation process of keratin polypeptides

Isoelectric point (pI) is defined as the pH value at which a particular molecule or surface carries zero net electrical charge. Isoelectric precipitation is a process in which proteins or amino acids are precipitated at pH value close to their isoelectric points [24,25]. In this study, the pH value of the prepared wool hydrolysis solution was adjusted to pH 3.22 with 1 mol/L NaOH, a thick layer of precipitate was observed at the bottom of the solution after 24 h. After the precipitate was collected and denoted as keratin polypeptides (KP3), the rest wool hydrolysis solution was adjusted to pH 5.55. The present precipitate was also collected and denoted as keratin polypeptides (KP5). In order to wash away salt and any other impurities, KP3 and KP5 were dispersed in DI water and centrifuged 3 times, respectively. Finally, KP3 and KP5 suspensions were spray-dried to obtain keratin polypeptide powders, respectively.

2.2.4. XRD measurement

The differences on the crystal structures of wool fibers, KP5 and KP3 were compared by X-ray diffractometer (XRD, D8 Advance, Bruker AXS, Germany). The patterns were obtained by a diffractometer with a Cu Kα radiation source. The 2α Bragg angles were scanned over a range of 10–80° using a 0.05° step size and 10°/min scan speed.

2.2.5. Surface morphology

The surface morphology of KP5 and KP3 was observed by scanning electron microscope (SEM, JEOL, JSM-6490, Japan). In the preparation step, the samples were adhered directly onto an aluminium stub with a thin self-adherent carbon film and then coated with a thin layer of gold.

2.2.6. TG-DSC measurement

The phase change temperature and decomposition temperature were measured by thermogravimetryand differential scanning calorimetry (TG-DSC, Netzsch STA 449C, Burlington, Germany) at a heating rate of 10 °C/min over a temperature range of 30 °C to 400 °C.

2.2.7. FTIR measurement

The microstructures of KP5 and KP3 were examined by Fourier transform infrared spectroscopy (FTIR, Perkin Elmer 1720, Perkin Elmer, USA) in the transmission mode with the wave number ranging from 4,000 to 400 cm^{-1}. KBr pellets were prepared by gently mixing the sample powders with KBr.

2.3. Biocompatibility examination

2.3.1. Cell culture

Firstly both KP3 and KP5 powders were mixed in PBS solution. Then the polypeptide-PBS suspension was diluted by the medium to a series of concentrations settled as: 5 mg/ml, 1 mg/ml, 0.1 mg/ml, 0.01 mg/ml and 0.001 mg/ml. A human foreskin fibroblast cell line, HFF-1, was obtained from American Type Culture Collection (ATCC) and cultured in T25 culture flasks in an incubator at 37 °C and 5% CO_2. Dulbecco's modified medium, DMEM (Invitrogen), was supplemented with 10% foetal bovine serum, FBS (Invitrogen), 1% penicillin (Invitrogen), 1% streptomycin (Invitrogen). All cells used in this study were at passage 10 or less. Cells were harvested by adding 0.25% trypsin with EDTA (Invitrogen). The trypsin was neutralized by adding DMEM media with 10% fetal bovine serum. Then the cell suspension were centrifuged and re-suspended for subculture or for the particular experiments.

2.3.2. Cell number counting for cell viability

Cells were seeded into 24-well plates at the density of 2×10^4/mL and cultured for 24 h. Then the prepared KP3-PBS and KP5-PBS mediums were added to incubate for another 24 h. Then cells were centrifuged (10000 r/min), re-suspended into the medium contains with 0.4% trypan blue. Cells numbers were counted by hemocytometer. The cell numbers were normalized by the controls and expressed as the percentage of cell viability.

2.3.3. Alamar Blue assay for cell viability

Cells were seeded into 24-well plates at the density of 2×10^4/mL and cultured for 24 h. Samples were added to incubate for another 24 h and then media was removed from each well. The Alamar Blue solution (100 μl) mixed with 1 mL medium was added to each well and cells were incubated for 4 h. The optical absorbency was obtained by Micro-plate reader (Infinite F200, TECAN) with a wavelength of 570 nm. The absorbency values were normalized by the controls and expressed as the percentage of cell viability.

3. Results and discussion

3.1. Acidic hydrolysis of wool and isoelectric precipitation of keratin polypeptide

In the process of acidic hydrolysis of wool fibers, disulfide bond (S–S) and partial peptide bonds of keratin in wool were split apart.