International Journal of Biological Macromolecules 85 (2016) 442–450

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac

Fabrication of duck's feet collagen–silk hybrid biomaterial for tissue engineering

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ARTICLE INFO

Article history:
Received 10 July 2015
Received in revised form
27 December 2015
Accepted 28 December 2015
Available online 31 December 2015

Keywords:
Collagen
Duck's feet
Silk

ABSTRACT

Collagen constituting the extracellular matrix has been widely used as biocompatible material for human use. In this study, we have selected duck's feet for extracting collagen. A simple method not utilizing harsh chemical had been employed to extract collagen from duck's feet. We fabricated duck's feet collagen/silk hybrid scaffold for the purpose of modifying the degradation rate of duck's feet collagen. This study suggests that extracted collagen from duck's feet is biocompatible and resembles collagen extracted from porcine which is commercially used. Duck's feet collagen is also economically feasible and it could therefore be a good candidate as a tissue engineering material. Further, addition of silk to fabricate a duck's feet collagen/silk hybrid scaffold could enhance the biostability of duck's feet collagen scaffold. Duck's feet collagen/silk scaffold increased the cell viability compared to silk alone. Animal studies also showed that duck's feet collagen/silk scaffold was more biocompatible than silk alone and more biostable than duck's feet or porcine collagen alone. Additionally, the results revealed that duck's feet collagen/silk hybrid scaffold had high porosity, cell infiltration and proliferation. We suggest that duck's feet collagen/silk hybrid scaffold could be used as a dermal substitution for full thickness skin defects.

1. Introduction

Collagen is the main structural protein of the various connective tissues in animals such as ligaments, fascia, bones, blood vessels and skins. The use of collagen-based biomaterials in the field of tissue engineering has extensively grown over the past few decades because collagen has major advantages in being biodegradable, biocompatible, easily available and highly versatile [1]. Collagen has been widely used in regeneration of tissues such as cartilage, bone, nerve and skin. However, it has some drawbacks due to its difficult and complex extraction process and high cost [2].

In general, collagen used in tissue engineering is commonly extracted from skin or bone tissue of bovine and porcine. These porcine and other animal collagens are unsuitable for some religious and ethnic communities [3]. Therefore, it is of great interest to explore alternative collagen sources such as chicken, duck's feet or fish [2–8]. Also, industry is constantly searching for upgraded production methodologies.

The annual consumption of duck in Korea is about 30 million tons. However, most of byproducts of duck are wasted because their utilization is low. Study of the extraction and characteristic of collagen from duck's feet can increase the value-added potential of this byproduct and its utilization in the field of tissue engineering. Some studies have reported that duck's feet could be an alternative collagen source [2–4].

Collagen is a naturally derived polymer, which is beneficial to cell seeding and cell attachment, however, collagen application

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http://dx.doi.org/10.1016/j.ijbiomac.2015.12.086
0141-8130/

as a scaffold is limited by poor mechanical strength and rapid biodegradation [2,8,9]. Silk, a natural polymer produced by *Bombyx mori* silk-worm, consists of two proteins: fibroin and sericin. Fibroin, the main component of silk protein has diverse applications in the biomedical field due to its high tensile strength, controllable biodegradability, low antigenicity and noninflammatory characteristics [9,10]. Silk has been used to improve the mechanical properties of collagen-based membrane [9,11,12]. Also, silk displays excellent long-term degradation behavior [10]. Biodegradable synthetic polymers such as poly(lactic acid) (PLA), poly(glycolic acid) (PGA), and poly(lactic-*co*-glycolic acid) (PLGA) can also be used, however, such synthetic polymers are relatively poor at stimulating cell seeding [8,13]. Therefore, we fabricated duck's feet collagen/silk hybrid scaffold for the purposes of improving the mechanical properties and modifying the degradation rate of duck's feet collagen.

In this study, we extracted collagen from duck's feet for application in scaffold biomaterial. The characterization of duck's feet collagen was analyzed and compared with collagen extracted from porcine which is commercially used. Also, we evaluated the feasibility of duck's feet collagen/silk hybrid scaffold. We hypothesized that collagen/silk hybrid scaffolds have the advantages of both collage and silk. In addition, we suggested that the duck's feet collagen/silk hybrid scaffold could be used as dermal substitution for full thickness skin defects [14].

2. Materials and methods

2.1. Materials

Duck's feet were provided by Joowonori (Jincheon-gun, Chungcheongbuk-do, Korea) and stored at −20 °C. Collagen in the form of freeze-dried sponge was harvested after its extraction and separation from the duck's feet. NIH3T3 fibroblasts were purchased from ATCC (Manassas, VA, USA). Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fatal bovine serum (FBS), 1% antibiotics/antimycotics and trypsin were obtained from Welgene, Fresh Media™ (Dalseogu, Daegu, Korea). The cytotoxicity test was performed using cell counting kit from CCK-8, Enzo Life Sciences, Switzerland.

2.2. Collagen extraction

To remove the blood from duck feet, eighty grams of frozen samples were washed with tap water for 24 h at room temperature. Fat was removed by adding 0.5 M NaOH at a ratio of 3:1 of NaOH to the sample. Then, 5% citric acid was mixed in at ratio of 3:1 of acid to the sample and stirred at 4 °C for 72 h. After the acid treatment, solution was centrifuged at 12,000 rpm, 4 °C for 10–30 min. Supernatant was collected and precipitated with 100% EtOH. Collagen was harvested by centrifuging the sample at 3500 rpm, 4 °C for 5 min. The colla-

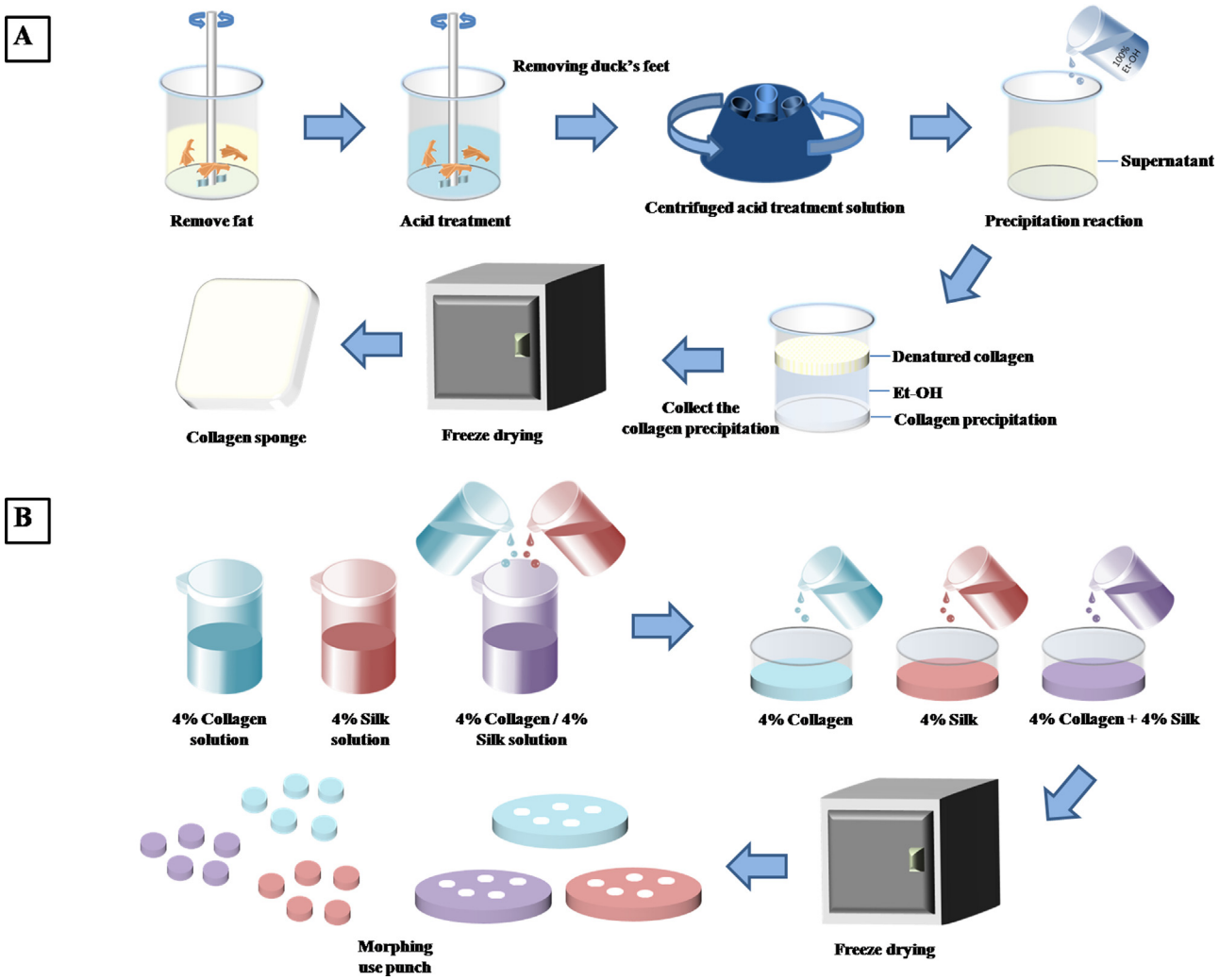

Fig. 1. Schematic process of the (A) extraction of the duck's feet collagen, using acid and (B) preparation for the duck's feet collagen, silk and duck's feet collagen/silk hybrid scaffolds using freeze drying method.