

Characteristics and Micromorphology of Designed Regenerative a Cellular Dermal Collagen Particles Derived from a Combined Procedure of Supercritical Carbon Dioxide Fluids and Protease Treatments at Low Temperatures

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Abstract

A new regenerative collagen particle with extra-cellular matrix (ECM) was designed and prepared from porcine dermal. A specific tissue-cutting machine was designed to prepare extra thin tissue membranes, which could be used for preparing the regenerative collagen particles with ECM. Furthermore, a novel procedure combining supercritical carbon dioxide fluids technology and protease treatments at low temperatures was employed. Characteristics of the resulting regenerative collagen particles with ECM were observed by determining morphology, thermal property and mechanical property for clinic application of medical devices.

Keywords: Collagen; Morphology; Extra-cellular matrix; ECM

Introduction

For the development of medical devices design, selections of suitable materials for biomedical applications such as polynorborene, poly (vinylidene fluoride), polymethacrylate, polymeric resins and

biomaterials could be substantially considered and employed [1–4]. The modification could be considered to change the microenvironment of materials for specific need [5]. A series of regenerative acellular dermal collagen particles with extra cellular matrix (ECM) were designed and prepared by using a novel procedure of

supercritical carbon dioxide fluid and protease at low temperatures. Tissue-engineering was important and popular which combine medical applications and engineering materials knowledge. The preclinical evaluations of the resulting regenerative collagen particles with extra cellular matrix (ECM) must be carried out by determining the morphology, thermal property, and mechanical property for clinic application of orthopedics.

Methods

Selection of ISO9001 quality certification and ISO14001 environmental certification, the steadily thickness of about 5mm of dermal tissue could be obtained from porcine skin by using a designed tissue-cutting machine (Taiwan PARSD Pharm. Tech. Consulting Ltd Co. and Kuin Biotech. Ltd Co.) Then, supercritical carbon dioxide fluid was used with carbon dioxides (99.999%, 20 MPa) for 6 hours. A series of samples were first soaked in 0.5M acetic acid for 2hrs, then soaked for 2hrs with 2% NaOH and 3% Triton X-100 with mixer,

followed by proteinase (4,5,10,15 ug/ml) at low temperature (4°C) for 2hrs, The samples were washed with PBS at the intermediate interval under ultrasonic wave to remove residual fat and organic particles.

Results

For preparing designed regenerative collagen membrane cap with ECM, dermis of skin was hydrolyzed by enzymes of different concentration after supercritical carbon dioxide fluid (SCF), in this way it can gain state completely and clearly. From the Fourier transform infrared spectroscopy analysis, absorptions bands at 1453, 1400, 1337, 1238, 1202, and 1080 cm^{-1} may be attributed to the $\nu(\text{CH}_2)$, $\nu(\text{CH}_3)$, $\nu(\text{C-N})$, and $\nu(\text{N-H})$ absorptions of collagens (Figure 1). Amides I and amides II absorptions could be found at 1632 and 1552 cm^{-1} respectively. The absorption band at 2900~3000 cm^{-1} $\delta(\text{C-H})$ peak value of the fatty acid tends to slow down, demonstrating the effectiveness of the supercritical carbon dioxide fluids and protease treatments.

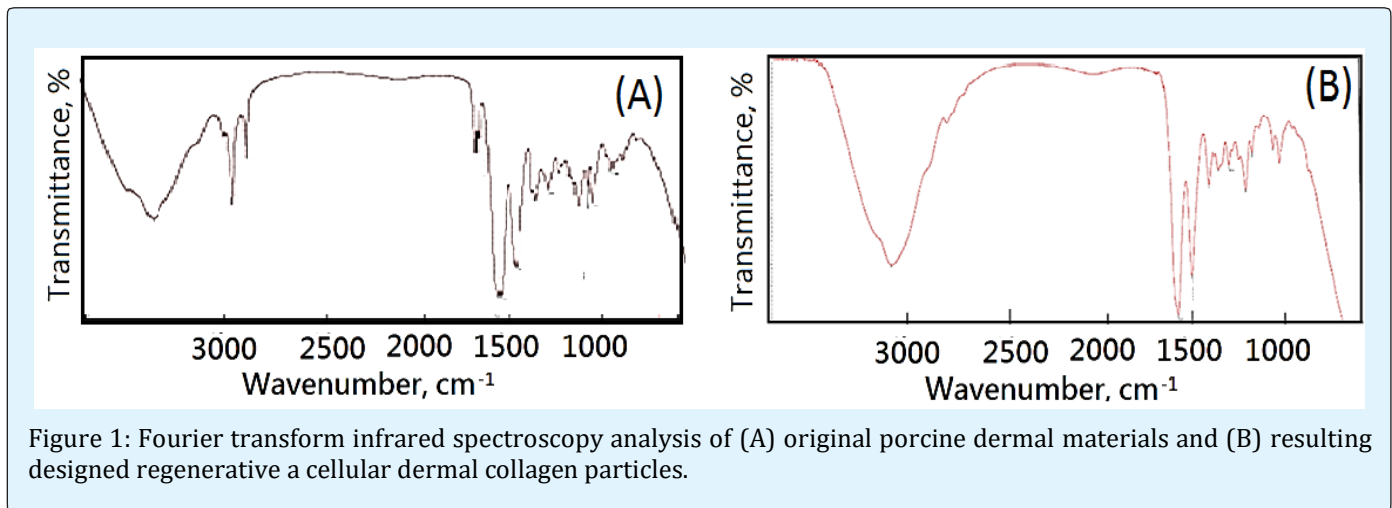


Figure 1: Fourier transform infrared spectroscopy analysis of (A) original porcine dermal materials and (B) resulting designed regenerative a cellular dermal collagen particles.

Furthermore, the micro-structure of resulting new regenerative a cellular dermal collagen particles with extra-cellular matrix (ECM) could be characterized by scanning electron microscope (SEM) (Figure 2). The helical micro structures of a cellular dermal collagen

could be observed in SEM as shown in Figure 2(B). The SEM results revealed effect in cellular dermal collagen particles with extra-cellular matrix (ECM) of porcine dermal tissue *via* supercritical fluid at low temperature.

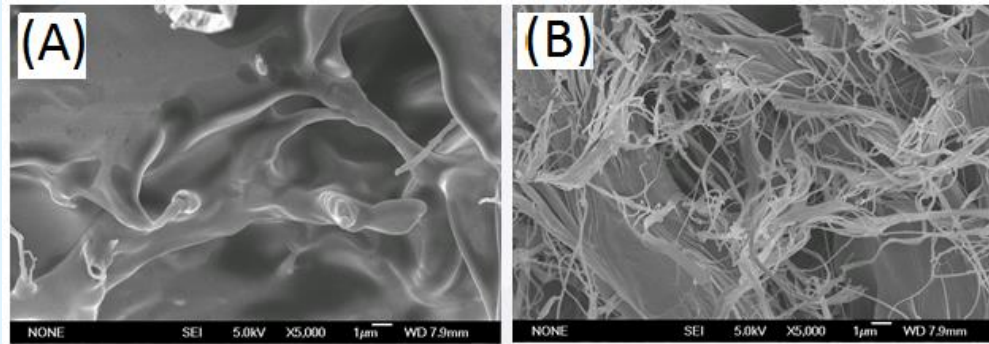


Figure 2: SEM analysis of (A) original porcine dermal materials and (B) resulting designed regenerative acellular dermal collagen particles.

Conclusion

In this study, a series of new designed regenerative collagen particles with extra-cellular matrix were obtained from porcine dermal by using a novel procedure combining supercritical carbon dioxide fluids technology and protease treatments at low temperatures. An extra-cellular matrix and integrity scaffold-structure could be obtained and observed by SEM. This study provides a simple and time-saving method to removing tissue and form decellularized structurally intact collagen scaffold. The T_{dmax} values of the resulting regenerative collagen particles with ECM were observed over 350°C, which imply high thermal stability of collagen scaffolds. Hence, the designed collagen scaffolds could be a potential application for minimally invasive surgeries as regenerative collagen particles with extra-cellular matrix.

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