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Influence of modified polyester on the material properties of collagen-based biocomposites and in vitro evaluation of cytocompatibility

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ABSTRACT

The cytocompatibility of composite materials collagen (Col)/poly(hydroxyalkanoate) (PHA) and collagen/maleic anhydride-grafted PHA (PHA-g-MA) was investigated in this study. Col was homogeneously dispersed in the PHA-g-MA matrix as a result of condensation reactions. Mechanical characterisation indicated that the improved adhesion between Col and PHA-g-MA enhanced the tensile strength of the composite compared with that of PHA/Col. PHA-g-MA/Col composites were also more water-resistant than PHA/Col composites. Collagen and cell proliferation analysis indicated that PHA and PHA-g-MA and their composites were biocompatible with respect to FB proliferation. Cell-cycle and apoptosis assays by FBs on the PHA series composite samples were not affected by DNA content related to damage, i.e. rapid apoptosis/necrosis was not observed, demonstrating the potential of PHA/Col or PHA-g-MA/Col membranes for biomedical material applications.

1. Introduction

With improved human longevity and living standards, the medical industries market continues to expand rapidly, prompting research into new biomedical materials [1–3]. Biomedicine spans many domains and includes the research areas of biology, medicine, and material science, with applications to many areas, including general wound dressings for external use, drug-cladding materials, contact lens materials, and tissue engineering [4,5]. Biomedical materials in medical substrates can provide information on cell growth, proliferation, and bioactivation, contributing to the generation of healthy histiocytes [6,7].

Collagen (Col), the leading protein in the human extracellular matrix, has emerged as an important biomedical material in recent years due to its biocompatibility, biodegradability, nontoxicity, and low allergic reactivity. It exists in various tissues in vivo, especially in the skin, joints, muscle tendons, cartilage, bones, and basilar membrane [8,9]. Eighteen types of Col have been identified. Type I Col, an important structural protein, provides the environment for cell growth and is responsible for the elasticity and mechanical strength of tissues [10,11]. In this study, Col was mixed with aliphatic polyester to improve the applicability of Col to biomedical packaging products, medical beauty treatments, and surgical materials fabrication.

At present, biodegradable polymers are being actively developed worldwide; research has shown that these biodegradable polymers can be decomposed by microorganisms in nature into CO_2, water, and other harmless residues after use [12,13]. Researchers of medical material have mainly focused on materials that can be biodegradable polymers, such as polylactic acid (PLA) [14], polycaprolactone (PCL) [15], polyglycolide (PGA) [16] and poly (3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) [17]. As for Polyhydroxyalkanoates (PHA), a new class of microbial biopolymers [18,19], it is characterised by nontoxicity, as well as good biocompatibility, mechanical properties, and processability [20,21]. Because of its good biocompatibility, flexible mechanical strengths, and superior elastic property, PHA have been developed for applications as medical implants, drug delivery matrices, and devices to support cell growth [22]. Also, PHA can be made into dental fillings, biological sutures, tissue engineering for wound dressings, drug delivery carriers and fracture fixation materials [23–25]. The absence of risk of toxic substances penetrating from it into the human body has led it to become commonplace in the biomedical materials market [26]. To enhance the function of PHA-based materials in biomedical engineering, it is combined with collagen powder to create a PHA/collagen composite material.

In this study, composite materials consisting of PHA blended with Col filler were investigated in an effort to enhance the applicability of PHA in biomedical applications. To reduce the incompatibility between Col and the PHA matrix, MA was grafted onto PHA as an interface compatibility agent [27]. This allowed PHA and Col to chemically bond to each other, thereby greatly improving the mechanical properties of the resulting composite. Bulk structural changes in the composites, induced by the MA moiety, were identified using Fourier transform infrared spectrometry (FTIR). Additionally, the biocompatibility of the

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composites was evaluated based on cell proliferation, collagen quantification, apoptosis, and the cell cycle.

2. Experimental

2.1. Materials

Commercial-grade PHA (EM 5400F) was obtained from Shenzhen Ecomann Biotechnology Co., Ltd. (Shenzhen, China). Collagen (Col), maleic anhydride (MA), benzoyl peroxide (BPO), and dimethyl sulphoxide (DMSO) were purchased from Sigma-Aldrich Chemical, Inc. (St. Louis, MO, USA). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolelium bromide (MTT) was acquired from Promega (Madison, WI, USA). Foetal bovine serum and Dulbecco's modified Eagle's medium (DMEM) were purchased from Gibco-BRL (Gaithersburg, MD, USA). Binding buffers and Annexin V-FITC apoptosis detection kits were obtained from Becton Dickinson (Franklin Lakes, NJ, USA). All buffers and other reagents were of the highest purity grade commercially available.

2.2. PHA-g-MA copolymer

The grafting of MA onto molten PHA. In preliminary testing using 240 mL dichloromethane as a solvent, a mixture of 6.0 g MA and 1.8 g BPO was four equal doses at two-minute intervals added to the 54.0 g PHA. The grafting reactions were performed in a nitrogen (N_2) atmosphere at 85 °C. Preliminary results revealed that reaction equilibrium was attained in less than 12 h. Thus, reactions were allowed to progress for 12 h while stirring at 60 rpm. The product (4.0 g) was dissolved in 200 mL of refluxing dichloromethane at 85 °C and the solution was filtered through several layers of cheesecloth, and was then dried in a vacuum oven at 80.0 °C for 24 h. The dichloromethane-insoluble product that remained on the cheesecloth was washed using acetone to remove any unreacted MA. The dichloromethane-soluble product in the filtrate was extracted using 600 mL of cold acetone.

The MA loading of the dichloromethane-soluble polymer (expressed as grafting percentage) was calculated from the acid number and was determined as follows. First, about 2.0 g copolymer was heated in 200 mL refluxing dichloromethane for 2 h. The hot solution was then titrated immediately with 0.03 N ethanolic potassium hydroxide (KOH) solution, which was standardized against a solution of potassium hydrogen phthalate, with phenolphthalein used as an indicator. The acid number was calculated using Eq. (1) below, and the grafting percentage was calculated using Eq. (2) [28].

$$\text{Acid number}(\text{mg KOH/g}) = \frac{V_{KOH}(\text{mL}) \times C_{KOH}(\text{N}) \times 56.1}{\text{polymer}(\text{g})} \quad (1)$$

$$\text{Grafting percentage}(\%) = \frac{\text{Acid number} \times 98.1}{2 \times 561} \times 100\%. \quad (2)$$

The grafting percentage was found to be 1.06 wt.% when BPO and MA loadings were maintained at 0.3 wt.% and 10 wt.%, respectively.

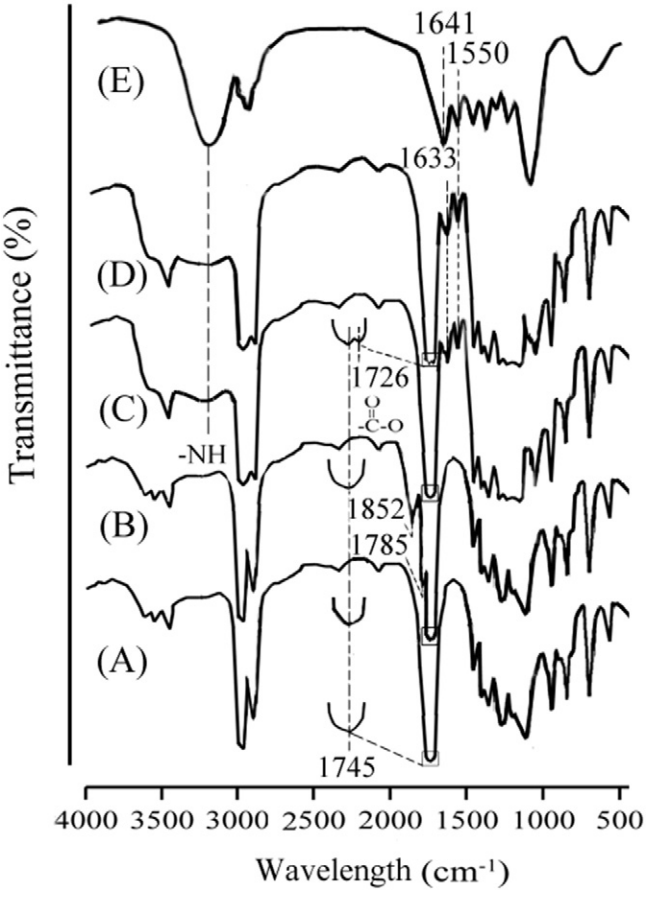

Fig. 1. Fourier transform infrared (FTIR) spectra of (A) poly(hydroxyalkanoate) (PHA), (B) maleic anhydride-grafted PHA (PHA-g-MA), (C) PHA/collagen composite (PHA/Col (10 wt.%)), (D) PHA-g-MA/Col (10 wt.%), and (E) Col.

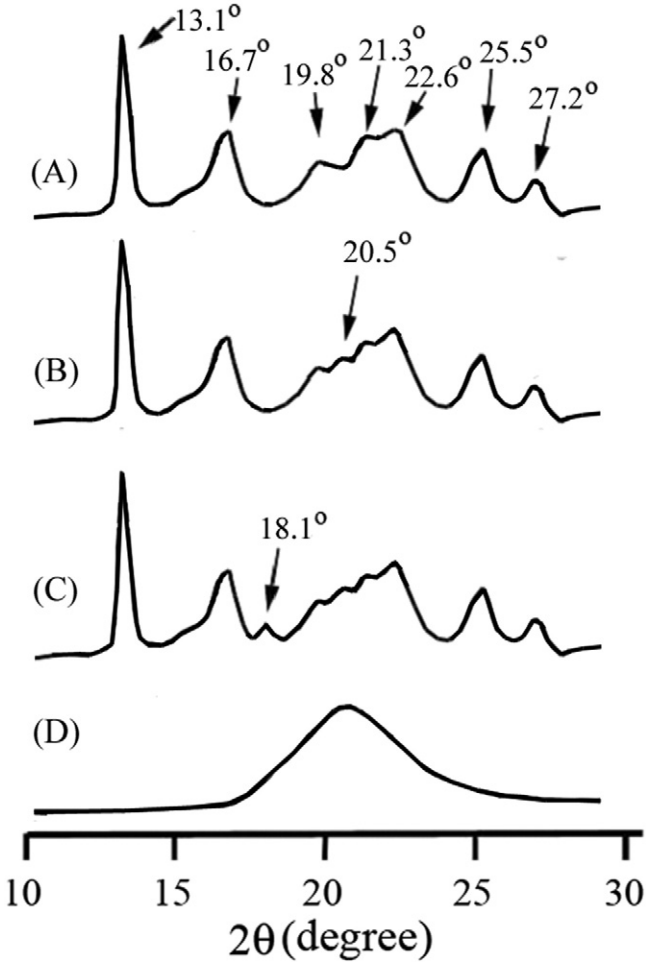

Fig. 2. XRD patterns for (A) PHA, (B) PHA/Col, (C) PHA-g-MA/Col, and (D) Col.