Materials Letters ∎ (∎∎∎∎) ∎∎∎–∎∎∎

Contents lists available at ScienceDirect

Materials Letters

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

Preparation and characterization of keratin-based biosheet from bovine horn waste as wound dressing material

Sivakumar Singaravelu, Giriprasath Ramanathan, M.D. Raja, Sagar Barge, Uma Tirichurapalli Sivagnanam*

Bioproducts Lab, CSIR-Central Leather Research Institute, Adyar, Chennai, India

ARTICLE INFO

Article history:
Received 8 January 2015
Accepted 19 March 2015

Keywords:
Biomaterials
Biomimetic
Composite materials

ABSTRACT

The preparation of a skin substitute with improved mechanical property and biocompatibility is needed for the advancement of tissue engineering. This is a first time report on the extraction of keratin (HK) from bovine horn and its use in the preparation of a composite biosheet by blending with chitosan (CH). The prepared biosheet exhibited better thermal and mechanical properties. Surface morphology revealed that the biosheet supported fibroblast cell attachment, and this result was corroborated with the MTT assay. Sustained release of the mupirocin (MP) from biosheet was observed during *in vitro* drug release studies. The enhancement of cell adhesion and proliferation of NIH 3T3 fibroblast has shown better biocompatibility with the HK-based biosheet. Thus HK-based biosheet have good potentials and could be used as wound dressing material.

1. Introduction

Tissue engineering promotes the wound healing process as it mimics the function of the extracellular matrix, thus it improves and maintains the biological function of the material. It therefore plays a pivotal role in restoring the function of tissue or whole organs [1]. Throughout the world, the bovine horn, a by-product of the slaughter house and meat industry remains unused and lying as biowaste. Keratin (HK), a fibrous structural protein, is abundantly present in horns [2]. HK is a valuable biopolymer in the field of biomedicine as it exhibits properties such as biodegradability, biocompatibility [3] and good mechanical strength. Keratins are rich in cysteine residues which form covalent disulfide bonds and give rise to more durable structures [4]. The blending of HK with suitable polymer (chitosan) becomes an essential to achieve a biomaterial with ideal mechanical properties. The presence of cell binding motifs in keratin and the mucoadhesive nature of the chitosan make the blended material more suitable for tissue engineering application. Mupirocin (MP) was incorporated in HK-based material which absorbs exudates and simultaneously enhances the bioavailability of the drug at the wound site which in turn accelerates the healing rate. The HK-based material provides release of the drug continuously in sustained manner which lessens the dressing frequency and reduces the trauma to the patients.

2. Materials and methods

Bovine horn was collected from the slaughterhouse at Perambur, Chennai. Chitosan (CH) (low molecular weight) and all other chemicals were purchased from Sigma-Aldrich, Bangalore, India, unless specified otherwise. The mouse NIH-3T3 fibroblast was obtained from the National Centre for Cell Science (NCCS), Pune, Maharastra, India.

Extraction of HK from bovine horn: The HK from bovine horn was extracted by the modified Shindai method (Briefly in Supporting information S1) [4–6]. Subsequently, the extracted solution was filtered and centrifuged. The supernatant was dialyzed extensively against deionized water using a dialysis bag (12,000 Da) for 3 days to get the HK dialysate.

Fabrication of keratin–chitosan (HK–CH) biosheet: The extracted HK and 2% (w/v) CH solutions were mixed in different stoichiometric ratios (Table 1). 1.5 ml of ethylene glycol was added as plasticizer. Finally, the mixture was poured into a polyethylene tray (measuring 16 cm × 18 cm) and air dried at room temperature to get the HK–CH biosheet. Similarly the drug loaded biosheets (HK–CH–MP) was prepared by incorporation of 50 mg of MP.

Physicochemical characterization: The prepared biosheets were characterized using Fourier Transform Infrared Spectroscopy (FTIR-ABB 3000 spectrometer) The Amide I region (1700–1600 cm^{-1}) was

* Corresponding author. Tel.: +91 44 24420709, mobile: +91 99400 81005; fax: +91 44 24911589.
E-mail address: suma67@gmail.com (U.T. Sivagnanam).

http://dx.doi.org/10.1016/j.matlet.2015.03.088
0167-577X/

curve fitted into Gaussian line shapes for secondary structure analysis by Origin 6.0 software (Origin Lab, USA). Absorption band positions of individual components were used to identify α-helix, β-sheet, β-turn, and disordered structure of the protein. The percentages of different types of secondary structures were determined by adding the sum of absorptions for each and expressing their sums as a fraction of the total Amide I band area in Fig. 2(d). Scanning electron microscopy (SEM) was employed to study the surface morphology of the biosheets. Thermal properties were studied by Differential scanning calorimetry (DSC) and Thermo gravimetric analysis (DTA) (universal V4.4A TA instruments). Mechanical properties such as elongation at break and tensile strength were assessed using a Universal Testing machine (INSTRON model 1405). The swelling study and *in vitro* drug release studies were done according to the procedure followed from Naveen et al. [7–8].

Biocompatibility study: NIH 3T3 fibroblast cells were seeded over biosheets and were maintained in Dulbecco's modified Eagle's medium (DMEM) with 10% Fetal bovine serum (FBS), supplemented with antibiotics at 37 °C in a humidified atmosphere of 5% CO_2. At the end of 24 h, cell viability was assessed by standard 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) assay [9].

Evaluation of antimicrobial activity: The HK–CH and HK–CH–MP (0.3 mg/cm^2) biosheet were cut into 1 cm^2 and evaluated against gram-positive *Staphylococcus aureus* (ATCC 11632) and gram-negative *Escherichia coli* (ATCC 10536) using agar well diffusion method [10].

Table 1
Tensile properties of biosheet at various stoichiometric ratios of HK and CH.

Sample no.	HK:CH ratio	Mean tensile strength (MPa)	Mean elongation at break (%)	Mean Young's modulus (MPa)
1.	1:1	7.40 ± 0.437	16.03 ± 0.548	0.461 ± 0.635
2.	1:2	16.3 ± 0.872	9.37 ± 0.482	1.732 ± 0.723
3.	1:3	21.14 ± 0.864	6.19 ± 0.548	3.145 ± 0.962

3. Results and discussion

Physiochemical characterization: The FTIR of CH, HK, and HK–CH biosheets are depicted in Fig. 1(a). The strong absorption band at 2600–3300 cm^{-1} and 1142 cm^{-1} corresponds to the OH and polysaccharide structure of chitosan. The amide I and II bands were observed at 3300–3400 cm^{-1} and 1550–1650 cm^{-1}, corresponding to the peak of HK with peptide bonds (O=C–N–H) were attributed by major peaks held in the above mentioned region with NH bending and CH stretching between the same, signifying the interaction between HK and CH biosheets [11]. For the secondary structure analysis, the Amide I band spectrum was curve fitted into the Gaussian line shapes (Fig. 2(b)) with absorption region with individual peak representing α-helix (1650–1657 cm^{-1}), β-sheet (1612–1645 cm^{-1}), β-turn (1655–1675 cm^{-1}), and disordered structure (1640–1651 and (1670–1697 cm^{-1}). Calculated peak area for the HK showed 29.04% α-helix, 37.91% β-sheet, 26.08% β-turn and 6.95% disordered structure. The increase in β-sheet content is also key factor for the mechanical property of HK [12].

TGA thermograms of HK–CH and HK–CH–MP biosheet are shown in Fig. 1(c). Initial weight losses were due to the evaporation of water. The second weight loss of 63% for HK–CH–MP and 45% for HK–CH biosheet was observed at 200–400 °C due to denaturation of

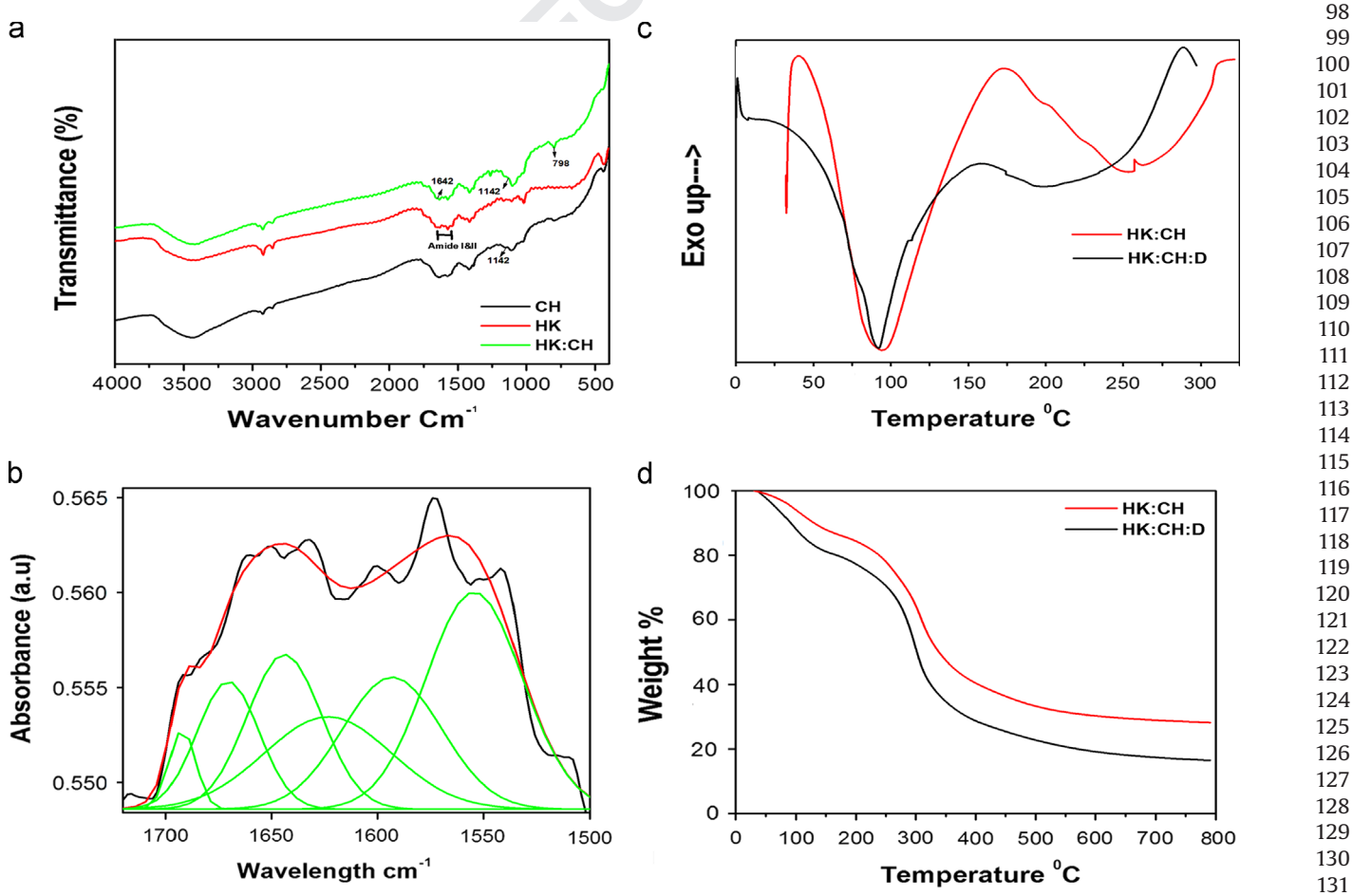

Fig. 1. (a) FTIR spectra, (b) secondary structure analysis of HK by FTIR, (c) DSC, (d) TGA of biosheets.

Please cite this article as: Singaravelu S, et al. Preparation and characterization of keratin-based biosheet from bovine horn waste as wound dressing material. Mater Lett (2015), http://dx.doi.org/10.1016/j.matlet.2015.03.088