Carbohydrate Polymers 114 (2014) 312–320

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Bacterial cellulose/acrylic acid hydrogel synthesized via electron beam irradiation: Accelerated burn wound healing in an animal model

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ARTICLE INFO

Article history:
Received 22 May 2014
Received in revised form 20 July 2014
Accepted 5 August 2014
Available online 23 August 2014

Keywords:
Bacterial cellulose
Acrylic acid
Burn wound
Wound healing

ABSTRACT

Natural polymer-based hydrogels are of interest to health care professionals as wound dressings owing to their ability to absorb exudates and provide hydration for healing. The aims of this study were to develop and characterize bacterial cellulose/acrylic acid (BC/AA) hydrogels synthesized by electron beam irradiation and investigate its wound healing potential in an animal model. The BC/AA hydrogels were characterized by SEM, tensile strength, water absorptivity, and water vapor transmission rate (WVTR). The cytotoxicity of the hydrogels was investigated in L929 cells. Skin irritation and wound healing properties were evaluated in Sprague-Dawley rats. BC/AA hydrogels had a macroporous network structure, high swelling ratio (4000–6000% at 24 h), and high WVTR (2175–2280 g/m^2/day). The hydrogels were non-toxic in the cell viability assay. *In vivo* experiments indicated that hydrogels promoted faster wound-healing, enhanced epithelialization, and accelerated fibroblast proliferation compared to that in the control group. These results suggest that BC/AA hydrogels are promising materials for burn dressings.

1. Introduction

Health care personnel are faced with the difficulty of treating an increasing number of patients with wounds and burns (Mogoşanu & Grumezescu, 2014). Among the most complex types of burn to manage are partial- (second-degree) and full-thickness (third degree) burns, which require specialized dressings (Pham, Greenwood, Cleland, Woodruff, & Maddern, 2007). Numerous types of dressings are used to cover the wound surface in order to encourage healing. Hydrogel dressings have generated the greatest interest as dressings for burns because they produce an ideal hydration environment for healing (Yang, Zhu, Liu, Chen, & Ma, 2008). Hydrogels also have the ability to absorb exudates, and their transparency facilitates observation of the healing process (Boateng, Matthews, Stevens, & Eccleston, 2008). Additionally, hydrogels, three-dimensional polymers, have been widely investigated because of their biocompatibility with the human skin (Ito, Yoshida, & Murakami, 2013).

Natural product-based polymers are now widely used in regenerative medicine as dressings for wounds and burns, owing to their biocompatibility, biodegradability, and similarity to the extracellular matrix (Huang & Fu, 2010). Cross-linked natural polymers commonly used in hydrogel preparations include polysaccharides, homoglycans, chitin and chitosan, alginates, α-glucans, and β-glucans (Mogoşanu & Grumezescu, 2014). Recent studies have shown that bacterial cellulose (BC), synthesized by *Acetobacter xylinum* sp., has potential for use in wound dressings and artificial skin (Czaja, Krystynowicz, Bielecki, & Brown, 2006). BC and plant cellulose have similar chemical structures, the crystallinity, mechanical strength, and absorption capacity of BC are greater than those of plant cellulose, which has led to the utilization of BC in the biomedical field (Shah, Ul-Islam, Khattak, & Park, 2013). Due to its various unique properties, BC has been recommended as an alternative dressing for partial-thickness burns (Fu, Zhang, & Yang, 2013).

Natural product-based polymers are commonly cross-linked with synthetic polymers to ensure that the hydrogel can entrap water and prevent dissolution of the hydrophilic polymer chains in an aqueous environment. Numerous crosslinking methods have been developed for the production of hydrogels. Current techniques include chemical crosslinking, polymerization with crosslinking agents, and high-energy radiation-induced crosslinking. The

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http://dx.doi.org/10.1016/j.carbpol.2014.08.025
0144-8617/

irradiation-induced cross-linking technique provides sterilization and hydrogel crosslinking in a single step. The physical properties of hydrogels produced in this manner are dependent on the degree of crosslinking and polymer composition (Soler, Rodríguez, Correa, Moreno, & Carrizales, 2012). This method also provides an alternative to the use of chemical initiators and crosslinkers, which can be harmful and difficult to remove (Yang et al., 2008). Poly(vinyl alcohol), poly(ethylene glycol), and poly(acrylic acid) are common synthetic polymers that are cross-linked to form hydrogels by using a high-energy irradiation process.

In our previous study, BC was combined with AA (acrylic acid) at several ratios was used to fabricate hydrogels by exposure to accelerated electron beam (EB) irradiation at different doses. The Fourier transform infrared spectroscopy (FTIR) results revealed that AA had been successfully grafted onto the cellulose fibers. Morphological analysis showed that hydrogels prepared by the mixture of BC and AA had a highly macroporous sponge-like structure. Pore size in the hydrogels decreased as AA content and irradiation doses increased (Amin, Ahmad, Halib, & Ahmad, 2012). These hydrogels exhibited many promising features for an effective wound dressing.

The highly macroporous structure and water absorption capability of bacterial cellulose acrylic acid (BC/AA) hydrogels may be beneficial for exudate absorption and preservation of moisture in the wound area. Furthermore, the degree of crosslinking of these hydrogels can be fine-tuned by varying the irradiation dose. This can be helpful to controlling the mechanical strength and water absorption capacity of the hydrogels.

The objectives of this study were to develop and characterize BC/AA hydrogels synthesized by EB irradiation specifically used for wound dressing material and investigate its wound healing potential in an animal model. We studied in particular the physical and mechanical properties, and cytotoxicity of hydrogels fabricated by EB irradiation at doses of 35 kGy (H_{35}) or 50 kGy (H_{50}). Furthermore, the effects of H_{35} on normal skin and on healing of partial-thickness burns were evaluated in rats.

2. Experimental

2.1. Materials

BC was prepared from *nata de coco* (coconut water fermented by *A. xylinum*) that had been purified as reported earlier (Amin, Abadi, & Katas, 2014). AA and phosphate-buffered saline (PBS) were supplied by Sigma–Aldrich (USA). Distilled water was used to prepare aqueous solutions and dispersions. Petri dishes (90 mm × 13 mm) were used as molds for the preparation of hydrogels. Isoflurane was supplied by Piramal Healthcare (India).

2.2. Preparation of BC/AA hydrogels

The BC/AA hydrogel was prepared as described by Amin et al. (2012). Purified BC was ground to a powder (particle size 20–200 μm). AA was added to a 1% (w/v) dispersion of BC in distilled water to produce a 40:60 AA:BC mixture, which was mixed using a mechanical homogenizer (IKA Labortechink Ultra Turrax T25, Germany) at room temperature (26 °C) for 30 min to ensure thorough mixing. This mixture was poured into petri dishes (10 mL/dish) for exposure to electron-beam radiation at 35 and 50 kGy in air at the accelerator facility (NHV-Nissin High Voltage, EPS 3000, Japan) at the Malaysian Nuclear Agency.

2.3. Gel fractions of hydrogels

Freshly prepared hydrogels were cut into disks (1 cm diameter, 0.7–1.0 mm thick) and dried in an oven at 60 °C to a constant weight. The dried hydrogels were then subjected to extraction in distilled water with the distilled water replaced twice a day for 2 weeks. This ensured removal of un-reacted monomers so that only purified hydrogel would be subjected to analysis. The extracted hydrogels were dried in an oven at 60 °C to a constant weight. The gel fraction (%) was calculated using the following Eq. (1):

$$G_F(\%) = \frac{G_d}{G_i} \times 100 \quad (1)$$

where G_d is the initial dried weight before extraction and G_i is the constant dried weight of the hydrogels after extraction.

2.4. Film thickness

The film thickness of dried extracted hydrogels was measured using a micrometer (Digimatic Micrometer MDC-S, Mitutoyo Co., Japan) with 0.001 mm accuracy (Rhim, 2004). Five measurements were taken for each sample.

2.5. Water absorptivity study

The degree of swelling of the hydrogels was performed according to a previously described method (Wang, Zhu, Xue, & Wu, 2012). Dried and weighed hydrogels disks were immersed in 50 mL PBS (pH 7.4, 37 °C) and the swollen samples were weighed at specific intervals. The degree of swelling (2) and moisture content (3) in PBS were calculated using the following equations:

$$\text{Swelling ratio}(\%) = \left[\frac{H_s - H_d}{H_d}\right] \times 100 \quad (2)$$

$$\text{Moisture content ratio}\,(\%) = \left[\frac{H_s - H_d}{H_s}\right] \times 100 \quad (3)$$

where H_s and H_d are the weights of swollen and dried hydrogels. The experiments were performed in triplicate and the average of the results was reported.

2.6. Water retention properties

Moisture conservation in the hydrogels was evaluated by a water retention test (Lin, Lien, Yeh, Yu, & Hsu, 2013). After measuring the initial dry weights (M_{dry}), the hydrogels were immersed in PBS for 24 h. The swollen hydrogels were then wiped with filter paper to remove surface water and placed in petri dishes at room temperature. The samples were weighed (M_{wet}) after specific periods. The moisture retention ratio was calculated using the following Eq. (4):

$$\text{Moisture retention ratio}\,(\%) = \frac{M_{wet} - M_{dry}}{M_{dry}} \times 100 \quad (4)$$

2.7. Water vapor transmission

The water vapor transmission (WVT) of the hydrogel was measured using previously described methods (Lin et al., 2013). Briefly, hydrogels (diameter 1.1 cm) were mounted on the brim individual glass vials containing 20 mL of distilled water. The hydrogels were fastened to the vials with rubber rings, and the vials were placed in a humidity chamber (Terchy HRM 80 FA, Taiwan) at 37 °C and 84% humidity. The evaporation of water through the hydrogel was determined by weighing the vials once per hour for 8 h, and then at 24 and 48 h. A weight decrease was taken to indicate the loss of water. The profile of weight loss was plotted versus time for each sample. The water vapor transmission rate (WVTR) was calculated by dividing the daily weight loss of water by the area of the vial opening.