Food Hydrocolloids 53 (2016) 75–83

Contents lists available at ScienceDirect

Food Hydrocolloids

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

# Physical properties of bacterial cellulose composites for wound dressings

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ARTICLE INFO

*Article history:*
Received 6 August 2014
Received in revised form
2 December 2014
Accepted 8 December 2014
Available online 16 December 2014

*Keywords:*
Bacterial cellulose
Chitosan
Composite
Physical property
Wound dressing

ABSTRACT

To test various formulas and techniques for manufacturing dry-fabricated bio-film (DFBF) that exhibits physical properties advantageous to the use of the DFBF in wound dressings, a DFBF was fabricated by adding chitosan (Chi) and alginate (Alg) to homogenized bacterial cellulose (BC) obtained from vinegar pellicles in vinegar brewing byproducts in this study. The results revealed that the degree of oxidation in DFBF manufactured using hydrogen peroxide oxidized BC (HOBC), with 0.092% carboxyl group content, was lower than that in DFBF manufactured using periodic acid oxidized BC (POBC), but DFBF made using HOBC exhibited higher elongation, rehydration, swelling ratios, and water vapor transmission than that fabricated using POBC. A DFBF composite gel with 98.5% water content possessed appropriate fluidity for molding. After 10 min of rinsing cross-linked HOBC, 72 ppm of calcium remained in the final DFBF, which not only prevented cell toxicity but also demonstrated desirable mechanical and rehydration properties. Overall, the modified DFBF possessed a high rehydration ratio of 51.69% and could absorb and gradually release naringin by up to 80% within 24 h. This modified DFBF has the potential for exudate absorption and the controlled release of medicinal substances at the initial stage of healing when used in wound dressings.



## 1. Introduction

Artificial materials with biomimetic behavior are essential in the biomedical field. Scaffolds based on nanofibers (NFs) mimic the natural extracellular matrix and its nanoscale fibrous structure. Several approaches have been described for developing materials based on NFs from synthetic or natural polymers (Ashammakhi, Ndreu, Yang, Ylikauppila, & Nikkola, 2007). Bacterial cellulose (BC), secreted by *Gluconacetobacter xylinus*, has been presented as a biocompatible scaffold with a nanoscale fibrous structure for the engineering of cartilage and blood vessels, wound dressings, and guided tissue regeneration among other applications (Chiaoprakobkij, Sanchavanakit, Subbalekha, & Pavasant, 2011; Fernandes et al., 2010; Wiegand, Elsner, Hipler, & Klemm, 2006; Wu et al., 2014).

BC has attracted increasing attention as a wound-dressing material, because of its high purity, hydrophilicity, structure-forming potential, chirality, and biocompatibility (Kawecki, Krystynowicz, Wysota, Czaja, Sakiel, 2006; Keshk, 2014). Because of its fine network structure, it also exhibits controlled-release functionality, a critical skin-barrier function in wound healing (Czaja, Krystynowicz, Bielecki, & Brown, 2006).

Depending on synthetic conditions, BC has a water-holding capacity ranging from 60 to 700 times its dry weight. The cellulose in typical statically cultured pellicles is approximately 1% of the total weight, with the rest being water (Okiyama, Shirae, Kano, & Yamanaka, 1992; Yamanaka et al., 1989). One reason for this hydrophilicity is that the cellulose ribbons are assembled extracellularly in the liquid medium; abundant micelles are then formed that trap large quantities of liquid. In addition, the hydrophilicity of the cellulose pellicle is partially a result of the extensive interior surface area of the interstitial space of the undried pellicle. However, BC exhibits poor rehydration after drying, because of high crystallinity (Huang, Chen, Lin, Hsu, & Chen, 2010). This limits its use as a biofilm. In this study, two hydrophilic materials, chitosan and alginate, were added to a dehydrated BC composite to increase its rehydration ability.

Investigation of the use of BC in a liquid-loaded pad for wound care began in the early 1980s and led to some BC products in the wound-care market. For example, a purified gelatinous BC

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http://dx.doi.org/10.1016/j.foodhyd.2014.12.009
0268-005X/

membrane was commercialized as an artificial skin, or wet wound dressing (Fontana, Souza, Fontana, Toriani, & Moreschi, 1990). A high mechanical strength in its wet state, substantial permeability for liquids and gases, and low skin irritation indicated that the BC gelatinous membrane was superior to conventional gauze as an artificial skin for temporary covering of wounds (Fontana, Franco, Souza, Lyra, & Souza, 1991). BC composites fabricated by blending chitosan, polyethylene glycol (PEG), and gelatin were developed for a possible tissue engineering scaffold and wound-dressing material (Ougiya, Watanabe, Morinaga, & Yoshinaga, 1997). The BC composites were prepared by immersing a wet BC pellicle in chitosan, PEG, or a gelatin solution followed by freeze-drying.

An ideal wound-dressing material should maintain a moist environment at the wound interface, allow gaseous exchange, act as a barrier to microorganisms, and remove excess exudates. It should also be nontoxic, non-allergenic, and non-adherent in the sense that it can be easily removed without trauma; and it should be made from a readily available biomaterial that requires minimal processing, possesses antimicrobial properties, and promotes wound healing (Shuangyun, Wenjuan, & Gu, 2008). Chitosan and alginate were the best materials to control the microenvironment on the wound surface including certain bioactive products that stimulate some part of the healing cascade (Paul & Sharma, 2004; Sudheesh Kumar et al., 2010; Yang, Yang, Lin, Wu, & Chen, 2008). Furthermore, oxidized celluloses containing carboxylic groups represent a new class of biodegradable materials, and have been accepted for use in humans to stop bleeding during surgery and to prevent the formation and reformation of postsurgical adhesions (Bowman & Cooke, 1994).

However, the BC used in the composites or wound dressings in the aforementioned studies were prepared using cultured pellicles. The high production costs and low efficiency in yield limit the use of this BC to high-value-added applications (Chang, Chen, Lin, & Chen, 2012; Chawla, Bajaj, Survase, & Singhal, 2009; Hungund et al., 2013). In the present study, homogenized BC was economically gathered from the film wastes of a vinegar factory for use as the raw material. It could also be obtained from the offal from “nata” or “biocellulose facial mask” industries. The end-product, a DFBF, was composed of the homogenized BC, chitosan (Chi) and alginate (Alg). To investigate the usefulness of this DFBF for wound dressings, various fabrication techniques (oxidation treatment, water content adjustment, and rinsing time) were adapted with regard to specific physical properties (tensile properties, hydrophilicity, and water vapor transmission) and biocompatibility.

## 2. Materials and methods

### 2.1. DFBF preparation

The BC used to prepare the DFBF was obtained from solid byproducts of vinegar brewing (Sunny Way Biotech Co., Ltd., I-Lan, Taiwan). Based on Huang et al. (2010), the vinegar film was immersed in 0.5 N NaOH for 10 min and then in 0.5 N NaOH for 24 h at room temperature to purify it, then rinsed with deionized water to achieve a neutral pH. The BC was homogenized using a seven-speed blender (Waring Commercial, Torrington, CT, USA) at 3500 rpm for 3 min and oxidized by hydrogen peroxide ($H_2O_2$, Nippon Chemical Industrial Co., Ltd., Tokyo, Japan) or periodic acid ($H_5IO_6$, Mallinckrodt Chemicals, St. Louis, MO, USA) for 6 h to obtain hydrogen-peroxide-oxidized BC (HOBC) or periodic-acid-oxidized BC (POBC), respectively. According to the optimized conditions of fabricated composites film with BC from Yang (2011), the BC or oxidized BC (OBC) was blended with sodium alginate (Alg, Food Chemifa, Tokyo, Japan) and chitosan (Chi, DD 83%, 297 kDa, Ohka Enterprises Co., Ltd., Kaohsiung, Taiwan) at a ratio of BC:Alg:Chi = 2:1:1 in 98.0%, 98.5% or 99.0% water content to form a basic DFBF gel solution. We molded the gel solution, cross-linked it with 3% (w/w) calcium chloride for 2 h in a 5-mm-thick wet-fabricated pellicle (WFP), and rinsed it with phosphate-buffered saline (PBS, pH = 7.2). Finally, the rinsed fabricated film was freeze-dried, to obtain the desirable rehydration ability, and the resultant DFBF was stored in a dry cabinet.

### 2.2. COOH content analysis

According to Kumar and Yang (1999), about 0.5 g of the dried sample was accurately weighed and dispersed in 50 ml of a 2% weight by weight solution of calcium acetate for 30 min. The suspension was then titrated with standardized 0.1 N NaOH solution using phenolphthalein as an indicator. The volume of NaOH solution consumed was corrected for the blank. The carboxylic content in the sample was calculated from the following relationship:

$$\text{COOH content} = \frac{N \cdot V \cdot M_{WCOOH}}{\text{Weight of the sample (mg)}} \times 100\%$$

where $M_{WCOOH}$ is the molecular weight of COOH, $N$ is the normality of NaOH, and $V$ is the volume of NaOH in mls consumed in titration, after correcting for the blank.

### 2.3. Fourier Transform Infrared (FT-IR) Spectroscopy

The FT-IR spectra of freeze-dried samples were recorded on a Paragon 500 (Perkin Elmer, Waltham, USA) in absorption mode in the range of 4000–450 $cm^{-1}$. Thirty-two scans were performed to establish accuracy.

### 2.4. Rheological properties

The rheological properties of HOBC/Chi/Alg gel solution were determined in a dynamic rheometer (Rheometer AR-550, TA Instruments, New Castle, Delaware, U.S.A.) using a small amplitude oscillatory test. The dynamic rheometer was equipped with parallel-plate geometry of 40 mm diameter. Gap and strain were set at 2.0 mm and 1.0%, respectively. Steady shear tests were performed over a shear rate range of 0.065–65 1/s and conducted between 0.01 and 10 Hz at 25 °C in frequency sweeps. Three replicate scans were conducted, with storage modulus ($G'$), loss modulus ($G''$), tan$\delta$ and magnitude of the complex viscosity ($\eta^*$) recorded.

### 2.5. Mechanical properties of DFBF

The mechanical properties of rehydrated DFBF were determined since the rehydration would weaken the toughness of wound dressing. The tensile strength and elongation at break of rehydrated DFBF were measured by TPA (TA.XT2, Stable Micro Systems, Godalming, Surrey, England), equipped with a 5-kg load cell and a cylindrical probe (P/36R, 35 mm diameter). The dumbbell-shaped sample was placed on an ASPR probe, the spacing and extension speed were 50 mm and 1.0 mm/s, respectively, to determine the tensile properties. Five replicates were performed and calculated as:

$$\text{Tensile strength (kPa)} = F/A$$

$$\text{Elongation at break (\%)} = 100 \times \Delta L/L$$

where F and A represent the maximum force (N) to break composite and area ($m^2$) of sample, respectively. $\Delta L$ and L represent the elongation (mm) and original length (50 mm) of sample, respectively.