Journal of Siberian Federal University. Chemistry 2023 16(3): 350–359
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EDN: KOBOQZ

УДК 66.017

Synthesis and Functionalization of Bacterial Cellulose Nanocrystals from Kombucha Tea for Wound Dressing Applications

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Received 29.04.2023, received in revised form 30.06.2023, accepted 07.07.2023

Abstract: In this study, bacterial cellulose nanocrystals and aldehyde functionalized bacterial cellulose nanocrystals were synthesized from kombucha tea. Bacterial cellulose derived from kombucha tea is a biopolymer synthesized by a symbiotic consortium of bacteria and yeast (SCOBY). The main goal of this work was the synthesis and chemical modification of cellulose nanocrystals from bacterial cellulose isolated from kombucha tea. The hydrolysis of bacterial cellulose using sulfuric acid resulted in bacterial cellulose nanocrystals. Aldehyde modified bacterial cellulose nanocrystals were synthesized using periodate oxidation in order to acquire new properties such as a non-toxic crosslinking agent with other biopolymers. The bacterial cellulose nanocrystals and dialdehyde bacterial cellulose nanocrystals were characterized by FT-IR spectroscopy, X-ray diffraction, thermal analysis and particle size distribution. The synthesized bacterial cellulose nanocrystals and the dialdehyde derivative are excellent materials that could be used as potent wound dressing materials and scaffolds for tissue engineering applications.

Keywords: cellulose nanocrystals, periodate, bacterial cellulose, kombucha tea, biopolymers.

Citation: Elangwe C. N., Morozkina S. N., Uspenskaya M. V., Nguyen T. H., Dada K. S. Synthesis and functionalization of bacterial cellulose nanocrystals from kombucha tea for wound dressing applications. J. Sib. Fed. Univ. Chem., 2023, 16(3), 350–359. EDN: KOBOQZ

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Синтез и функционализация нанокристаллов бактериальной целлюлозы из чайного гриба для раневых повязок

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Аннотация. В этом исследовании нанокристаллы бактериальной целлюлозы и нанокристаллы бактериальной целлюлозы, функционализированные альдегидными группами, были синтезированы из чайного гриба. Бактериальная целлюлоза, полученная из чайного гриба, представляет собой биополимер, синтезированный симбиотическим консорциумом бактерий и дрожжей (SCOBY). Основной целью данной работы был синтез и химическая модификация нанокристаллов целлюлозы из бактериальной целлюлозы, выделенной из чайного гриба. В результате гидролиза бактериальной целлюлозы серной кислотой были получены нанокристаллы бактериальной целлюлозы. Нанокристаллы бактериальной целлюлозы, модифицированные альдегидными группами, были синтезированы с использованием перийодата для приобретения новых свойств, таких как нетоксичный сшивающий агент с другими биополимерами. Нанокристаллы бактериальной целлюлозы и нанокристаллы диальдегидной бактериальной целлюлозы были охарактеризованы с помощью ИК-Фурье-спектроскопии, рентгеновской дифракции, термического анализа, и определены распределения частиц по размерам. Синтезированные нанокристаллы бактериальной целлюлозы и ее функционализированное альдегидными группами производное являются превосходными материалами, которые можно использовать в качестве эффективных перевязочных материалов для ран и каркасов для приложений тканевой инженерии.

Ключевые слова: нанокристаллы целлюлозы, перийодат, бактериальная целлюлоза, чайный гриб, биополимеры.

Цитирование: Элангвэ К. Н., Морозкина С. Н., Успенская М. В., Нгуен Т. Х., Дада К. С. Синтез и функционализация нанокристаллов бактериальной целлюлозы из чайного гриба для раневых повязок. Журн. Сиб. федер. ун-та. Химия, 2023, 16(3). С. 350–359. EDN: KOBOQZ

Introduction

Despite of the fabrication of various wound dressings from a wide spectrum of materials for the management of chronic and acute wounds, there are still challenges in developing high-quality wound dressings with excellent mechanical properties, non-toxic and economically viable. Wound dressings that are currently used include films, bandages, foams, patches and hydrogels. Currently, hydrogels are gaining a lot of attention due to their high-water holding capacity. Naturally derived polymers such as polysaccharides have been employed to improve the mechanical properties of hydrogels [1]. Cellulose is one of the most abundant natural polymers derived from plants, as well as algae, fungi and bacteria. It

consists of a linear homopolysaccharides comprising of glucose units linked with β-1,4-glycosidic bonds. Recently, cellulose synthesized from bacteria known as bacterial cellulose has been more attractive due to its fascinating properties.

Bacterial cellulose (BC) from kombucha tea is a biopolymer synthesized by a symbiotic consortium of bacteria and yeast (SCOBY) with many advantages over plant cellulose, such as high degree of purity (unlike plant cellulose, it is free from hemicellulose, lignin and pectin), high crystallinity index and high-water retention capacity [2, 3]. Kombucha tea or mushroom tea is a sweetened beverage usually prepared by the fermentation of black tea [4]. In order to reach the objectives of climate change and sustainable development goals of 2030 [5], the focal point of research is directed towards bacterial cellulose instead of using plant derived cellulose. Bacterial cellulose is a promising starting material for the synthesis of cellulose nanocrystals or nano-whiskers. In addition to its biocompatibility and good mechanical properties, it can be functionalized in order to acquire new properties such as antibacterial response and as a non-toxic crosslinking agent with other biopolymers for the synthesis of biocompatible wound dressings, tissue engineering scaffolds and drug delivery systems [5, 6]. Several methods have been used for the production of cellulose nanocrystals such as mechanical, enzymatic, TEMPO oxidative, hydrochloric acid hydrolysis, and sulfuric acid hydrolysis. However, sulfuric acid hydrolysis is widely used for preparing cellulose nanocrystals because it results in nanoparticles (100–1000 nm) with high degree of crystallinity. The main goal of this research was the synthesis and chemically modification of bacterial cellulose nanocrystals from Kombucha or mushroom tea for biomedical applications such as nanocomposite hydrogels wound dressings and tissue engineering scaffolds with improved mechanical properties.

Materials and Methods

Kombucha SCOBY (symbiotic culture of bacteria and yeast) was purchased from an online store, sulfuric acid (98 % purity, “NevaReactiv”), sodium periodate (95 % purity, “NevaReactiv”), black tea, sugar, hydrogen peroxide (98 % purity, “NevaReactiv”), sodium hydroxide (95 % purity, “NevaReactiv”). All reagents were of analytic grade.

Synthesis of Bacterial Cellulose from Kombucha Tea

The production of bacterial cellulose (BC) was carried out by the fermentation of kombucha tea using SCOBY (Symbiotic Culture of Bacterial and Yeast) as the starting material. In a glass jar, a mixture of 5 bags black tea (11.5 g) and 100 g/L of sugar were mixed in the glass jar of hot water. The solution was allowed to cold below 40 °C followed by the addition of SCOBY for the fermentation. The glass jar was covered with a transparent cloth to avoid contamination of the medium. The fermentation process was allowed in the dark for 21 days at the room temperature. The new kombucha pellicles or biofilms formed at the solution surface was isolated from the kombucha tea. The pellicles were washed with distilled water, and later with hot water at 70 °C, and dried in an oven at 70 °C for 24 h.

Purification of Bacterial Cellulose

The dried bacterial cellulose was ground using a blender into fine powder. 10 g of BC powder was dissolved in 200 ml of 0.5 M NaOH solution at 70 °C for 3 h under magnetic stirring. It was then washed in hot water at 80 °C for 4 h and later bleached in 100 ml of 1.5 w% aqueous hydrogen peroxide

at the room temperature for 4 h. Finally, it was washed six times in cold water until neutral pH and allowed to dry for two days at the room temperature.

Preparation of Bacterial Cellulose Nanocrystals

The preparation of bacterial cellulose nanocrystals (BCNC) was done by acid hydrolysis. The dried bacterial cellulose was blended to obtain fine powder. The powdered BC was mixed with water in a 200 ml beaker and then 98 w% sulfuric acid was gradually poured into the beaker to obtain a final acid concentration of 64 w%. The mixture was then heated at 55 °C for 60 min under magnetic stirring, and the bacterial cellulose/acid ratio was 1:18 (g: ml). After the acidic treatment time is over, the reaction was stopped by pouring out into a cold water (ten-folds). The colloidal suspension of cellulose nanocrystals was separated and centrifuged at 3500g for 10 min, and washed several times with distilled water by centrifugation until the pH was about 6. The obtained bacterial cellulose suspension was sonicated for 15 min to disperse the nanocrystals and later placed in a dialyzed membrane and dialyzed for 5 days. The purified BCNC was stored in a refrigerator at 5 °C for further use. Fig. 1 shows the various stages of BCNC preparation.

Synthesis of Dialdehyde Bacterial Cellulose Nanocrystals

The previously obtained BCNC suspension were oxidized using sodium periodate. Sodium periodate was placed in the 1.0 w% suspension of BCNCs at a weight ratio of 0.6:1 (NaIO4: BCNC). The flask was covered with Aluminum foil and the mixture was stirred at the room temperature for 2 h. 600 µl of Ethylene glycol was added into the reaction mixture to quench it. The oxidized BCNC suspension was dialyzed for 5 days and then stored at 5 °C in a refrigerator.

Determination of Particle Size Distribution

The particle size distribution of BCNC and dialdehyde bacterial cellulose nanocrystals (DABCNC) was determined by the HORIBA LB-550 dynamic light scattering particle size analyzer. LB-550 measures particle size from 1 nm to 6 microns.

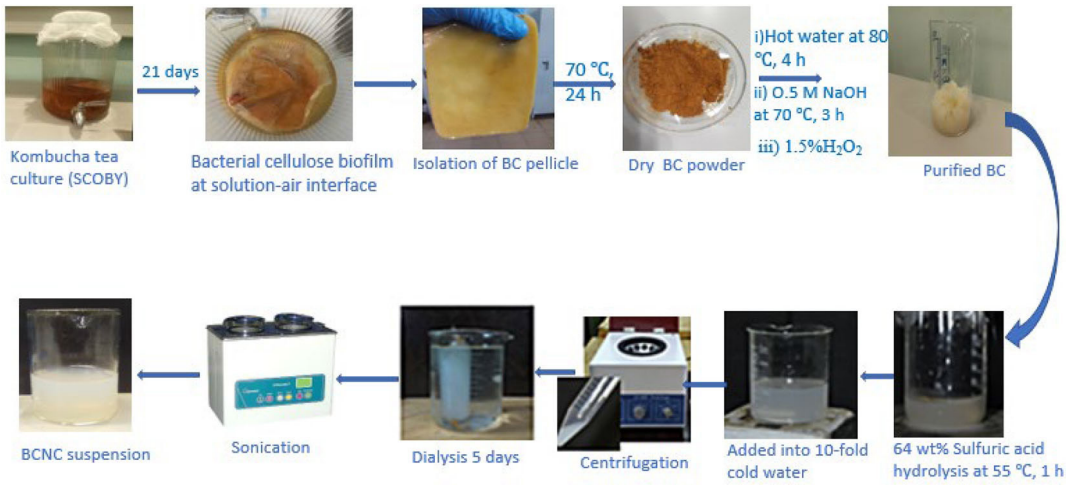

Fig. 1. Preparation of bacterial cellulose nanocrystals from kombucha SCOBY

Thermal Analysis

Thermogravimetric analysis (TA) was carried out with a TG 209 F1 Libra (Netzsch, Germany). The samples of BCNC and DABCNC were prepared by lyophilization. The samples were heated from 25 to 900 °C with a heating rate of 10 °C/min under nitrogen atmosphere with a flow rate of 50 ml/min. Derivative of TA (DTG) curves were also recorded, which express the weight loss rate as a function of temperature.

Fourier Transformed-Infrared Spectroscopy

Fourier-Transform infrared spectroscopy was performed on a Tensor 37 spectrometer (Bruker, Germany) equipped with a diamond coated ZnSe crystal to determine the change in chemical functional groups. FTIR spectra of bacterial cellulose nanocrystals and dialdehyde bacterial cellulose nanocrystals samples were recorded in the range of 4000 to 500 cm-1 with 32 scans.

X-Ray Diffraction

The crystallinity index (CI) of the samples was determined by XRD spectrum using a DRON-8 diffractometer with Cu K radiation (1.5419) at the room temperature. The samples were freeze-dried and mounted on a copper target on a sample holder with the test voltage of 40 kV, current of 100 mA at 2 theta scale. The angular range was from 5 to 70°. The crystallinity index (CI) was calculated using the equation 1:

$$\text{Crystallinity index}(\%) = \frac{\text{Area of crystalline peaks}}{\text{Area of all peaks (crystalline + amorphous)}} \quad (1)$$

Results and Discussion

Acid hydrolysis and functionalization of bacterial cellulose

Bacterial cellulose nanocrystals were obtained by sulfuric acid hydrolysis of bacterial cellulose to degrade the amorphous regions of the microfibrils (Fig. 2). Sulfuric acid hydrolysis is the most effective approach for commercial production of cellulose nanocrystals with low cost and time consumption. It is reported that during acid hydrolysis the hydronium ions penetrate the cellulose and cleave the amorphous regions of the bacterial cellulose [7].

The surface of BCNC has abundant hydroxyl groups which can easily be functionalized to other functional groups. Particularly, aldehyde modified cellulose nanocrystals were obtained via periodate oxidation at the room temperature for 2 h. Periodate oxidation of bacterial cellulose nanocrystals breaks the C 2-C 3 bond (Fig. 3) of the glucose repeating units producing vicinal dialdehyde groups that can react with other functional groups. Dialdehyde bacterial cellulose can be an interesting material for covalent immobilization of biopolymers containing the amine group such as chitosan, gelatin and collagen via Schiff base reaction. Immobilization with biopolymers containing amine group could be employed in wound dressing, drug delivery and tissue scaffolds.

Particle size distribution

The particle size measurement was conducted at the room temperature. It was observed that the average particle size distribution by intensity (Z-average) of the derived BCNC and DABCNC were

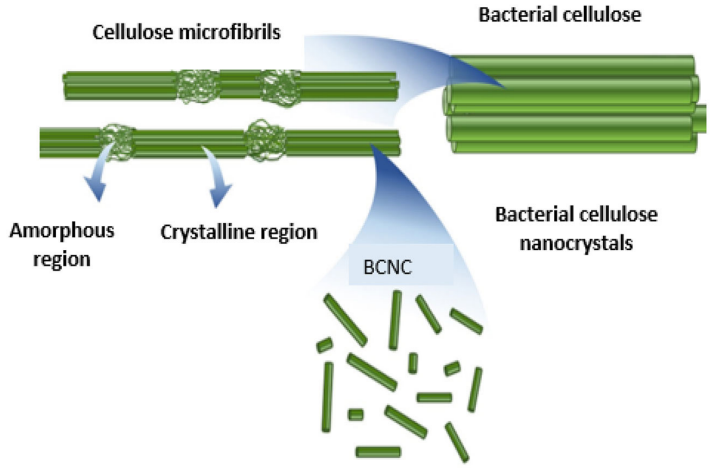

Fig. 2. Schematic illustration of acid hydrolysis of bacterial cellulose to bacterial cellulose nanocrystals

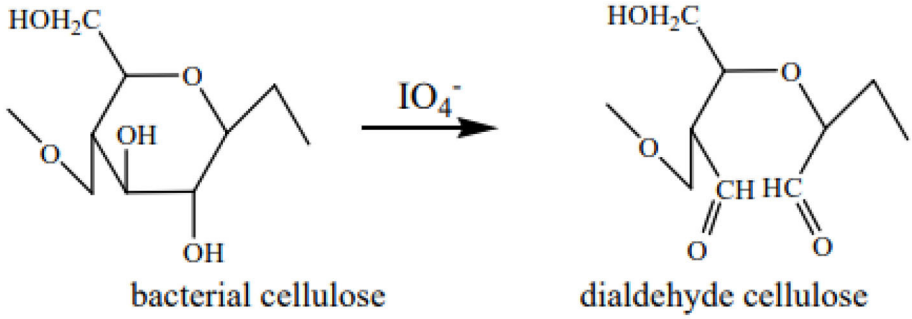

Fig. 3. Periodate oxidation of BCNC to DABCNC

104.0 and 92.8 nm respectively. As depicted in Fig. 4, the average particle size distribution of DABCNC is significantly smaller than that of BCNC. This was attributed to further degradation of BCNC as side reaction. During periodate oxidation, parts of beta-1–4 glycosidic bonds in BCNC chains containing reductive end groups were cleaved [8].

Fourier-transform infrared spectroscopy (FTIR)

The chemical structures of BCNC and DABCNC were examined using FTIR. In the spectra, characteristics of bacterial cellulose vibrational bands were identified (Fig. 5). The absorption peaks at 3330, 2897, 1650, 1107 cm-1 were attributed to the vibrational frequencies of OH stretching, CH_2 stretching, bending deformation of OH and C-O stretching respectively. The peaks at 1022 and 1392 confirmed the presence of C-O-C stretching and CH_2 bending vibrations [9].

Thermal Analysis

The thermogravimetric curves of both BCNC and DABCNC are presented in Fig. 6a. The first stage of weight loss was observed at about 98 °C due to the presence of volatile compounds. In the second stage the loss of weight was as a result of the materials degradation such as dehydration,

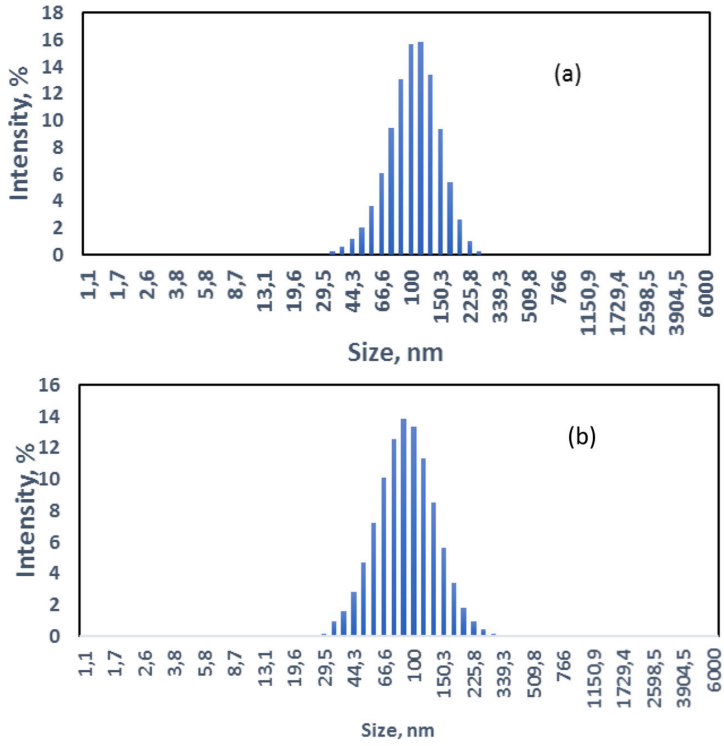

Fig. 4. Particle size distribution of (a) BCNC, (b) DABCNC

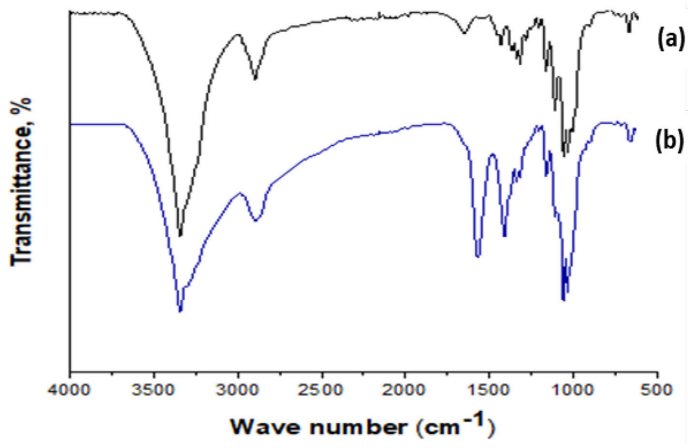

Fig. 5. FTIR spectra of (a) DABCNC, (b) BCNC

depolymerization and glycosidic unit decomposition. The third stage at about 400 to 900 °C could be attributed to the formation of carbon residues. The maximum degradation temperature of BCNC and DABCNC samples were possible to identify by derivation of thermogravimetric (DTG) (Fig. 6b) which were 341.6 °C and 276.9 °C respectively. It was demonstrated that DABCNC sample had lower degradation temperature. Although oxidation of BCNC reduces the thermal stability of the material, this does not limit its applications as it can withstand autoclave temperatures for the sterilization [9, 10].

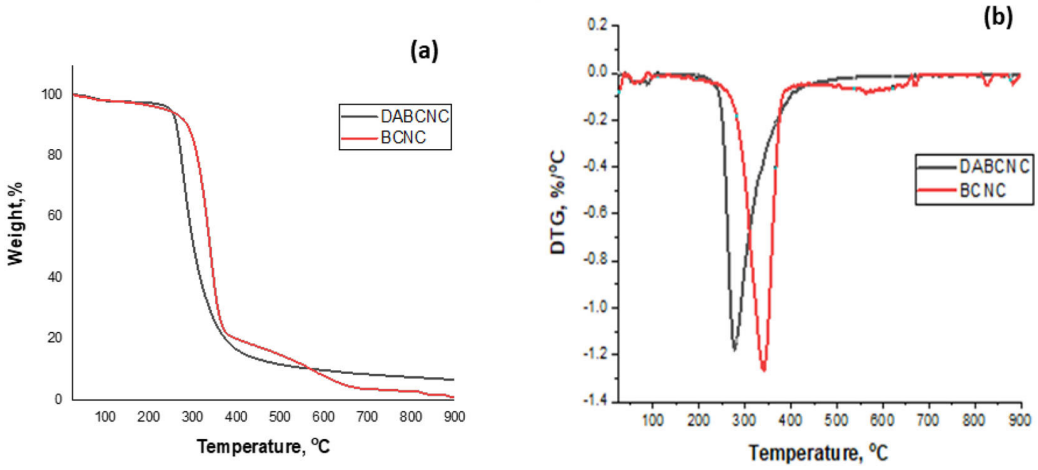

Fig. 6. (a) Thermogravimetric curves, (b) Derivative thermogravimetric curves (DTG) of BCNC and DABCNC

X-ray diffractometry

X-ray diffractograms of BCNC and DABCNC are shown in Fig. 7. It was observed that the diffractogram depicted three peaks for both samples near 14.5 theta, 16.9 theta and 25.7 theta of crystalline cellulose of type 1 [11]. The curves demonstrated that periodate oxidation did not change the configuration of bacterial cellulose nanocrystals because the peak positions and shapes of the crystals did not change. However, after periodate oxidation the crystallinity index (CI) of DABCNC was significantly smaller than BCNC whose CI reduced from 90.6 to 81.4. The difference in CI of BCNC was in agreement with the change in the particle size distribution, which confirmed the degradation process during periodate oxidation [8]. This phenomenon is due to the opening of the glucopyranose rings and destruction of the ordered arrangement of the crystal structure. Studies from other researchers claim that three reaction takes place during periodate oxidation: a fast initial attack of

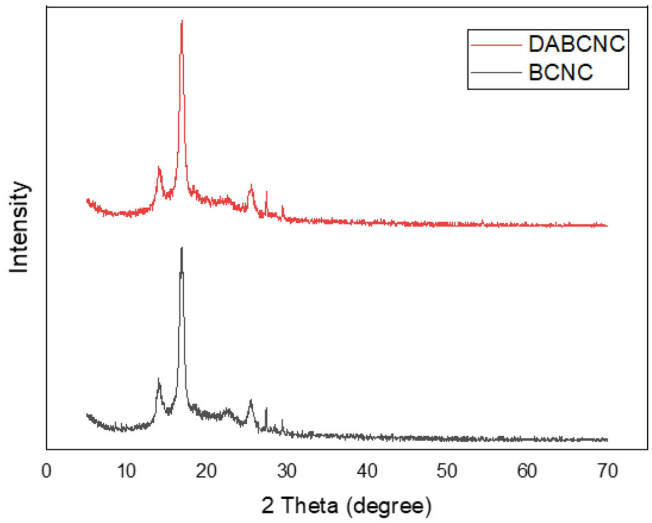

Fig. 7. XRD of BCNC and DABCNC

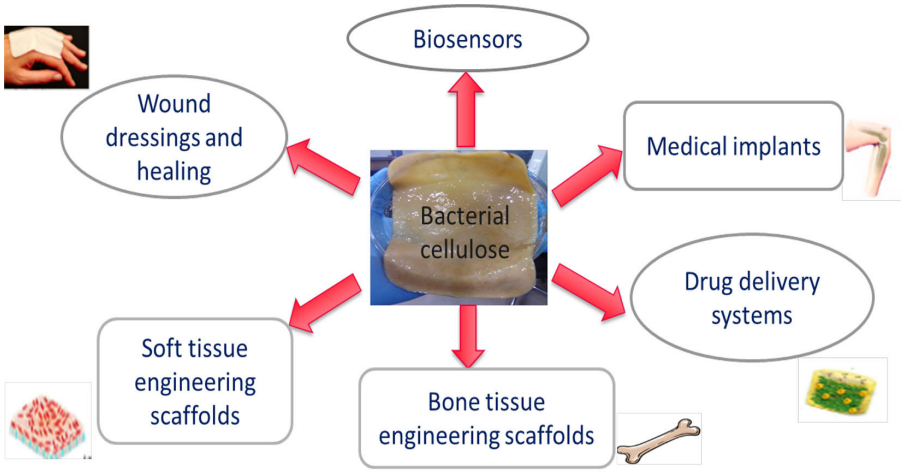

Fig. 8. Biomedical applications of bacterial cellulose

the periodate ions on the amorphous region of cellulose, a second gradual reaction due to the oxidation of the surface of the crystalline region, and a third extremely slow reaction attributed to the oxidation of the crystalline nucleus [10, 12].

Bacterial cellulose has better properties than plant cellulose and owing to its biocompatibility, nontoxic, biodegradability properties has been widely used in biomedical applications such as wound dressing, tissue engineering and drug delivery systems [13, 14, 15]. Fig. 8 demonstrates some of the common applications of bacterial cellulose.

Conclusion

Bacterial cellulose nanocrystals with excellent properties were successfully obtained from kombucha tea fermentation, and chemically modified to dialdehyde bacterial cellulose nanocrystals by periodate oxidation. Therefore, bacterial cellulose can be used as a promising cost-effective material and as a crosslinker in hydrogel synthesis. In the future, the industrial production of bacterial cellulose from kombucha tea and other culture media with high yield and green synthesis approach is expected to gain enormous attention in order to meet various trends in the modern world. The successful commercialization of cellulose nanocrystals derived from plants origin using sulfuric acid holds a good base for similar approach to be applied to bacterial cellulose. Because of its excellent properties, bacterial cellulose holds high clinical potentials to be utilized as wound dressings and skin tissue engineering scaffolds.

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