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Development of Bacterial Cellulose Herbal Wound Dressing

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Abstract

Wound dressings play an essential role in wound care and healing, but the price is expensive for patients who have to treat for a long time such as patients from burns, scalds, etc. The objective of the research is to develop a wound dressing made with bacterial cellulose and local herb that has antibacterial properties, is cheap, and can be created by yourself. The bacterial cellulose (BC) produced from *Acetobactor xylinum* using coconut water waste to reduce costs. Its structure was changed to bacterial cellulose nanocrystals (BCNC) by acid hydrolysis, dried in 2 methods: Oven dried at 50 °C (dy) or freeze dried (fd). Then, herbal dressings were produced as a composite BC with herbs in 2 forms: A solution, i.e. *C. Odorata* leaf extract solution and a colloid, i.e. *C. hystrix* essential oil nanocapsule. The results for the production of a wound dressing showed that fd-BCNC had good quality, good fiber dispersion, high porosity and no antibacterial activity. It is suitable as a primary or secondary wound dressing, that does not help in wound healing. When fd-BCNC was loaded with herbs in 2 forms, it was found to be stable at room temperature, water adsorption, water vapor permeability and inhibit *Staphylococcus aureus* and *Escherichia coli*. Especially fd-BCNC of nanocapsule of *C. hystrix* peel essential oil has the best antibacterial effect. Therefore, you can apply this developed wound dressing to herbs used specifically for healing wounds. Moreover, we believe that this research will be a database that can be expanded to commercial production.

Keywords: Herbal wound dressing, Bacterial cellulose, Bacterial cellulose nanocrystal, Freeze drying, herbal, *C. Odorata* leaf, *C. hystrix* peel essential oil, Nanocapsule

Introduction

Treatment of burns, scalds, or accidents require wound dressings that promote healing and prevent infection. The ideal wound dressing should keep the wound moist, gas exchange between the injured tissue and the environment, limit bacterial overgrowth, easy to remove, non-toxic and non-allergic [1]. Traditional dressing such as gauze, cotton wool, plaster, bandages (natural or synthetic), help prevent bacterial infection and absorb secretions but must be changed often. Generally traditional wound dressings are indicated for the clean and dry wounds with mild exudate levels or used as secondary dressings. They have been replaced by modern wound dressings such as semi-permeable film, semi-permeable foam, hydrogels, hydrocolloid and alginate. These dressings are focused to keep the wound from dehydration and promote healing. Moreover, the last type of modern wound dressing is bioactive dressings and is produced from biomaterials. These dressings are known for their biocompatibility, biodegradability and non-toxic such as collagen, hyaluronic acid, chitosan, alginate and elastin [2]. Bacterial cellulose (BC) is another natural hydropolymer with good properties for wound dressing. Due to its intrinsic properties: hydrophilicity, very high purity, porosity, biocompatibility, controlled release of drugs. It's a never-ending material and eco-friendly [3]. However, wound dressing from BC alone does not present several desirable characteristics, such as antibacterial, anti-inflammatory and wound healing [4]. This research is also interested in the development of new and effective wound dressings for antibacterial and wound healing. We produce BC from waste coconut water to reduce costs [5]. Then improve internal properties by acid hydrolysis and drying. Moreover, we loaded BC with herbs such as *Chromolaena Odorata* (Bitter Bush) and *Citrus Hystrix* (Kaffir Lime) which have been used in traditional medicine for a long time as herbal remedies for skin diseases [6,7]. *C. Odorata* is a weed that is commonly found in tropical. The fresh leaf of *C. odorata* (**Figure 1(a)**) showed that they were rich in alkaloids, flavonoids, tannins, saponins, terpenoids, anthraquinones, cardiac glycosides and carbohydrates [8]. While, *C. hystrix* (**Figure 1(b)**) is native to Thailand and Southeast Asia. The results showed that the essential oil composed of monoterpene hydrocarbon, aldehyde and ester with

sabinene, β-pinene, D-limonene and β-citronellal being the main components [9]. It is also an antioxidant, antibacterial and anti-leukemia [10].

Therefore, we chose to develop BC and BC/herbal, in which the herbal form is a solution and colloid. To be a model for producing a wound dressing that can be used as primary, secondary wound dressing and herbal wound dressing that can be used on specific wounds.

(a) (b)

Figure 1 (a) *C.odorata* and (b) *C.hystrix*.

Materials and methods

Materials

Plant materials: Coconut water wastes, fresh leaf of *C. Odorata* and *C. hystrix* peel were collected from Sakon Nakhon provinces in Thailand. *Acetobacter xylinum*, *Staphylococcus aureus* and *Escherichia coli* were purchased from Thailand institute of scientific and technological research (TISTR) in Thailand. Sodium hydroxide (NaOH), sulfuric acid (H_2SO_4), hydrogen peroxide (H_2O_2), acetic acid (CH_3COOH), ethanol (C_2H_5OH), dimethyl sulfoxide (C_2H_6OS; DMSO), sodium dodecyl sulfate ($NaC_{12}H_{25}SO_4$; SDS) and chitosan (Ch) were purchased from Merck Ltd., United States.

Methods

Production of bacterial cellulose

Bacterial cellulose (BC) was synthesized by *Acetobacter xylinum* using coconut water wastes as culture medium. Then statically incubated at room temperature for 7 days. After this time, bacterial cellulose films were formed in the air/liquid medium interface. The films were treated with 0.3 M NaOH at 80 °C for 2 h to remove bacterial cells, remains of the culture medium and treated with distilled water to pH 7 [11]. It was hydrolyzed by 0.1 M H_2SO_4 in a ratio of 1:20 g/mL with continuous stirring for 1 h at 85 °C followed by the oxidation of H_2O_2 to obtain the bacterial cellulose nanocrystal (BCNC) [12].

Drying of bacterial cellulose

Twenty gram each of the BC and BCNC were made into 8×16 cm sheets and dried by 2 different drying processes: Oven dried at 50 °C (dy) or freeze dried (fd) [13]. Product properties include water absorption (ASTM D570 standard test method), water vapor permeability (ASTM E96 standard test method), room temperature stability (ASTM D2756-02 standard test method) and surface structure (scanning electron microscope; SEM) were analyzed to choose the best bacterial cellulose wound dressing.

Preparation of herbal

C. Odorata extract solution (pe) and *C. hystrix* peel essential oil nanocapsule (eo). Fresh leaf of *C. Odorata* was extracted with 95 % C_2H_5OH by maceration at room temperature for 7 days. The extract was filtered and the solvent was evaporated using a rotary evaporator, and finally the crude extract was formed. It was prepared as a solution to the desired concentration in 10 % DMSO and in water by sonication [14]. They were analyzed for UV-Visible spectrum to select the best solvent. Peel of *C. hystrix* is extracted through the process of steam distillation until obtaining essential oil [15]. Then, essential oil nanocapsule were prepared by mixing essential oil, chitosan (Ch) in 1 % CH_3COOH and SDS, surfactant in various ratios, as the **Table 1** below. Then adjusted to a total volume of 100 mL with 1 % CH_3COOH. After sonicated for 30 min, white colloid was formed. They were analyzed for the zeta potential (zeta potential analyzer) to select the best parameters for the essential oil nanocapsule [16].

Table 1 The ratio of mixtures in the production of essential oil nanocapsule.

Formula No.	SDS (M, in 10^{-3})	Ch (ppm)	Essential oil (g)
1		50	0.5
2		50	1.0
3		50	1.5
4		100	0.5
5	5	100	1.0
6		100	1.5
7		150	0.5
8		150	1.0
9		150	1.5
10		50	0.5
11		50	1.0
12		50	1.5
13		100	0.5
14	10	100	1.0
15		100	1.5
16		150	0.5
17		150	1.0
18		150	1.5
19		50	0.5
20		50	1.0
21		50	1.5
22		100	0.5
23	20	100	1.0
24		100	1.5
25		150	0.5
26		150	1.0
27		150	1.5

DS = sodium dodecyl sulfate; Ch = chitosan

The prepared herb was determined for stability at room temperature by assaying its absorbance over time, inhibition of *Staphylococcus aureus* (gram-positive) and *Escherichia coli* (gram-negative) by agar diffusion and MIC and MBC determined by broth micro dilution (only for *C. Odorata* leaf extract solution).

Production of herbal bacterial cellulose wound dressing

Dehydrated BC and BCNC were immersed in a solution of *C. Odorata* leaf extract and *C. hystrix* peel essential oil nanocapsule at room temperature for 3 h and dried in a desiccator. The percentage absorption was calculated by weighing and the absorbent was confirmed by comparing the absorbance spectra before and after absorption. The absorbance of *C. Odorata* leaf extract solution was measured at wavelengths of 400 - 700 nm, while *C. hystrix* peel essential oil nanocapsule was measured at a wavelength of 600 nm, which was used to measure turbidity. Then, they were analyzed for properties including water absorption (ASTM D570 standard test method), water vapor permeability (ASTM E96 standard test method), room temperature stability (ASTM D2756-02 standard test method), surface structure (scanning electron microscope; SEM analysis) and antimicrobial activity of *Staphylococcus aureus* and *Escherichia coli* to choose the best bacterial cellulose wound dressing for each herb.

Results and discussion

Production of bacterial cellulose

The BC production using coconut water waste as culture medium. After incubation for 7 days, bacteria were found to grow to form a thick white gel-like BC on the surface of coconut water waste. The BC yield with dry weight of 3.24 g/L of culture media and moisture content of 98.44 %. The production of BCNC by hydrolysis of BC with H_2SO_4 to form a white suspension, yield 72.79 as shown in **Figure 2**. The results of this study suggest that the coconut water waste is rich in nutrients, which is suitable for the growth of microorganisms [17]. In addition, BC can be produced by simple fermentation conditions and indicating that a renewable resource of high-purity. The BCNC produced were esterified the hydroxyl (OH) groups of cellulose with sulfate groups (OSO_3) to yield a negatively charged surface nanocrystals of cellulose sulfate that can provide ionic stabilization through pulsed electric. Consequently, it can help prevent aggregation of nanocrystals driven by both inter and intramolecular hydrogen bonds in the structure of BC fibers, resulting in a stable dispersion of nanocrystals [18].

Figure 2 (a) bacterial cellulose (BC) and (b) bacterial cellulose nanocrystal (BCNC).

Drying of bacterial cellulose

The produced BC and BCNC went through 2 different drying processes: oven dried at 50 °C (dy) or freeze dried (fd) as shown in **Figure 3(a)**. The results showed that the reduction of the high-water content of BC causes a thickness reduction of the samples (≈89 %), whereas BCNC looks like a foam and leads to a thickness reduction of only ≈10 %. In addition, in fd there is a higher porosity due to the hydrogen bond network structure of the BC upon freeze drying by sublimation in very cold air and vacuum chamber, the pore geometry of BC is preserved [19]. While heat removal of water decreases the external surface area and the closure of pores [20]. The morphology by SEM (**Figure 3(b)**), the produced BC showed a surface consisting of many intertwined fibers formed and randomly arranged. Whereas the produced BCNCs showed a surface consisting of short fibers, various pore sizes and the distribution of tiny white dots. It results from the hydrolysis of acids to sugar molecules in the amorphous chains of cellulose that are broken and agglomerated.

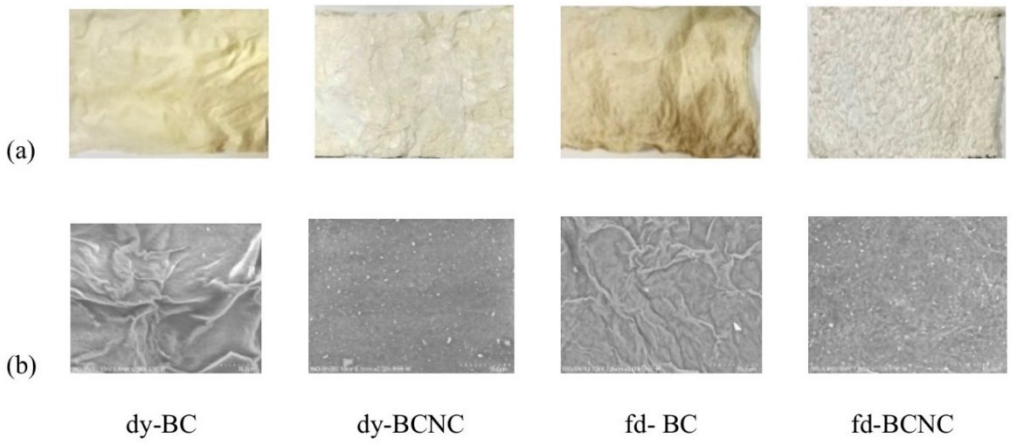
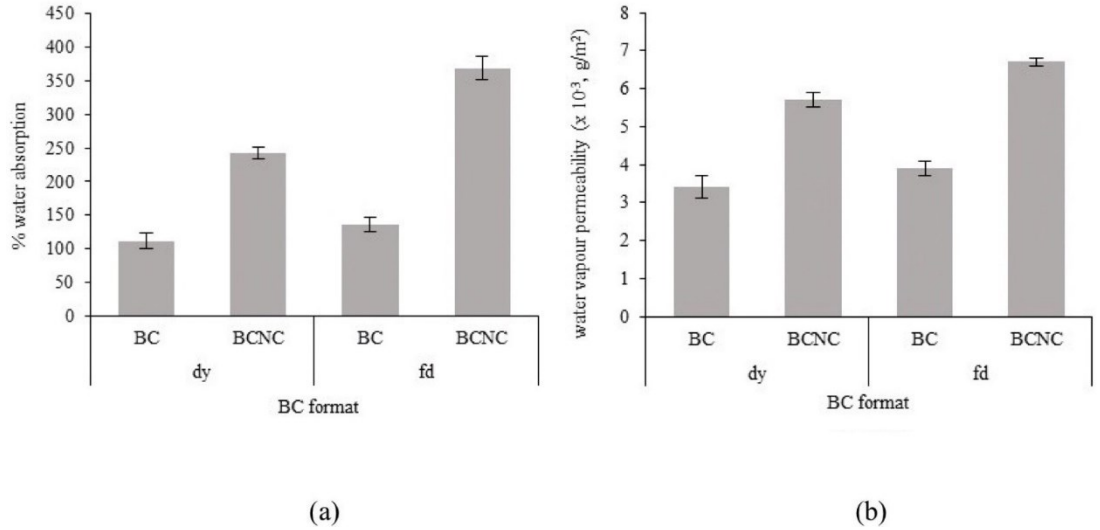


Figure 3 (a) BC film dried and (b) SEM micrographs (2000×) of BC film dried.

Figure 4, fd-BCNC showed better water absorption and water vapor permeability than all samples. It was stable after storage at room temperature for 30 days, but fd-BCNC was not antibacterial against both *Staphylococcus aureus* and *Escherichia coli*. Therefore, the superior properties of fd-BCNC were selected as a primary or secondary wound dressing that does not have wound healing properties.

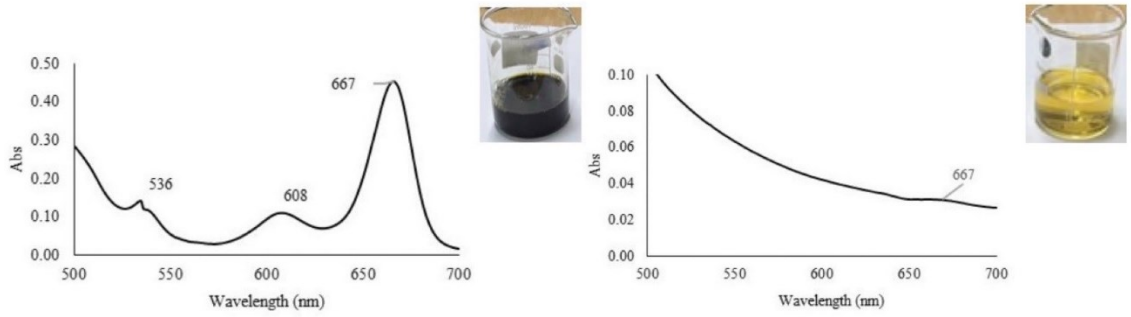


(a) (b)

Figure 4 (a) water absorption and (b) water vapor permeability of the investigated BC produced.

Preparation of herbal

The crude C_2H_5OH extract of *C. Odorata* leaf was a dark green slurry and its extraction yield was 4.42 %. It could be dissolved in 10 % DMSO but less soluble in water. Visible absorption spectra shown 3 peaks at 536, 608 and 667 nm (**Figure 5(a)**), while, in water shown single peak at 667 nm (**Figure 5(b)**). Indicated that the crude ethanol extract contained terpenoids (400 - 550 nm) and chlorophyll (600 - 700 nm), while the aqueous content was very small [21]. Therefore, we chose 10 % DMSO as the solvent of this extract.



(a) (b)

Figure 5 Visible absorption spectra of extract of *C. Odorata* leaf (a) ethanol and (b) water.

C.hystrix peel essential oil is obtained by steam distillation. It is a clear yellow oil, with a bergamot aroma, yield of 2.44 % of fresh peel weight. It is prepared as nanocapsule, a white opaque colloid. The best formula is number 18 consisting of 1.5 g of *C. hystrix* peel essential oil, 50 ppm of chitosan and 10×10^{-3} M of SDS, all mixed in 100 mL of 1 % CH_3COOH. The result was the most negative zeta potential, −96.8 mV and the smallest particle size, 107.9 nm (**Figure 6**). A low zeta potential indicates that there is no force acting on the particles to clump together, the stability of the colloid [22].

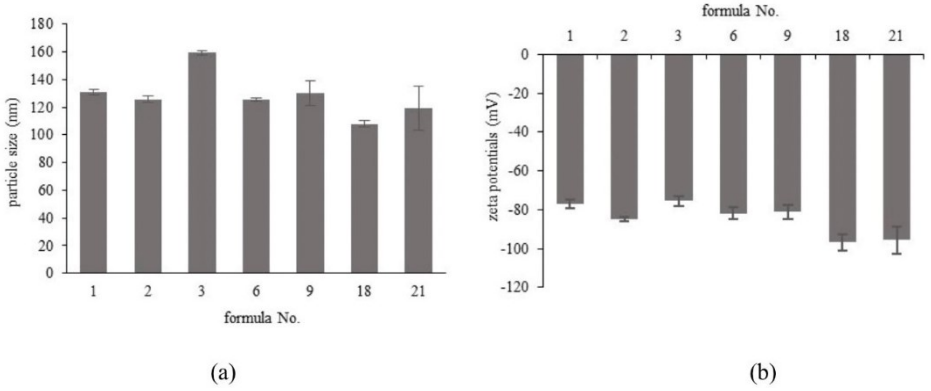


Figure 6 (a) hydrodynamic sizes and (b) zeta potentials of *C. hystrix* peel essential oil nanocapsule.

The solution of the crude C_2H_5OH extract from *C. Odorata* leaf and *C. hystrix* peel essential oil nanocapsule were found to be active against *Staphylococcus aureus* and *Escherichia coli*, was comparable to penicillin V 0.01 mg/mL and olfoxacin 0.01 mg/mL, respectively (**Table 2**). In addition, test results of the crude C_2H_5OH extract from *C. Odorata* leaf showed that the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of 2.5 mg/mL and 100 mg/mL, respectively (data not shown).

Production of herbal bacterial cellulose wound dressing

All formulations of wound dressings have the herbal absorption capacity of more than 200 %, especially fd-BCNC has the highest. It was found to be 449.77 % for the *C. Odorata* leaf extract solution and 342.96 % for *C. hystrix* peel essential oil nanocapsule. Due to the highly porous surface area of fd-BCNC, the herb both in solution and colloid form can migrate into it, confirmed through dry-weight analysis (data not shown). The fd-BCNC/herbs composite, it was shown that the water absorption and water vapor permeability were lower than that of all samples as shown in **Figures 7(a)** and **7(b)**. Compared with commercial wound dressings, the results showed water absorption (214 %) and water vapor permeability (3.50×10^{-3} g/m^2) were approximately equal to fd-BCNC/herb. As result, explaining the loading these herbs onto fd-BCNC through the pores, the pores are narrowed and fewer pores. These observations are consistent with the SEM images (**Figure 8**), resulted the water absorption and water vapor permeability were reduced compared to pre-loading the herbs. Moreover, all formulations of herbal bacterial cellulose wound dressing have inhibited against both *Staphylococcus aureus* and *Escherichia coli* especially fd-BCNC of *C. hystrix* peel essential nanocapsule has the best antibacterial effect. In contrast, commercial wound dressings showed no inhibition of the bacteria (**Table 2**). The fd-BC/herbs composite as herbal wood dressing were an interaction between the cellulose structure and herbs. And the structure of cellulose with amphiphilic property (The chain consists of hydrophilic parts. and hydrophobic) can also form micelles to encapsulate colloidal particles [23]. For these reasons, herbal wound dressing was stable in use, confirmed by its stable weight when stored at room temperature for 30 days.

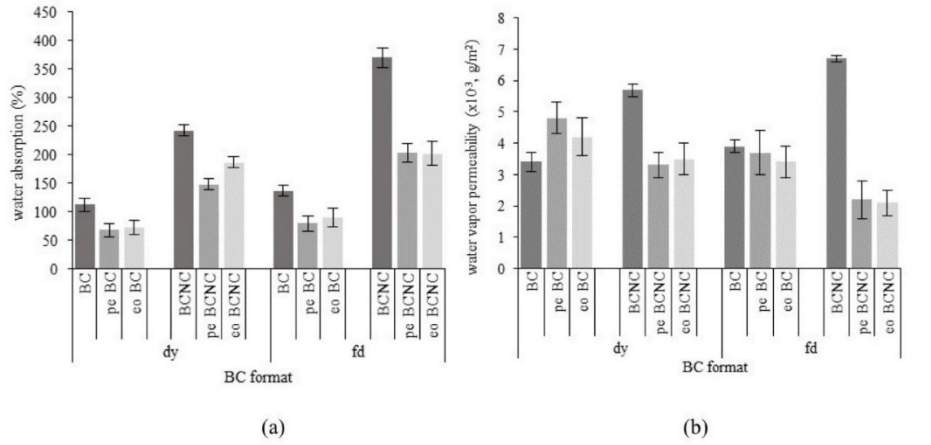


Figure 7 (a) water absorption and (b) water vapor permeability of the investigated fd-BC/herbs composite.

Table 2 Antibacterial analysis of samples.

Samples	microorganism/ clear zone diameter (cm)	
	Staphylococcus aureus	***Escherichia coli***
Penicillin V (0.01 mg/mL)	1.1±1.25	-
Plfoxacin (0.01 mg/mL)	-	1.6±1.51
C. odorata extract	1.2±5.03	1.2±3.07
C. hystrix peel essential oil nanocapsule	2.1±0.21	2.0±0.11
pe fd-BCNC	1.0±0.00	1.7±0.12
eo fd-BCNC	1.8±0.06	2.0±0.00
Commercial wound dressings	NI	NI

NI = No inhibition; ± Standard Deviation

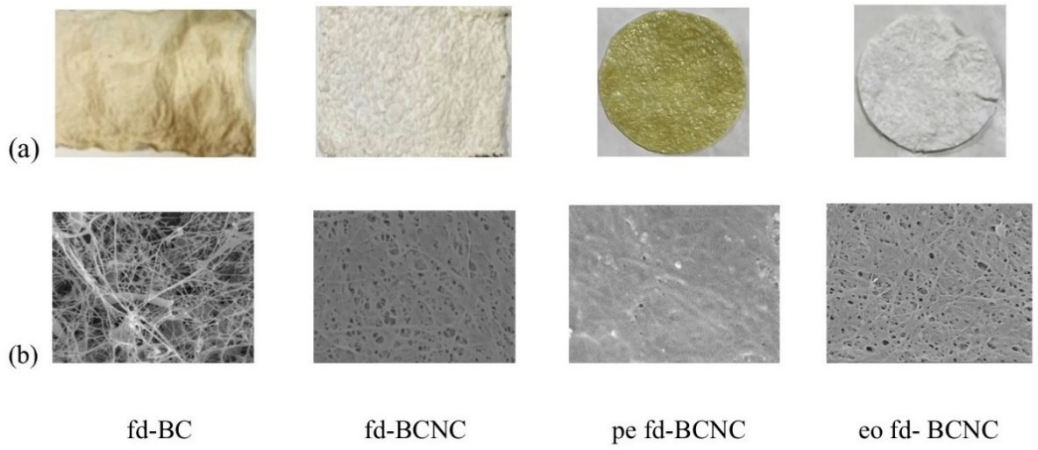


Figure 8 (a) BC film freeze dried and (b) SEM micrographs (50000×) of BC film freeze dried.

The wound dressing and herbal wound dressing are shown in **Figure 9**.

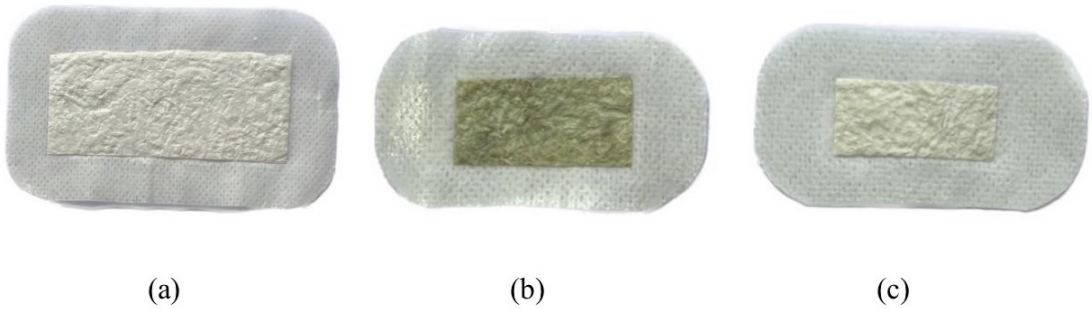


Figure 9 The applications of wound dressing; (a) fd-BCNC, (b) pe fd-BCNC and (c) eo fd-BCNC.

Conclusions

The fd-BCNC is produced from low-cost BC by acid hydrolysis. It can be used as a primary and secondary non antibacterial wound dressing. In addition, the composite of fd-BCNC/herbs: The *C. Odorata* leaf extract in solution form and *C. hystrix* peel essential oil nanocapsule in colloidal form could be used as antibacterial wound dressing.

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