In Vitro Investigation of Bacterial Cellulose/Turmeric Extract (BC-TE) Nanocomposite for Burn Wound Dressing

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Abstract: Bacterial cellulose is an interesting polymer material for using as a wound dressing. Bacterial cellulose has an excellent physical and chemical properties such as high biocompatibility, microporosity, transparant, non-toxic, and also it provides moist environment that made this polymer ideal for wound dressing. However, bacterial cellulose itself has no antimicrobial activity to prevent wound infection. To achieve antimicrobial activity, turmeric extract (TE) were impregnated into bacterial cellulose by immersing bacterial cellulose (BC) in turmeric extract solution to produce BC-TE nanocomposite. A scanning electron microscope (SEM) was used to examine the surface morphology of BC and BC–TE nanocomposite. Antimicrobial tests in vitro indicated that BC–TE nanocomposite showed excellent antibacterial activity against *Staphylococcus aureus*, and *Escherichia coli* with the inhibition zone of 12.45 mm and 10 mm, respectively

1 INTRODUCTION

Burns wound are the most painful wound and can cause trauma. More than 300,000 people die every year around the world and 90% of these deaths are caused by complications due to burns that occur in many middle and lower income countries (Peck, 2011). First-degree burns usually heal without complications while partial burns and full burns are more complex so a clinical challenge must be faced (Pham et al, 2007). Common complications in the treatment of burns arise because the area of the wound that is too large causes a long treatment time. Contamination of wounds from the external environment, air, water and hands of health workers (Mehta et al, 2014). The unavailability of modern medicine and minimal handling makes this become a serious health problem. Ointment is less effective in treating burns. Good treatment of burns must have an effective wound covering material that can create an optimal environment for regenerating the outer skin and prevent infection of chronic wounds and water loss (McLoughlin, 1995).

Bacterial Cellulose (BC) is one of the promising polymer compounds produced by several types of bacteria such as *Acetobacter*, *Rhizobium*, *Agrobacterium*, *Aerobacter*, *Achromobacter*, *Azotobacter*, *Salmonella*, *Escherichia*, and *Sarcina* species (Ullah, Santos, & Khan, 2016). BC has excellent physical and chemical properties such as high biocompatibility, hydrophilicity, microporosity, transparent and non-toxic which makes it ideal in the treatment of wounds and skin substitutes (Gelin et al, 2007). However, bacterial cellulose does not have good antimicrobial activity to prevent infection. The versatile biomedical characteristics of bacterial cellulose can be used to make the latest wound dressing by combining active molecules such as antimicrobials to improve wound healing properties. (Maneerung, Tokura, & Rujiravanit 2008).

Turmeric is one type of medicinal plant that has many benefits and is found in many parts of Indonesia. Turmeric contains curcumin which can accelerate wound healing. Curcumin can increase reepithelialization, suppress inflammation, increase tissue collagen density and increase proliferation of fibroblasts (Simanjuntak, 2012). The nature of turmeric that can heal wounds has been reported since 1953. The results showed, with turmeric the rate of wound healing increased 23.3% in rabbits and 24.4% in mice (Van Schraelen, 2011). Turmeric

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has pharmacological effects, such as accelerate blood circulation and vital energy, eliminates menstrual blockage, anti-inflammation, facilitates labor, high antibacterial activity, facilitates the release of bile (cholagogum), unleash fart and mousturizier (astringen) (Melin and Soleha 2016).

Composite is a very interesting material because it combines materials with different properties to produce new materials with better properties. Polymer nanocomposites are defined as polymers containing materials smaller than 100 nm. Nanocomposites are categorized in nanotechnology if the resulting composite reflects the superiority of nanomaterials, which is a significantly improved performance. (Rudin and Choi, 2013).

2 MATERIALS AND METHODS

2.1 Materials

Acetobacter xylinum, *Escherichia coli* and *Staphylococcus aureus* were purchased from Microbiological Resources Centre, Thailand Institute of Scientific and Technological Research Nutrient broth (Approximate formula*per liter: Beef extract 3.0 g and Peptone 5.0 g) was purchased from Difco. Analytical grade D-glucose anhydrous was purchased from Ajax Fine-chem. Yeast extract powder and agar powder were bacteriological grade and purchased from HiMedia. Laboratory grade calcium carbonate and analytical grade silver nitrate were purchased from Fisher Scientific. Laboratory grade sodium borohydride was purchased from CARLO ERBA. Analytical grade sodium hydroxide anhydrate pellet and sodium chloride were purchased from Aldrich Chemical. Analytical grade glacial acetic acid was purchased from CSL Chemical. Ethanol was commercial grade and used without further purification.

2.2 Synthesize of Turmeric Extract

Turmeric used waa a fresh turmeric. The process of making turmeric extract methanol is carried out based on Yacob et al. (2010) using maceration and evaporation methods. First, fresh turmeric washed, drained, and dried for 3 days until the turmeric is completely dry. After turmeric has completely dried, dried turmeric was mashed until it become powder. Turmeric powder soaked with ethanol until homogeneous and then turmeric macerated for 3 x 24 hours. Maseration of turmeric powder was filtered using whatman filter paper No.42. Fractions containing volatile solvents, were concentrated with the help of rotary evaporator. The concentrated extract was unloaded to sterilized collecting tube

2.3 Production of Bacterial Cellulose

2.3.1 Culture-Medium

Culture medium used for the fermentation of *A. xylinum* to produce bacterial cellulose consisted of 5 % glucose, 0.5 % bacto-peptone, 0.2 % disodium phosphate, 0.1 % monocalium phosphate and the addition of glacial acetic acid until the pH of the culture medium reached 4. The solution was stirred for 1 hour at 90^{o} C and followed by autoclave at 121^{o} C for 30 minutes.

2.3.2 Culture Condition

Pre-inoculum for all experiments was prepared by transferring a single *A. xylinum* colony grown on liquid culture medium into a 100 mL beaker glass filled with liquid culture medium, then inoculated in an incubator at 28 for 7 days with a rotational speed of 100 rpm.

2.3.3 Purification of Bacterial Cellulose

After incubation, bacterial cellulose pellicles produced on the surface of each liquid culture medium were harvested and purified by soaking them in 2.5 % NaOH for 24 h, then soaking them in 2.5% NaOCl for 24 h and finally thoroughly washed in tap water until bacterial cellulose pellicles became neutral and then immersed in the distilled water prior to use.

2.3.4 Impregnation of Turmeric Extract Into Bacterial Cellulose

The impregnation of turmeric extract into bacterial cellulose using ex-situ method. Turmeric extract were impregnated into bacterial cellulose fiber by immersing bacterial cellulose pellicles in turmeric extract until all the surface of bacterial cellulose pellicles covered with turmeric extract. After then bacterial cellulose pellicles impregnated for 24 h, and then the nanocomposite rinsed with water to remove turmeric sludge, the obtained nanocomposite were frozen at and dried in a vacuum at -52^{o} C.

2.4 Characterization

The morphology of bacterial cellulose was observed by using JEOL JSM-5200 scanning electron microscope (SEM) operating at 20 kV at a magnification of 10000.

2.5 Antimicrobial Activity Studies

Antimicrobial activities of freeze-dried bacterial cellulose/turmeric extract (BC-TE) nanocomposite have been investigated against *E. coli* as the model Gram-negative bacteria and *S. aureus* as the model Gram-positive bacteria. The antimicrobial activities of freeze-dried bacterial cellulose/turmeric extract (BC-TE) nanocomposite were carried out by disc diffusion method. This method was performed in disc diffusion method on the media Muller Hitton Agar (MHA). Silver sulfadiazine is used as a standard drug (positive control). Bacterial cellulose nanocomposite / turmeric extract is placed on sterile filter paper and also bacterial cellulose. Then placed on gelatin media containing the selected bacteria then incubated at 37° C for 24 hours. The inhibition zone was measured to determine the antimicrobial ability of bacterial cellulose / turmeric extract nanocomposite.

3 RESULTS AND DISCUSSION

3.1 Morphology of Bacterial Cellulose

Figure 1 shows the SEM micrographs of bacterial cellulose and bacterial cellulose/turmeric extract nanocomposite. As shown in Figure 1a BC had a fibrous network with highly porous structure. As shown in Figure 1b, turmeric extract particles attached on the BC composite membrane, it is showed that the network of BC was well retained after impregnation of turmeric extract into BC.

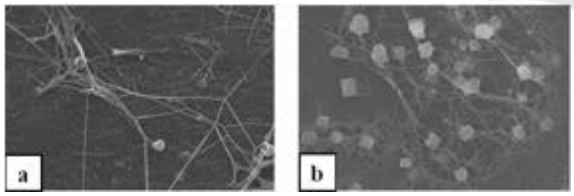

Figure 1: SEM image of (a) surface morphology of bacterial cellulose (b) bacterial cellulose/ turmeric extract nanocomposite

3.2 Antimicrobial Activity Studies

The antibacterial activity of freeze-dried bacterial cellulose/turmeric extract nanocomposite for E. coli and S. aureus was measured by the disc diffusion method. It was found that the freeze-dried bacterial cellulose/turmeric extract nanocomposite exhibit an inhibition zone. The growth inhibition ring of E. coli and S. aureus was 12,45 and 10 mm, respectively. The growth inhibition zone with the pure bacterial cellulose as control of E. coli and S. aureus was 6,5 mm and 0 mm, respectively. (see Fig. 2 a and b). This clearly demonstrates that the antimicrobial activity is only due to turmeric extract impregnated inside bacterial cellulose and not due to individual bacterial cellulose.

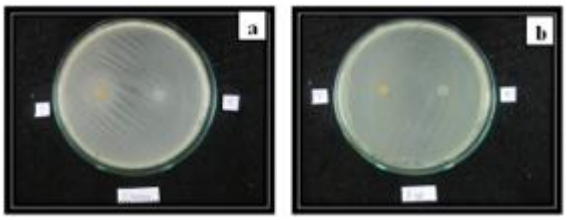

Figure 2: Antimicrobial activity against (a) Escherichia coli and (b) Staphylococcus aureus

Table 1: Antimicrobial activity

E.coli		*S. aureus*	
Impregnated BC	Pure BC	Impregnated BC	Pure BC
12.45 mm	6.5 mm	10 mm	0 mm

4 CONCLUSIONS

To summarize, we succeeded in the ex situ synthesis of bacterial cellulose/ turmeric extract nanocomposite. The preparative procedure is surprisingly simple. It can provide a facile approach toward manufacturing of nanocomposites, antimicrobial materials and other useful materials. The freeze-dried bacterial cellulose/turmeric extract nanocomposite exhibited a strong antimicrobial activity against both S. aureus (Gram-positive bacteria) and E. coli (Gram-negative bacteria), which are general bacteria that found on the contaminated wound. A recent study showed that impregnation, instead of coating the wound dressing with turmeric extract improved the antimicrobial activity of the wound dressing and lowered possibility of the normal human tissue damage. This is probably due to the slow and continual release of turmeric extract and then was slowly changed our physiological system and interact with bacterial cells, thus turmeric extract will not be so high enough to cause the normal human cells damage and can prolonged the antimicrobial effect.

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