

Enlarge Bacterial Cellulose Pore by Adding Aloe Vera Extract as Potential Material for Skin Tissue Engineering

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Abstract: Bacterial cellulose (BC) is a abundant natural biopolymer which due to its biocompatibility, it has a big potential to be used in biomedical material applications such as a scaffolding for skin tissue engineering. But, pristine BC still has some lacks desired properties, which limits its uses as a scaffolding or a template for growing fibroblast cell. Therefore its properties need to be boosted up to formed a better material that allow cellular penetration and several key requirement for tissue engineering. Here in this study a biocomposite was produce by loaded aloe vera extract in to medium in static culture during cellulose fiber biosynthesis using strain bacteria *Acetobacter xylinum*. BC/aloe biocomposite were characterized using FTIR and SEM analyses. Interaction between BC fibers and aloe vera extract was proved by peak in region 1643 cm⁻¹ which is implied the intermolecular interaction of BC and the amino groups of aloe vera gel in FTIR. It is also supported by SEM analysis that showed Aloe vera extract was covered the BC fiber. Additionally, due to its biocompatibility BC/aloe composite was potentially using as scaffold material for skin tissue engineering.

1 INTRODUCTION

Bacterial cellulose (BC) is an abundantly available biopolymer and has been shown to be biocompatible with living tissues. BC is also considered suitable for biomedical applications such as wound dressing, due to its mechanical characteristics and porous structure. The three dimension fibers structure of bacterial cellulose was identical can mimic the skin extracellular matrix when dried with a freeze dryer (Gea et al., 2018). Furthermore, it may also impregnated with other modifiers such drugs to modulate its properties through enhancing BC properties such as antimicrobial properties, which is low for pristine BC. Never dries and high hydrophilic properties of BC, also become the desired property to aplicate BC as wound dressing, because it has been shown that when the wound is continuously moisturised, wounds heal better and faster. This feature of BC means that it can act as a wound dressing and skin tissue scaffold successfully (Kucińska-Lipka, Carayon and Janik, 2015).

Unfortunately, these features alone do not allow BC to fulfill the require to be a good wound dressing or skin-tissue replacement, as wound dressing must also mimic the wound bed extracellular matrices (ECM), decrease scar formation and increase wound healing (Meng et al., 2019). BC also has some disadvantages to be incorporated as a scaffold, due to biodegradability and inadequate pore diameters. Two different approaches were used in BC-based composite production to obtain the desired properties as a scaffolding material: in situ (inside medium culture during BC synthesis) and ex situ (outside medium culture after BC gels harvested) modifications. BC properties, such as antimicrobial, biodegradability or antioxidant activity, reported can be improved by modifying or adding various compounds into the BC fibers (Keskin, Urkmez and Hames, 2017).

Biopolymer-based scaffold materials such as proteins, (gelatin, fibrin and collagen) and polysaccharides, (chitosan, hyaluronic acid, dextran,

alginate, cellulose and aloe vera) were used to improve BC properties mimic to ECM.

For regenerative medicine, composite based on BC can be used to restore and rebuild hard and soft tissues, such as vascular tissues, bone, skin and cartilage (Dutta, Patel and Lim, 2019). Aloe vera is a succulent plant, also known as *Aloe barbadensis* Miller that is commonly used for cosmetic, pharmaceutical and biomedical uses. Aloe vera gel known consists of acetylated glucomannan polysaccharides that form in a long chain, a complex of amino acids and other carbohydrates. It also contains ascorbic acid, 99 percent of water, salicylic acids, antioxidant (vitamin E and vitamin A) (Venugopal and Mary, 2014).

Because of its low toxicity, biocompatibility and biodegradability characteristic, aloe vera gained significant attention in tissue engineering. There have been significant recent advances in the development of aloe vera for tissue engineering applications. Aloe Vera has been revealed to possess many biologically active elements. Bioactive components in aloe vera have effective antibacterial, antioxidant, immunomodulatory and anti-inflammatory properties that supported growth cell and tissue regeneration (Rahman, Carter and Bhattarai, 2017). Gel inside aloe vera leaf as an abundant natural material is known suitable to be impregnated with other biomaterials in tissue engineering in aims to improve scaffold biological, porosity and biodegradability properties for support the cell growth of new tissue implanted in the human body (Tran, Hamid and Cheong, 2018). But, pure aloe vera is not suitable for becoming a material for scaffolding because it has not become a template for growth cells. In order to make it a scaffold material, it is important to combine aloe vera with other polymers such as cellulose. In this research, we concentrated on the in situ manufacturing of BC-Aloe composite and characterization of BC-Aloe potential as a skin tissue engineering scaffolding material.

2 METHOD

2.1 Production of Pristine BC and in situ BC-Aloe Composite

Acetobacter xylinum, the bacterial strain that used for the production of BC gels in this study was obtained from the Material and Polymer Postgraduate Laboratory of Universitas Sumatera Utara, Indonesia. Ekstrak aloe vera was purchased from PT. Bali Extract Utama. For in situ production,

pure Aloe Vera (1% w/v) was added to Hestrin & Schramm (HS) medium that contain 20 g/L glucose, 2.7 g/L disodium hydrogen phosphate, 5 g/L peptone, 1.15 g/L citric acid and 5 g/L yeast extract. Then starter of *Acetobacter xylinum* was inoculated in the HS medium at a concentration of 1 % v/v. It left for inoculation at 30 ° C for 14 days in static condition. After completion of the inoculation process, composite BC-Aloe gels were harvested from the medium surface. After that, composites were purified in a solution of 2.5 M NaOH and wash with distilled water until reach neutral pH. The same procedure was follow to produce pristine BC without adding aloe vera into the medium. Then pristine BC, BC-aloe dried using freeze dryer.

2.2 Characterization of Pristine BC and BC-Aloe Composite

Fourier transform infrared (FTIR): FT-IR spectroscopy of BC and BC-Aloe composite were characterized using a Fourier transform infrared spectrophotometer in the frequency range of 400 to 4,000 cm^{-1} (FTIR 8400S, Shimadzu, Tokyo, Japan). Scanning electron microscope (SEM): SEM EDX EVO 10 car MA Zeiss Bruker operated at 20 kV was used to analyse the surface morphology of Pristine BC and BC-Aloe composite.

3 RESULT AND DISCUSSION

3.1 FTIR Analysis

In order to analyse the emergence of any peak changes or new peaks or that could be due to interactions between aloe vera gel and cellulose Fourier transform infrared (FTIR) spectroscopy of the BC and BC-Aloe composite was performed. The FTIR spectra of all samples were displayed in Figure 1. The BC FTIR spectrum show that the intense absorption was in the region band at 1642.9 cm^{-1} , which has been assigned to carbonyl groups (Amaturrahim, S.A; Gea, 2018), while the band at 1635 cm^{-1} was observed as the characteristic band for the absorption of aloe vera. C-O stretching was assigned to the bands at 1543–1635 cm^{-1} which overlap with NH bending. In addition the absorption band of NH deformation was at 1565–1540 cm^{-1} . In Figure 1 the BC-Aloe presents a new peak of 1543 cm^{-1} in FTIR spectra. The new peak suggested there are intermolecular interaction among the cellulose chain of the BC and the amino groups in the aloe vera gel. This accepted that aloe vera gel have

intermolecular bonding with cellulose fibril as supported in SEM study (Saibuatong and Phisalaphong, 2010).

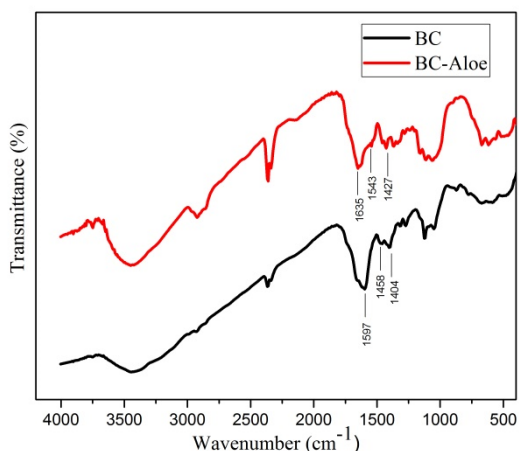


Figure 1: The FTIR Spectra of BC and BC-Aloe Composite.

3.2 Surface Morphology

Figure 2 shows the difference between (a) BC pristine film and (b) dried BC-Aloe composite film. From the picture it can be seen that the BC pristine film looks more transparent than the BC-Aloe composite film. This can occur due to the addition of aloe into the medium culture or the manufacture of composites in situ making aloe vera extract trapped in the bacterial cellulose fibers during the inoculation process. This is also supported by the SEM results shown in Figure 3.

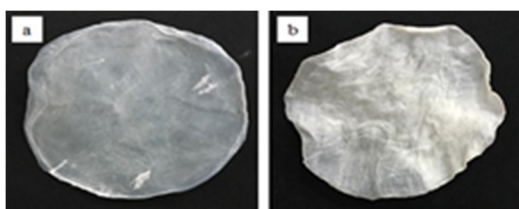


Figure 2: Photos of dried (a) Pristine BC and (b) BC-Aloe Composites.

SEM images in Figure 3 illustrate that there are differences in the surface morphology of Pristine BC and BC-Aloe composite at magnification 5000 X. The results of SEM analysis shows that in Pristine BC, BC fibers are still visible on the surface of the film but the addition of aloe vera into the culture medium during the process of inoculation and synthesis of BC by *Acetobacter xylinum* makes aloe vera trapped in BC fibers and covers the surface of BC. So that in Figure 3 (b) the BC fibers are no

longer visible. Through SEM image also seen. The entrapment of aloe vera extract in BC can make BC pores get bigger. This is in accordance with previous research which states the addition of aloe vera gel can disrupt the structure of BC and enlarge pores (Saibuatong and Phisalaphong, 2010).

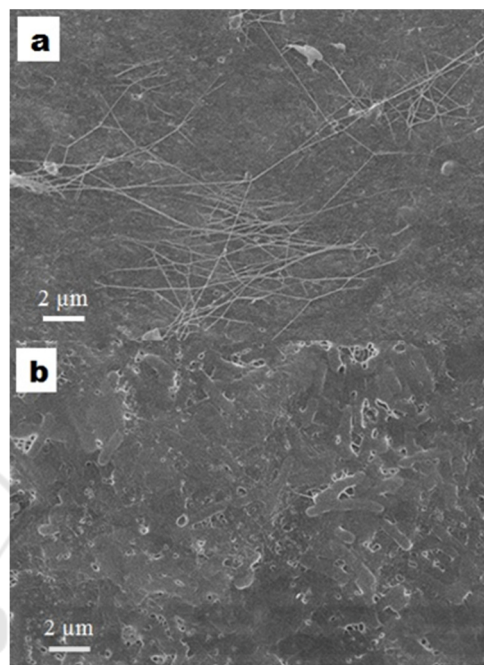


Figure 3: SEM images of surface morphology of (a) Pristine BC and (b) BC-Aloe.

4 CONCLUSIONS

Research shows that the use of *Acetobacter xylinum* strain bacteria to produce BC then modify the film by adding aloe vera gel to the culture medium during BC biosynthesis has improved and provided many beneficial effects for BC/Aloe composite. FTIR spectra of BC/Aloe shows that there are interaction between BC intermolecular with the amino groups of aloe vera extract. SEM image also showed that morphologically BC-Aloe composite was successfully produced. The BC-Aloe composite is expected to be used in a wide range of medical applications. Another supported characterization to proven BC-Aloe was potential material for tissue engineering is on progress

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