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Influence of Poly(lactic acid) Layer on the Physical and Antibacterial Properties of Dry Bacterial Cellulose Sheet for Potential Acute Wound Healing Materials

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Abstract: Dry bacterial cellulose nanofiber (BC) sheet coated with poly(lactic acid) (PLA) was developed and characterized towards acute wound healing applications. This new approach of PLA coating on BC revealed enhanced physical and antibacterial properties. Commercial BC sheets originated from the manufacturing of *nata de coco* jelly were dried and coated with the PLA at various concentrations of 2, 4, 6, 8, 10 and 12 % w/v for the purpose of improving the mechanical properties and followed by loading of antiseptic such as benzalkonium chloride (BAC). PLA has been proposed for the use of coating materials at a concentration of 8 %, the biocomposite sheet started exhibiting a low moisture uptake, prolonged swelling in simulated wound fluid solution and high tear (9.17 Nm^2/kg) and burst indices (32.5 $kPa \cdot m^2/g$). The 8 % PLA coating revealed porous fiber-like morphology as observed under scanning electron microscope. Therapeutic loading capacity of the BC/8 PLA was substantially higher than the pristine BC. Furthermore strong antimicrobial activities against *Staphylococcus aureaus* and *Escherichia coli* were observed for the BC/8PLA biocomposite film. These reports were clearly suggestive of the fact that synthetic biodegradable polymers, such as PLA, may be exploited for the synergistic combination with BC for antimicrobial and acute wound management. This new and modified fiber source material could reduce the dependency on plant based cellulose for more demanding biomedical applications such as wound healing materials, vascular graft, cartilage replacement, drug delivery and tissue engineering.

Keywords: Bacterial cellulose, Nanofiber, Poly(lactic acid), Biocomposite, Benzalkonium chloride

Introduction

Bacterial cellulose, otherwise also popularly referred to as BC, is a kind of unique non-plant cellulose that is being produced with the assistance of various kinds of bacteria such as *Acetobacter xylinum, Agrobacterium, Gluconacetobacter, Rhizobium, Achromobacter, Alcaligenes, Aerobacter, Azotobacter, Salmonella, Esherichia,* and *Sarcina* [1] under synthetic or non-synthetic culture medium through oxidative fermentation process. BC can be regarded superior compared to plant cellulose in terms of its biocompatibility, purity and crystallinity. It was shown that BC does not contain hemicellulose and lignin thus it purity is higher than that of plant based cellulose [2,3]. Moreover, the high degree of polymerization and 3D ultrafine network structure of BC contribute to its high mechanical strength [4]. In addition, the network structure of BC enables it to capture higher amount of water molecules, thus it has high water absorption capacity. It was also reported that bio-cellulose could be produced through a cell-free system [5,6].

In the food industry, the bacteria *Acetobacterxylinum* is known for producing white pellicle using fermentation process which is referred to as *nata de coco* using coconut water as the culture medium. *Nata de coco* is a popular traditional dessert in Southeast Asia particularly in the Philippines [7]. Though the primary end product of fermenting the bacteria is the *nata de coco* jelly, the uncooked BC can be utilized as various industrial products such as textiles, packaging and biomedical materials. Several potential usage of BC in biomedical applications are in bone tissue engineering [8,9], blood vessel and cartilage replacement [10,11] and wound dressing [12]. Wet form of BC membrane is being used for wound dressing due to its extensive ultrafine network that facilitate wound healing environment, improve the wound exudates absorption, reduce scarring and increase the re-epithelialization while at the same time reduce the healing times [13].

Though several commercialized BC based wound healing products are already in the market such as Bioprocess®, XCell® and Biofill®, they are designed for chronic wound healing; hence they are in their wet condition. Since the healing of acute traumas are known to happen with greater frequency in the regular life of a human being, the dry state of the BC sheet is more convenient for practical applications. Acute wounds are known to go through the ordinary stages of healing and showing the potential to be healed within a short span of 4 weeks (trauma or surgery), while chronic wounds are quite different in the sense such wounds do not get healed within the span of 4 weeks (ulcers of the lower extremities) [14]. Benzalkonium chloride (BAC) is a non-

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alcohol based antiseptic drug applied topically for wound healing. It can be classified as a quaternary ammonium compound having cationic amphiphilic features [15]. BACs have been used mainly for preventing topical wound infections and shows potential in treating musculoskeletal wounds [16] and have been used as preservatives for eye wash solutions [17]. It was shown previously that dry BC can be loaded with BAC and show reasonable release properties [18]. The main disadvantage of its dry form is that, once dried the BC ability to absorb or swell will be reduced and the moisture uptake of the BC from the environment becomes higher hence reducing the mechanical properties [19,20].

Hence it can be regarded to be an intuitive decision to enhance the BC sheets by availing the use of a biopolymer possessing sound and impressive mechanical strength apart from providing good porous surface morphology. Considering this, one of the most reliable thermoplastic biopolymer, that can be deemed suitable for this, is the PLA as this is a thermoplastic which consists of excellent mechanical strength, high biocompatibility, as well as high biodegradability characteristics. The production of PLA is being done eco-friendly and environmental friendly through fermentation process of sugars from renewable resources such as sugarcane and corn [21,22].

Earlier studies have shown that PLA coatings were able to impart desirable properties to the host substrate. PLA can be coated on biopulped pineapple leaf fiber showing porous coating morphology [23] that is suitable for packaging materials. Furthermore coating of PLA on medical metallic alloy and biaxially orientated poly ethylene terephthalate have shown increased in corrosion resistance and increased in roughness, respectively [24,25].

Previous reports showed that BC with addition of fillers such as clay [26], silver nanoparticle [27] and chitosan [28] possessed potential antibacterial activities and for wound healing. Properties of the reported BC composites varies with the type of fillers used. Enhancing the BC with a coating might be an alternative approach of effectively adding therapeutics. Since so far, the evidence of any kind of surface modified BC sheet was absolutely non-existent, we had thought and expected that the attributes of the dry BC sheet could be increased with the utilization of surface coating by using the PLA. Though the targeted applications of the BC/PLA sheets are in biomedicals, the outcome of this study can be applied to packaging, paper and textile products as well. Thus as it is evident here, the primary goals we worked with were to enhance the physical, uptake/release of BAC and antibacterial properties of the BC by coating with PLA for potential wound healing applications. Initially, the BC pellicle was fermented using *Acetobacter xylinum* with coconut water as the medium at a *nata de coco* manufacturing factory. Then it was dried and coated with PLA at various concentrations. Then the selected PLA formulation was loaded with BAC as the model antiseptic.

Experimental

Preparation of BC

The BC was supplied by Happy Alliance, Pvt. Ltd. (Malaysia), manufacturer of *nata de coco* jelly. Briefly, the *Acetobacter xylinum* was cultured in static condition in coconut water and citric acid at temperature of 27-30 °C for 5 days. The cultured BC sheets were being washed with distilled water for the purpose of removing medium component following which the same was also being boiled in 1 % NaOH, for two hours for of eliminating the attached cells as well as any kind of impurities. Average thickness of the obtained wet BC was 1 cm. The BC sheet is then oven dried at 38 °C for 24 hours to obtain dried forms.

Coating of PLA on BC

PLA granules (molecular weight between 195000 and 205000 g/mol) obtained from Nature Works LLC, USA were

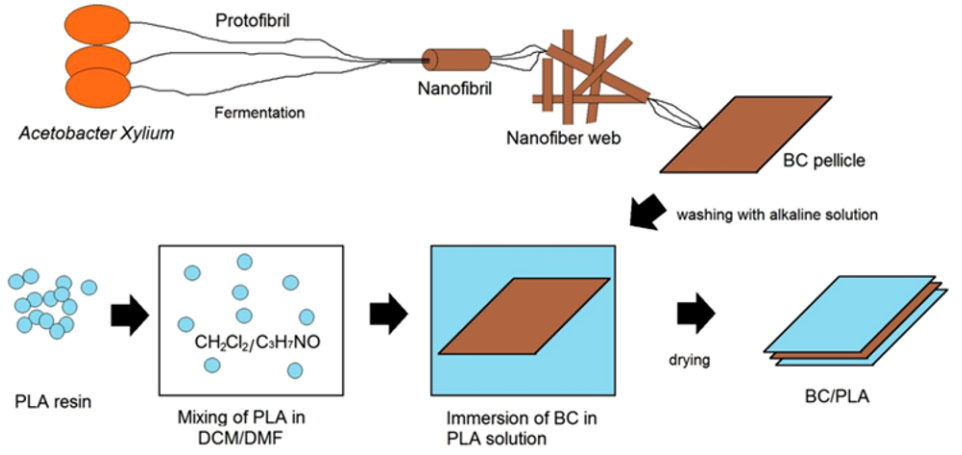

Figure 1. Schematic preparation of BC/PLA.

dissolved in a solvent which contained dichloromethane (Sigma Aldrich) and dimethyl formamide (Sigma Aldrich) in a ratio of 3:2 to produce different kinds of coating concentration such as (2 %, 4 %, 6 %, 8 %, 10 % and 12 % w/v). The PLA solutions were stirred at 500 rpm for 24 hours under room temperature. Next, the BC films were dipped in the solution for a time period of 5 minutes and being dried in a fume hood to speed up the drying process by allowing evaporation of the organic solvent under ambient condition. Finished BC/PLA films were weighted to in order to determine the amount of deposited PLA. The schematic process is depicted in Figure 1.

Fabrication of BC/PLA with BAC

A piece of 1 cm^2 of BC/PLA the dry sheet has been immersed in the solution of 25 m*l* of the benzalkonium chloride (BAC) solution (Sigma Aldrich) for the period of 24 hours. After this, the film was being gradually removed and was kept aside from the solution, and was finally immersed in distilled water briefly to remove excess free solution on the BC/PLA film. Next, filter paper was used for the purpose of removing any non-absorbed BAC and the film was subsequently freeze dried for 24 hours in order to obtain the biocomposite antimicrobial film of BC/PLA.

Characterizations

Tear Test

By employing the use of Elmendorf Tear method (American society for testing and materials, ASTM D-1922) [29], the tear index value can be obtained and equation (1) was used to calculate tear index, while equation (2) was needed to obtain average tearing force.

$$\text{Tear index} = \text{average tearing force } (N)/\text{average grammage } (kg/m^2) \quad (1)$$

$$\text{Average tearing force} = 16 \times 9.81 \times \text{average scale reading} \quad (2)$$

Bursting Test

According ASTM-D774 [30], Mullen type tester was performed for the bursting test and the burst index, which was calculated using equation (3). At a minimum, five replicates were being done to each sheet type in case of both of the mechanical tests.

$$\text{Burst index} = \text{bursting strength (kPa)}/\text{average grammage } (g/m^2) \quad (3)$$

Morphology

Scanning electron microscope (SEM) (Leo Supra 50VP Field Emission SEM, Carl Zeiss, Germany) was used to observe the sheet morphology. The dried BC coated PLA film was then cut into 1 cm^2 and placed on a sample grid. Then, the sample was sputtered coated with gold and examined.

Fourier Transform Infrared (FTIR)

The Perkin Elmer Spectrum System 2000 FTIR was used to characterize the functional structure of the BC coated PLA film by using wave range from 500-4000 cm^{-1}. A resolution of 4 cm^{-1} and averaging of 16 scans was employed.

Water Wetting Time

Then, the water wetting test was conducted on the BC/PLA samples in the size of 1.5 cm^2. One drop of distilled water was allowed to fall to the samples by using burette and the height between the burette and samples was controlled which is about 1 cm from the surface of samples. Then, the time taken to spread the droplet to 1 cm distance was being recorded with a digital stopwatch.

Water Contact Angle

Water contact angle images and determination were recorded using VCA optima, AST Products (USA). Five replications were conducted.

Moisture Absorption

The moisture absorption evaluation was employed using 3 replicates sheet samples of 2×2 cm. According to ASTM E-104 standards [31], a controlled environment with 53 % relative humidity (RH) was prepared and the samples were kept on the top of wired mesh and enclosed in controlled environment inside desiccators. The 53 % RH environment was prepared from saturated salt solution of magnesium nitrate (Sigma Aldrich). Following this, the weights of the samples were being recorded at the regular interval of 6 hours followed by 24, 48 and 72 hours intervals. The moisture absorption in percentage was calculated using equation (4),

$$M_t\,(\%) = (W_w - W_d)/W_d \times 100 \quad (4)$$

where M_t is the moisture absorption (%) of the sample, and W_w and W_d are the weights of the sample before and after its exposure in the controlled RH, respectively.

Degree of Swelling

As for the swelling studies, initial mass of prepared sheet were weighted. Samples were then immersed in 30 m*l* of simulated wound fluid (SWF) solution in order to observe the swelling behavior in solution that mimics wound environment. Mass of the samples was recorded at predetermined time interval. Swollen samples were freed of surface water using filter paper to remove excess solution and then weighed. The swelling (%) was calculated using equation (5),

$$\text{Swelling } (\%) = (W_f - W_i)/W_i \times 100 \quad (5)$$

in which W_f represent the weight of swollen sample at predetermined time and W_i is the initial weight of the film. The SWF solution was prepared according to Varaprasad *et al*. [32]. Briefly the solution simulated to the wound fluid consist of 0.68 g of NaCl, 0.22 g of KCl, 2.5 g of $NaHCO_3$ and 0.35 g of NaH_2PO_4 in 100 m*l* of distilled water at pH of 7.4.

Drug Loading and Release

As for the therapeutic loading capacity, the weight of the dry film was measured before immersing 25 m*l* of benzalkonium

chloride (BAC) solution for a period of 24 hours. After that, the sheet was being finally taken out from the solution and wiped with the use of filter paper for removing excess non-absorbed BAC. Next, the weight of the soaked film was measured. The drug loading capacity of BC/PLA film was calculated using equation (6),

$$\text{Drug upload capacity (mg/cm}^2\text{)} = (W_F - W_I)/\text{Area of dry film} \quad (6)$$

where W_F and W_I are the weights of the sample before and after being immersed in BAC solution, respectively.

In addition, the release behavior of the antimicrobial film was investigated. A piece of 1 cm^2 of BC/PLA dry film was placed in a beaker that is being sealed which contained 25 m*l* of deionised water. Following that the beaker was placed in incubator for 24 hours and the temperature was set to 37 °C. Then, the antimicrobial film was taken out from the system for every 2 hours and analyzed using ultraviolet-visible (UV-vis) spectroscopy. The BAC concentration was determined spectrophotometrically by measuring the absorbance value at 263 nm.

Antibacterial Test

The antibacterial properties of the BC/PLA dry film against *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) were investigated. Luria-Bertani medium solid agar Petri dish was utilized and the BC/PLA films were prepared by immersing in different concentration of BAC solution (0.06 %, 0.08 %, 0.10 %, 0.12 % and 0.14 %). Then, the dry antimicrobial BC/PLA dry films were sterilized by autoclaving the same at temperature of 121 °C for 20 minutes and was subsequently placed on *E. coli* and *S. aureus* cultured agar plates, respectively. The agar plates were incubated at 37 °C for 24 hours. By measuring the difference of the semi-diameter between the inhibition zone and the film, the antimicrobial activity of the films were determined.

Results and Discussion

Mechanical Properties

Tear and burst indices for neat BC film and prepared BC/PLA films are shown in Table 1. Tear index is being defined as the method of measuring the capability of sheet for withstanding tearing force while the burst index is used for the purpose of representing the degree of pressure the sheet can tolerate before rupture [33]. The result shows that the control neat BC film exhibit tear index value of approximately about 2.97 Nm^2/kg while the coating of the BC with 2 % of PLA solution showed significant enhancement to its tear index. Though the amount is minimal, the PLA component is able to coat the surface of BC forming a homogenous layer covering the nanocellulose surface. Increasing the concentration of PLA up to 8 % lead to maximum tear index of 9.17 Nm^2/kg. The biopolymer coating acts as mechanical barrier that strengthened the sheet by inhibiting the tear propagation [23]. Upon a higher coating concentration of 10 % (BC/10PLA), the rise of the tear index came to a halt because excessive PLA coating created weak spots during the mechanical testing that lead to surface detachment along with the weight of the PLA that relates to the specific weight relation of the testing [34].

Table 1. Tear and Burst Indices of BC and BC/PLA

Sample	Tear index (Nm^2/kg)	Burst index ($kPa{\cdot}m^2/g$)
BC	2.97±0.3	7.82±0.3
BC/2PLA	4.43±0.4	8.60±0.9
BC/4PLA	6.25±0.6	11.2±0.8
BC/6PLA	5.56±0.2	17.4±0.4
BC/8PLA	9.17±0.8	32.5±0.8
BC/10PLA	7.31±0.8	31.0±0.6
BC/12PLA	5.86±0.4	17.3±0.8

The burst index of the modified BC revealed similar improvements with the coating of PLA up to 8 %. The PLA layer had been utilized as a load bearing element, alongside with which it had also acted as a sufficient perpendicular pressure resistance of the burst test. Though reduction in tear and burst indexes were observed for 10 and 12 % PLA coated BC samples, the values of all the coated sheets were higher than that of the neat BC. This means that the mechanical interaction between BC and PLA is adequate enough to provide mechanical support. Both mechanical tests proved that by using an optimum concentration of the PLA solution (8 %), coated BC sheets with boasted mechanical properties could be accomplished. Furthermore we found out that 12 % PLA coating on the BC led to the creation of various kinds of difficulty in the sheet drying process due to the uneven PLA solution.

Morphological Analysis

Figure 2(a) is being used for the purpose of depicting SEM image of the control dry BC film. The sample is found to exhibit the distinguished surface features of cellulose nanofibers produced by bacterial fermentation process. A very uniform surface coating made up of PLA can be traced in the BC/4PLA sample, as it is shown in Figure 2(b). A close observation on the image revealed internal dimple-like patterns, with average internal diameters of 1.5 μm. This formation is a result of solvent evaporation [35]. Similar dimple formation was observed by coating of PLA on pineapple leaf fiber [23]. Electron image of sample BC/8PLA (Figure 2(c)) revealed that the PLA coating has transformed into more fibrous and porous morphology. Its corresponding cross sectional view (Figure 2(e)) indicated that the PLA is well attached to the BC with average PLA thickness of 43 microns. The existence of this porous PLA layer might open up potential applications such as wound dressings and external drug delivery materials. These micron

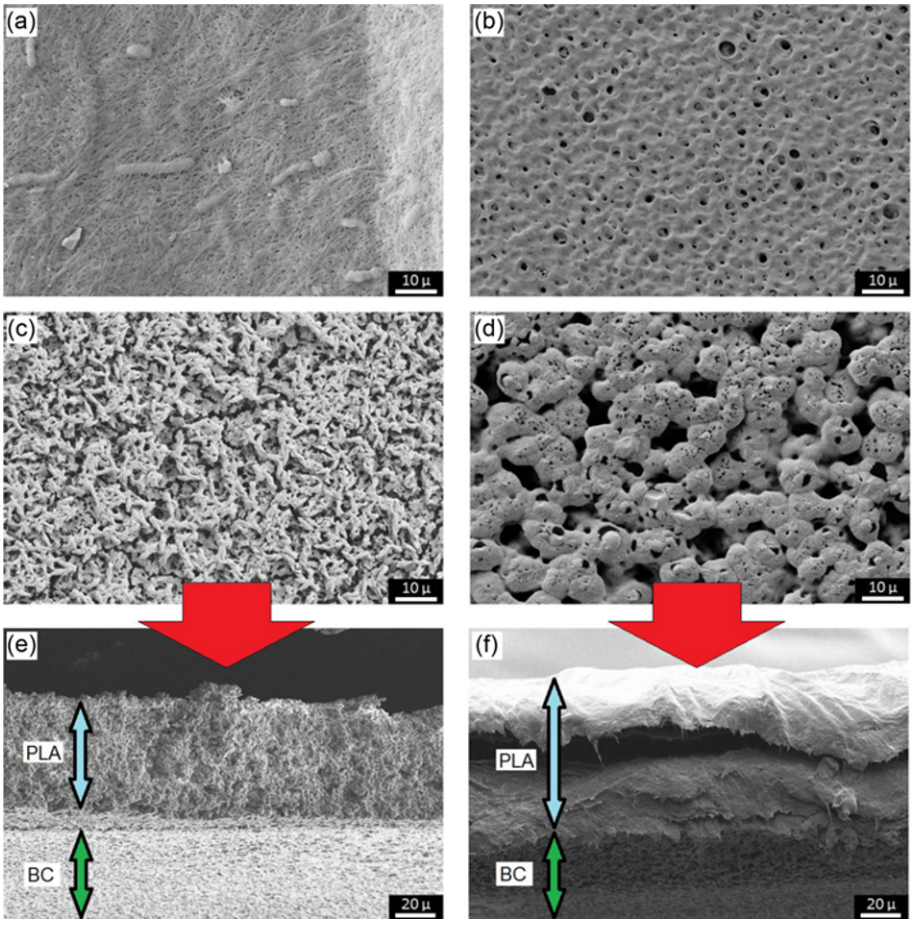

Figure 2. SEM images of (a) control BC, (b) BC/4PLA, (c) BC/8PLA, (d) BC/12PLA, and (e)-(f) cross sectional view of BC/8PLA and BC/12PLA.

sized pores provide suitable sites for the loading of antibacterial or therapeutics for biomedical purposes. Samples of BC/12PLA (Figure 2(d)) meanwhile revealed large and interconnected PLA beads. The cross section of the sample indicated that the PLA is detached from the BC sheet. High amount of PLA concentration lead to ineffective formation of PLA on the BC surface. This poor attachment of PLA is in agreement with the result obtained from the mechanical testing.

FTIR Spectroscopy

The structural features of BC and BC/PLA were evaluated by FT-IR analysis and are shown in Figure 3. The BC is in fact found exhibiting the intermolecular as well as the intramolecular H bonds of free OH in the cellulose at 3255 cm^{-1}, as well as the C-H stretching peak at 2919 cm^{-1}. This represents the typical spectrum of high purity cellulose [36]. Lower detection of the OH band for the BC/8PLA sample compared to neat BC as a result of the PLA coating limited the exposure of the cellulose surface. Noticeably new PLA related peaks emerged at 1720 and 1449 cm^{-1} which

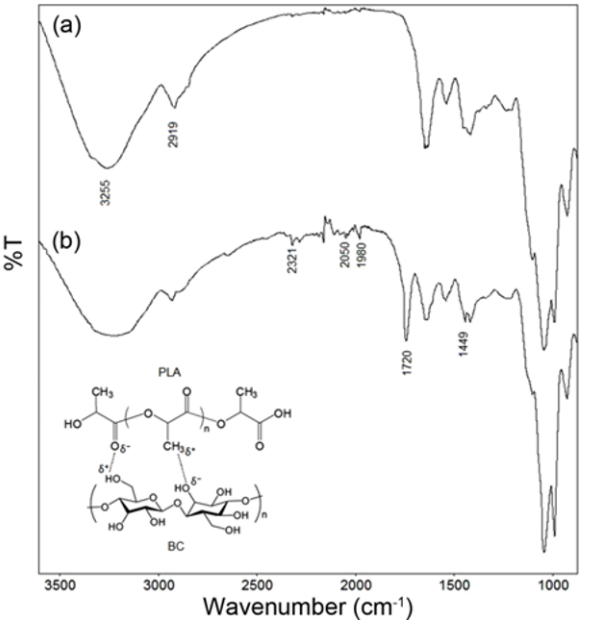

Figure 3. FTIR spectra of (a) control BC and (b) BC/8PLA.

corresponds to acetyl groups and CH_3 band of PLA, respectively [34] and this features indicated that the BC is being domineered by none other than the functional groups of the PLA. The proposed BC-PLA interactions are illustrated in the insert of Figure 3.

Water Wetting Time and Contact Angle

The results of the above mentioned water-wetting test of each of the samples after 1 s are shown in Figure 4(a) and (b). From the observation, the water droplet wetted the neat BC films instantaneously while the BC/8PLA sample delayed the wetting time up to 27 s. This was an indication that the PLA coating had been inducing effective water barrier for the BC surface or in other words the very existence of the hydrophobic PLA had succeeded in reducing the surface tension of the BC by providing it a high mismatch of surface tension between the water, thereby making it almost impossible for the water molecules to moisten the particular BC surface. This point is anything but suggestive of the fact that the water resistance of the BC can be improved by using PLA coating. The mean water contact angle of BC and BC/8PLA was at 35.40±1.1 ° and 51.78±1.72 °, respectively. Their corresponding water contact angle test images are shown in Figure 4(c) and (d).

Moisture Absorption and Degree of Swelling

Figure 5 presents the moisture uptake of the control BC as well as the BC/8PLA sheets. The PLA coated BC showed marginal reduction of moisture uptake up to 6 hours. However it can be seen that the extent of moisture uptake of the BC/8PLA at 24 hour interval is far less than that of control BC.

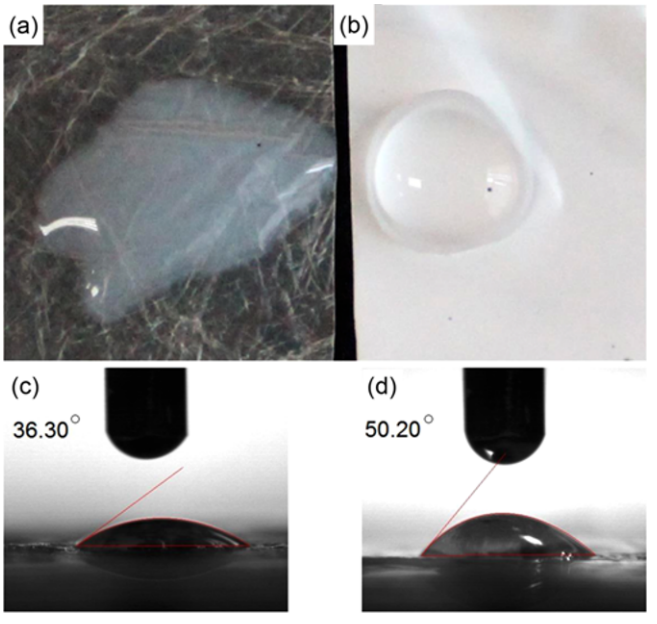

Figure 4. Water wetting test and its corresponding water contact angle of (a,c) control BC and (b,d) BC/8PLA.

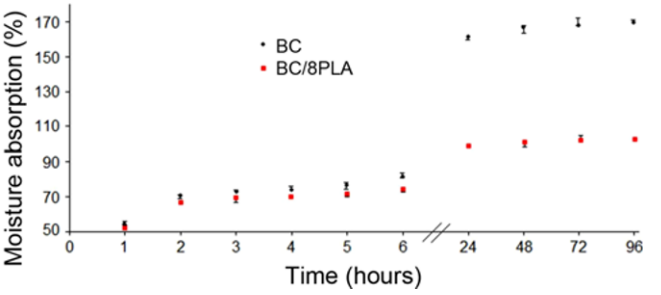

Figure 5. Moisture absorption of BC and BC/8PLA.

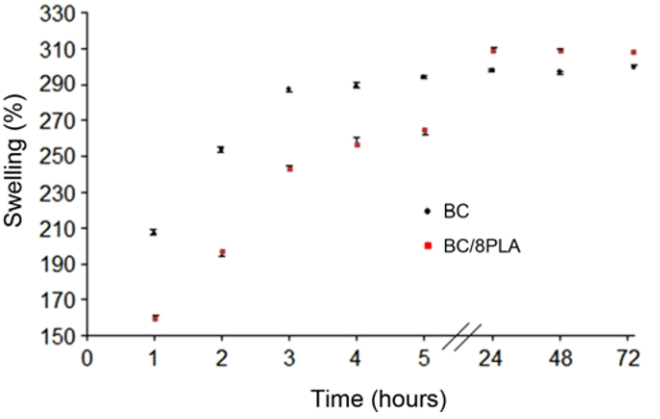

Figure 6. Swelling of BC and BC/8PLA in simulated wound fluid.

It can be explained that the hydrophobic features of the PLA layer coating on the surfaces of BC leads to the reduction mentioned above. Utilization of a biopolymer coating on the BC reduced the degree of moisture uptake, which had led to the preservation of the structural, mechanical as well as the functional attributes of the nanocellulose sheets.

The BC and BC/8PLA samples were evaluated for their swelling properties in a simulated wound fluid environment (SWF) (Figure 6). The BC sheets swelled up to 290 % at 3 hours and become stable up to 72 hours. The degree of swelling for the BC/8PLA was lower than the BC sheet due to the restriction of intake of the SWF ions into the BC on the surface. Interestingly, immersion of the sample from 24 to 72 hours showed that the degree of swelling is higher than the control BC. The porous PLA layers provide additional space for the SWF molecules to occupy. Moisture absorption and swelling profile of the BC/8PLA indicated that the modified sheet is suitable for applications that requires low surrounding moisture uptake and at the same time able to absorb wound fluid or water up to 72 hours.

Drug Loading Capacity

The drug loading capacity of neat BC and BC/8PLA under different concentration of BAC are shown in Figure 7. As the amount of BAC increased, the drug carrying ability of the BC as well as the biocomposite film increased. The drug

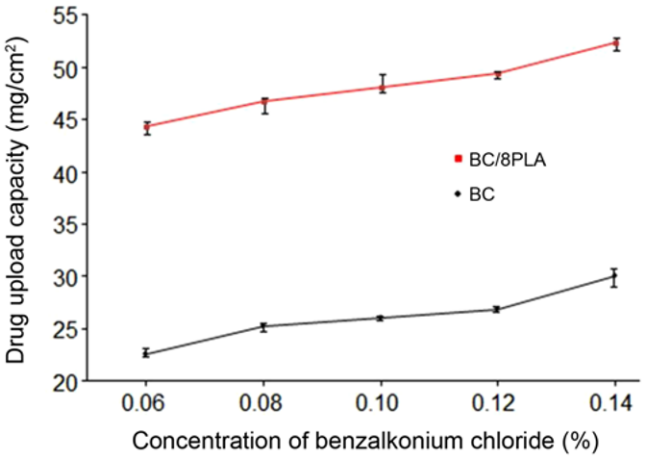

Figure 7. Drug loading capacity of BC and BC/8 PLA under different concentrations of BAC.

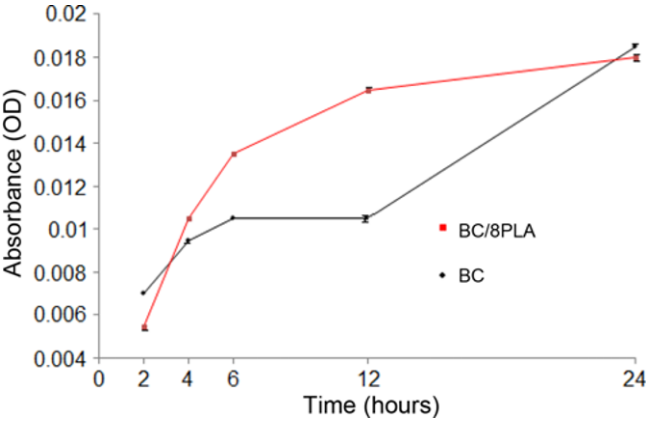

Figure 8. Benzalkonium chloride release from the BC/8PLA samples after loaded with 0.10 % drug solution.

loading capacity of BC and BC/8 PLA rose linearly when the feed concentration of BAC increased. Interestingly, the loading capacity of BC/8PLA was almost double compared to that of the neat BC. An earlier study showed that the loading capacity of pristine BC was only 0.145 mg/cm^2 with drug concentration of 0.128 % [18]. This encouragingly showed that coating of PLA on BC film enhanced the drug loading ability and could be more useful for antimicrobial loading. It was shown earlier that drug loading capacity varied based on the porous structure of the loading material [37]. This loading behavior can be corroborated with SEM image of Figure 2(c). More porous morphology was achieved with the addition of PLA layer and thus, enhancement in the drug loading capacity of the biocomposite film.

Release Behaviour

The main purpose of wound dressing is to protect wound from exogenous microorganisms, able to absorb exudate from the wound site as well as prevent scarring [38]. Thus, ideal wound dressing materials should able to preserve wound from infections and also able to enhance the wound healing process [39]. In addition, traditional inert wound dressing materials are not enough for the wound healing purpose because it does not help protecting wound from infection and thus requires extended wound healing period. BC itself does not contain any antimicrobial properties. Therefore bioactive dressings with antimicrobial properties such as BAC are needed [40]. A stable as well as prolonged release of bioactive agents is preferred for any wound healing applications. Release patterns of the drug molecules are depicted in Figure 8. It can be seen that a slight burst release occurred for the uncoated BC film at 2 hours. This was followed by quiet a stagnant release from 6 to 12 hours. The BC/8PLA sample however showed significant BAC release compared to its unmodified BC counterpart particularly during the 12 hour period. Moreover the slight bursting at the beginning (2 hours) was minimized. The PLA layers may function as a secondary component that facilitates the release of moieties to the surroundings. It can be said that drug efficiency of the dry BC film can be improved by modification with PLA, demonstrated by reducing the burst release at the early stage and with no fluctuation in therapeutic agent level [41].

Antibacterial Activity through Disc Diffusion Method

Disc diffusion test aims to check the antibiotic sensitivity of bacteria. If the antibiotic is able to prevent the bacteria growth or kill the bacteria, an area around the sample will be appeared and this is called inhibition zone. However, the inhibition zone diameter depends on the effectiveness of the antibiotic towards the bacteria itself [42]. Antimicrobial activities of BAC loaded BC/8PLA dry films against *S. aureaus* and *E. coli* with varying drug concentrations are shown in Figure 9. In addition, insert of Figure 9 shows the disc diffusion evaluation. BC samples without any BAC did not reveal any inhibition zone. As it can be seen, the antimicrobial properties against both bacterial enhanced as the concentration of BAC increased. However, antimicrobial activity of the drug loaded BC/8PLA film against *E. coli* reached saturation at 0.10 % concentration, higher concentration of the antibacterial solution did not display any further expansion of the inhibition zone. On the other hand, the inhibition zone of the loaded BC/8PLA towards *S. aureus* increased with the incorporation of the drug solution up to 0.14 %. It was reported earlier that the BAC loaded BC film displayed inhibition zone of around 1cm at loading of 0.077 % and becomes stable with higher concentrations [18]. In this report however, antimicrobial activity of the biocomposite BC/8PLA film increased with more incorporation of BAC. This enhancement is contributed by the presence of PLA layer on the BC surface that provides additional loading capacity and continuous release as discussed earlier. The difference in antimicrobial activity of the BC/8PLA

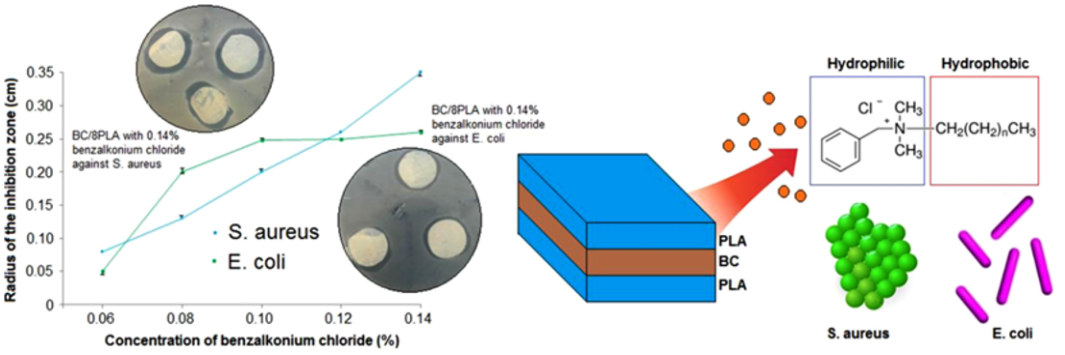

Figure 9. Antimicrobial activity of BC/8PLA films against *S. aureus* and *E. coli* at various BAC concentrations.

towards *S. aureaus* and *E. coli* are mainly due to the drug-bacteria surface relationship. In this case, the efficiency of BAC against gram positive bacteria (*S. aureaus*) is better compared to that of gram negative bacteria (*E. coli*) because of the quaternary ammonium compounds of BAC are positively charged derivatives of ammonium compounds (NR^{4+}) and consists of hydrophobic and hydrophilic regions that favourable to attack bacterial with less effective permeability properties such as gram positive bacteria [43]. The hydrophilic cationic region of BAC forms electrostatic interactions with the slightly negatively charged of pathogen's surface (peptidoglycan and techoic acid), then its hydrophobic part penetrates the hydrophobic bilayer membrane of the bacteria causing cell leakage and lysis [44]. The rupture of pathogen membrane affects the essential cell process such as adenosine triphosphate synthesis as well as solute uptake [45].

Conclusion

8 % of PLA concentration proved to be suitable for preparing BC/PLA sheets with antimicrobial properties. This was proven by the samples' high mechanical properties, good BC-PLA interaction, reasonable wetting time, preferable surface morphology on a microscopic level, low moisture uptake and prolonged swelling behavior in simulated environment. Antimicrobial BC/PLA dry film was successfully fabricated through a simple technique. The drug loading capacity of the BC dry film was found to have been enhanced by coating with PLA. The antimicrobial molecules were found to be released steadily from the dry film and it shows that the film has strong antibacterial properties against *Staphylococcus aureus* and *Escherichia coli*. It has been shown that the BC/PLA has the potential to be used as medical wound management sheet when it is coated with PLA. The primary benefit of the newly formed BC/PLA sheet is that the nanofiber source can be collected by fermentation of bacteria during the production of *nata de coco*. The ease of forming, as well as their economic value, presented led to the creation of the potential of these sheets to be produced on a large-scale as a secondary product of *nata de coco*.

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