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Development and evaluation of ciprofloxacin-bacterial cellulose composites produced through in situ incorporation method

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ABSTRACT

This paper describes the interaction and properties of bacterial cellulose (BC)–ciprofloxacin composites synthesized by in situ incorporation method. Ciprofloxacin's susceptibility to BC's producer, *Acetobacter xylinum 0416*, was first tested to determine its inhibitory activity against the bacteria. In situ incorporation method was performed by introducing 0.2% (w/v) ciprofloxacin into Hestrin–Schramm medium at the onset of exponential phase of *A. xylinum 0416* growth. Following a 10-day incubation at 28 °C, BC–ciprofloxacin composites were harvested and further characterised, while another BC–ciprofloxacin composite was harvested and purified prior to characterisation. The interaction between ciprofloxacin and BC was proven by the presence of quinolines and fluorine groups of ciprofloxacin on unpurified BC–ciprofloxacin composite and the reduction of crystallinity index as compared to the native BC. Moreover, deposited ciprofloxacin crystals on BC film and its composition were exhibited via SEM-Energy-dispersive X-ray analysis. Unpurified BC–ciprofloxacin film was determined to have strongly inhibited the following selected diabetic foot ulcer bacteria: *E. coli, K. pneumoniae* and *P. aeruginosa*. BC has the potential to be used as a wound dressing and a carrier for ciprofloxacin.

1. Introduction

Bacterial cellulose (BC) is defined as a nanoporous network polymer produced extracellularly by a number of bacteria including *Acetobacter, Pseudomonas, Azotobacter, Rhizobium, Xanthococcus, Aerobacter* and *Alcaligenes. Komagataeibacter xylinus*, also known as *Acetobacter xylinus* (or previously known as *Gluconacetobacter xylinum*), is the most frequently used bacteria for BC synthesis due to its strong ability to produce BC [10]. When compared to plant cellulose, BC is confirmed to be pure cellulose due to the absence of lignin and hemicellulose. BC possesses exceptional mechanical qualities, including flexibility, high crystallinity and elasticity, high water-holding capacity, a high specific surface area and degree of polymerization, and a low density. Additionally, it is known to have an exceptionally high permeability and porosity [10, 11].

While there are a variety of alternative wound dressing materials available, cotton wound dressings are the most frequently used when compared to gauzes, foams, gels, films, hydrocolloids, and hydrogels. However, cotton dressings dry the wound due to the fact that they are placed directly to the wound surface, making the removal procedure painful [24]. We must now admit that wound dressing is no longer a passive material. The wound dressing material must be capable of interacting effectively with the wound and facilitating the healing process. Moreover, an excellent wound dressing material should give protection against bacterial infection, maintain a moist environment, provide thermal insulation, promote enough oxygen circulation and allow for adequate liquid drainage and cell migration. Besides these requirements, wound dressings should be simple to apply and remove and free of allergens [24]. BC has attracted much interest as a novel form of wound dressing material [25, 41], and is thus considered the best

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candidate to meet the majority of the requirements. Interestingly, pure BC can be modified to meet all of the functional requirements for use as a wound dressing. BC's unique properties as a wound healing material are dictated by its high tensile strength, excellent flexibility, high water holding capacity, significant gas and liquid permeability, and high compatibility with living tissues. In terms of biocompatibility, BC is non-cytotoxic since no toxic substances are used in the manufacturing process, and no adverse reactions occur when BC comes into contact with the human body, either short or long-term, due to its histocompatibility and blood compatibility [10, 11, 41].

Nonetheless, BC lacks antibacterial capabilities and just acts as a physical barrier, preventing pathogens from reaching the wound site [35]. As a result, its efficacy as a therapy for extensively infected injuries will be limited. Therefore, additional modifications are needed to add antimicrobial activity to BC-based wound dressings. BC has demonstrated great promise as a cutting-edge wound dressing and drug delivery system. The high porosity and surface area of BC allow the introduction and release of antimicrobial agents, drugs and other biological functional materials [5] or in other words, BC can be used as a vehicle for drug delivery. When combined with BC, vaccarin, chitosan, zinc oxide nanocomposite, silver sulfadiazine, polyhexamethylene biguanide (PHMB), and propolis have all been demonstrated to be effective antimicrobial agents [3, 9, 15, 17, 23, 26, 37]. By grafting amoxicillin onto regenerated BC, Ye et al. [40] created a new biocompatible sponge with excellent antibacterial properties (RBC). The grafted RBC significantly enhanced the sponge's antibacterial activity against fungi, Gram-negative and Gram-positive bacteria, indicating that this sponge has a great deal of potential for clinical wound dressing applications. The use of drug-loaded BC in dental treatments such as teeth extraction and mucosal transplantation has also been studied in more detail [38]. Gupta et al. [12] recently described the characterization of curcumin silver nanoparticles loaded into bacterial cellulose hydrogels for wound management applications. Apart from their high cytocompatibility, these dressings exhibited antimicrobial activity against three common wound pathogens: *Staphylococcus aureus, Pseudomonas aeruginosa*, and *Candida auris*. When compared to cotton gauze, there is strong evidence that BC which has been loaded with antimicrobial agents such as povidone-iodine, polyhexamethylene biguanide and octenidine, exhibits a 1.5 fold increase in antimicrobial activity against both Gram-positive and Gram-negative bacteria [4].

BC wound dressing clearly reduced the time to cure or injury closure over standard care when applied to non-healing lower extremity ulcers [20]. Moreover, BC showed no allergic reaction or irritation to the wound site area after it was applied on the skin [36]. A number of BC-based wound dressings have been commercialised under the trademarks of NanodermTM, Bionext, Membracell, Suprasorb, Biofill, Gengiflex and Xcell. Other products, such as EXFILL, DERMAFILL, and CUTICELL EPIGRAFT, Biocel, Bionext®, and Membracel®, have also emerged, demonstrating the relevance of BC application in the medical field, as well as the potential for further development [21]. These success stories ascertained that BC could be used as a wound healing material.

In general, there are two methods for integrating antimicrobial properties into a BC material namely as in situ and ex situ methods. In situ method works by altering the BC formation during the fermentation process. The culture conditions may vary, and additives, reinforcement materials, or a carbon source may be added to the culture, thus changing the chemical composition of the BC composites. The additive materials are incorporated into the growing BC fibril networks, resulting in BC composites with both desired properties. In contrast, the purified BC pellicle is impregnated with various materials or compounds via an ex situ modification process to physically or chemically modify the BC sheet [33]. Despite of the fact that the ex situ method is less complicated than the in situ method, its primary disadvantage is its reversible nature, which is dependant on not only the nanomaterial's interaction with the BC matrix, but also on the physicochemical environmental conditions [31]. Meanwhile, the in situ method has been widely used as a scaffold material in the field of bone tissue engineering. The in situ method used in bone tissue engineering is slightly different than the in situ method used in wound healing. For instance, the BC is used as 3D structure scaffolds for cartilage cells and support cell adhesion and proliferation in tissue engineering application. The in situ strategy entails the incorporation of materials into BC culture media during the initial stages of the BC cultivation process. These materials then become integrated into the BC scaffold structure. As a result, a BC scaffold with uniform porosity will be produced. Following purification of the BC scaffold, it is seeded with the intended human cells in the presence of growth medium. On the contrary, in situ strategy for wound dressing application is simpler compared to tissue engineering. The antibiotic material is incorporated into the growing BC fibril network for a certain period of time until the complete composite is obtained, harvested and used as wound dressing. The limitation of the in situ modification approach is the incorporation of antibiotic materials that are also antibacterial against BC strains, as well as the insoluble nature of various materials in culture media [11].

Ciprofloxacin is the fifth largest generic antibiotic produced in the world [7]. It belongs to the fluoroquinolone family, a wide class of antibiotics with broad antibacterial spectrum which commonly used in many infections. Moreover, ciprofloxacin has been reported as an excellent antibiotic for wound dressing [34] and possesses strong activity against gram-negative bacteria [22]. Fluoroquinolones interfere with bacterial DNA gyrase, resulting in the degradation of chromosomal DNA and interference with cell division and gene expression [13].

A uniform Carbopol polymeric composite ciprofloxacin was successfully produced through mixing and homogenization in water with a drug/polymer ratio of 1:5 (w/w) (Sahoo et al. [29]. Meanwhile, another study used ultrasonication to create mucoadhesive suspensions with a variety of formulations, utilising the carbopol 934, carbopol 940, and hydroxypropyl methylcellulose polymers as carriers (Subhashree [28]). Recently, an ex situ method was used to produce a chitosan–BC patch of ciprofloxacin for wound dressing [7]. There is currently no report on BC–ciprofloxacin produced through in situ method. Thus, the purpose of this study was to determine the effect of ciprofloxacin on the growth of the BC producer strain *A.xylinum 0416* and to further investigate the interaction of ciprofloxacin and BC, as well as the properties of the BC–ciprofloxacin composites.

2. Materials and methods

2.1. Preparation of inoculum

Acetobacter xylinum 0416 used in this study was purchased from Malaysian Agricultural Research and Development Institute, Serdang, Malaysia. Hestrin–Schramm (HS) medium was prepared according to Faisul Aris et al. [9], with the following composition (w/v): 4% glucose, 0.5% granulated yeast extract, 0.5% peptone, 0.27% Na_2HPO_4 and 0.115% citric acid in 100 mL of distilled water. The pH of the medium was adjusted to 6.4 by 0.1 M HCl or 0.1 M NaOH prior to sterilisation at 121 °C for 15 min. 10% (v/v) of *A. xylinum 0416* seed culture was aseptically transferred into the sterile medium. The fermentation process was carried out at room temperature (±28 °C) for three days.

2.2. Determination of ciprofloxacin inhibition against A. xylinum 0416

A. xylinum 0416 cell suspension was prepared by adjusting its turbidity to match that of the 0.5 McFarland standard, which contained approximately 1×10^8 cfu/mL. HS medium agar was prepared similar to the previous section with additional of 4% (w/v) agar.

2.3. Determination of growth profile for A. xylinum 0416

HS medium was prepared based on the same protocols in inoculum preparation procedures. 10% (v/v) of the inoculum was transferred

aseptically to sterile HS medium. The static fermentation process was carried out at room temperature (±28 °C) for 15 days. The sampling was done at 24 h intervals, and the cell growth was determined based on the total protein content of the cells. The total protein assay has been determined to be a reliable and fast method for *A. xylinum* growth determination as our previous study has shown that average total cellular protein content of *A. xylinum* was 27.1% ± 6.8% by weight percent [1]. Based on this correlation, the growth of *A. xylinum* was estimated. Extraction of protein from *A. xylinum* cells was carried out through alkaline cell lyses prior to quantification of the total protein content of the cells as described by Lowry et al. [18]. The standard calibration curve was then developed based on bovine serum albumin at a concentration ranging from 1 to 1000 μg/mL. Spectrophotometric measurements were done using a microtiter plate reader at 750 nm wavelength. The glucose concentration in HS medium during fermentation process was monitored and measured using a glucose analyser. The BC yield was measured based on dry weight of BC obtained over glucose consumed.

2.4. Preparation of ciprofloxacin suspension

Ciprofloxacin was purchased from Solarbio, China. Ciprofloxacin was mixed with a predetermined volume of normal saline solution and then sonicated for 30 min at a frequency of 20 kHz, 40% amplitude, using the Sonic Vibra-Cell VCX 750 sonicator.

2.5. Production of BC–ciprofloxacin film via in situ modification method

HS medium was prepared as described in inoculum preparation procedures. 10% (v/v) of the inoculum was transferred aseptically to sterile HS medium. The static fermentation process was carried out at room temperature (±28 °C) for 10 days. A specific volume of ciprofloxacin suspension was added to the medium on the second day of fermentation to achieve a final concentration of 0.2% (w/v) of ciprofloxacin. For the control study, no ciprofloxacin suspension was introduced into the inoculated medium. The native BC pellicle (control) and BC–ciprofloxacin composites obtained at the end of fermentation process were then harvested and purified by immersing them into 0.1 M NaOH for 1 h at 80 °C [9]. A piece of the BC–ciprofloxacin composite was not purified to observe the effect of ciprofloxacin incorporation on the BC film.

2.6. Characterization of BC–ciprofloxacin composites

2.6.1. Scanning electron microscopy with energy-dispersive X-ray (SEM EDX) analysis

The morphological properties of native BC, purified and unpurified BC–ciprofloxacin composites were observed using SEM EDX Hitachi TM3030 Plus under the acceleration voltage of 15 kV. Prior to analysis, the samples were freeze-dried to remove moisture and then placed on a carbon tape and sputtered with gold particles. EDX spectroscopy was performed to examine the elemental and chemical composition of the samples.

2.6.2. Fourier transform infrared spectroscopy (FTIR)

Both native BC and BC–ciprofloxacin composites were frozen for 24 h in a freezer before they were freeze-dried for additional 48 h. FTIR spectroscopic measurements of the samples were recorded using a Thermo Scientific FTIR spectrometer. All spectra were acquired in the spectral range between 4000 and 400 cm^{-1} with a resolution of 4 cm^{-1}, averaging 16 scans [9].

2.6.3. X-ray diffraction (XRD)

XRD profiles of dried native BC, purified and unpurified BC–ciprofloxacin composites were obtained using a PANalytical X'PERT3 Powder Advance diffractometer under the voltage of 40 kV with Cu Kα radiation wavelength ($\lambda = 5406$ Å) and a filament current of 40 mA. The samples were scanned at ambient temperature in 2θ° range 5°–90°, with a step size of 0.020475° and scan speed of 0.5°/min. XRD patterns of the native BC, purified and unpurified BC–ciprofloxacin composites were shown by individual crystalline peaks extracted by a curve-fitting process from the diffraction intensity profiles. The concept of peak fitting programme OriginPro software was used, assuming Gaussian functions for each peak of the samples. The value of crystallinity index (CI) was calculated as follows:

$$CI = \frac{Area_{Crystalline}}{Area_{Total}} \times 100\%$$

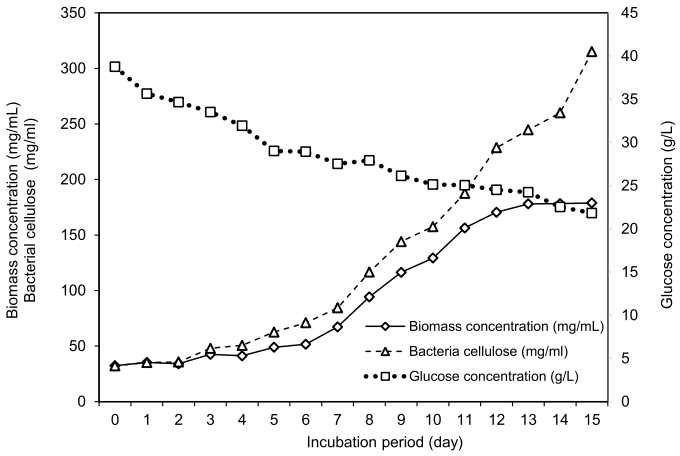

Fig. 1. Growth profile of *A. xylinum 0416* estimated through biomass concentration (mg/ml), bacterial cellulose (BC) production (mg/ml) and glucose consumption (g/l) during 15 h of static fermentation.

2.6.4. Antimicrobial study

The antimicrobial study of BC–ciprofloxacin composites was determined by Kirby–Bauer test method to measure the sensitivity of BC–ciprofloxacin against gram-negative bacteria, which are commonly found in diabetic foot ulcer: *Pseudomonas aeruginosa, Escherichia coli* and *Klebsiella* sp. A fresh bacterial suspension with a turbidity of 0.5 McFarland standard was prepared. The selected bacterial suspension was then streaked on nutrient agar plates using sterile cotton swab method. Purified, unpurified BC–ciprofloxacin and native BC discs were then placed on the plates and incubated at 37 °C for 24 h. The diameter of the inhibition zone appeared after incubation was measured in triplicates (mm).

3. Results and discussion

3.1. Growth profile of Acetobacter xylinum 0416

The time course of cell growth, bacterial cellulose formation, and glucose consumption in the static fermentation of *A. xylinum 0416* is depicted in Fig. 1. The data obtained from this experiment are critical in determining the optimal time to add ciprofloxacin to the fermentation medium. It was observed that the bacterial growth entered an exponential phase after two days of fermentation, during which the cells replicated at a rapid rate for the next twelve days. Parallel to this, the production of bacterial cellulose began on the second day of fermentation and steadily increased until the fermentation reached the stationary phase. As a result of this behaviour, BC is believed to be a mixed-growth associated product. A significant amount of glucose was found to be present in the fermentation broth, which prevented the cells from entering their death phase during the 15-day fermentation period, as observed previously. It is anticipated that BC will continue to be

Fig. 2. Growth of *A. xylinum 0416* colonies around the ciprofloxacin discs reflecting negative inhibition effect.

produced in a gradual manner until the glucose has been completely consumed. Further investigation of ciprofloxacin's inhibitory effect on *A. xylinum 0416* reveals that the BC producer strain is resistant to the antibiotic (Fig. 2). This finding indicates a favourable outcome for antibiotic incorporation into BC film during the fermentation process.

3.2. Morphology of BC–ciprofloxacin composites

Fig. 3 illustrates the morphological structures of purified native BC, unpurified BC–ciprofloxacin composites, and purified BC–ciprofloxacin composites. According to our findings, purified native BC has a distinct structure with numerous networks and porous structures of the BC fibrils, as illustrated in Fig. 3(a). BC possesses these characteristics, which qualify it as a possible antibiotic carrier. The presence of ciprofloxacin particles on the unpurified BC composite was clearly visible in Fig. 3(b). However, there was limited space for ciprofloxacin to penetrate through the fibrous network since the bacterial cells were actively growing and producing cellulose fibre extracellularly. The presence of numerous growing cells within the cellulose networks has also been shown to restrict access to the pores, thereby limiting ciprofloxacin particle penetration into the BC networks. Additionally, the unpurified BC pellicles have a pore size of approximately 20 μm, complicating the incorporation of larger ciprofloxacin particles into the BC networks. The average particle size of ciprofloxacin is estimated to be approximately 50 μm. It is obvious that the size of the ciprofloxacin particles is significantly larger than the size of the BC network pores, implying inefficient antibiotic incorporation into the BC network pores. The current method of preparing ciprofloxacin by ultrasonication at a frequency of 20 kHz, 40% amplitude for 30 min was unable to further reduce the particle size of ciprofloxacin, implying further optimization.

On the other hand, there is absence of ciprofloxacin particles

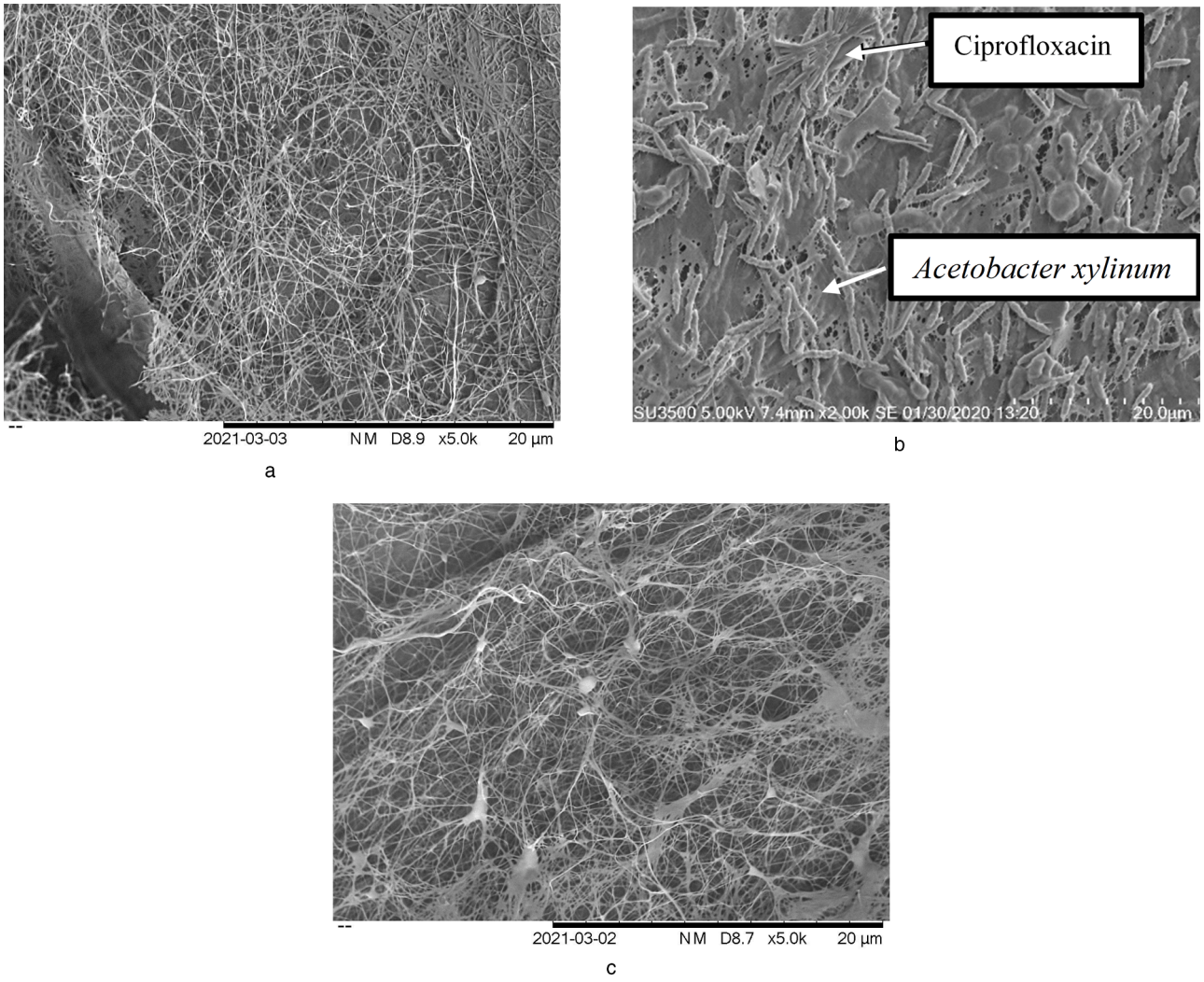

Fig. 3. Morphological structures of (a) native BC, (b) unpurified BC–ciprofloxacin and (c) purified BC–ciprofloxacin composites under SEM micrograph.

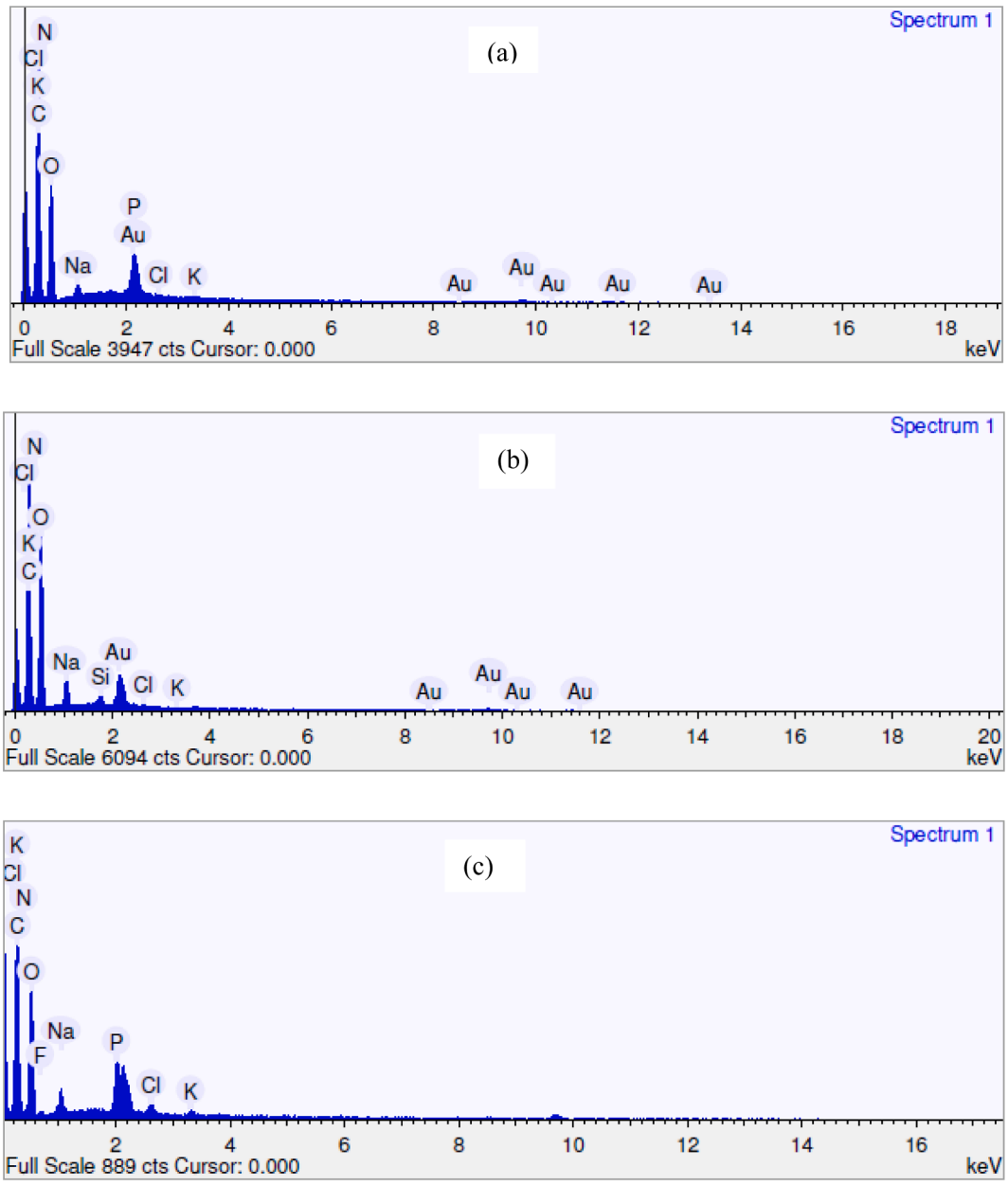

Fig. 4. Energy-dispersive X-ray (EDX) spectra of (a) native BC, (b) purified BC–ciprofloxacin and (c) unpurified BC–ciprofloxacin composites.

attached to the BC networks after the film was purified as can be seen in Fig. 3(c) (purified BC–ciprofloxacin composite). The EDX analysis confirmed the absence of ciprofloxacin on the purified BC–ciprofloxacin composite as shown in Fig. 4. In the case of unpurified BC–ciprofloxacin composite, the EDX analysis revealed the presence of element like fluorine characteristics for ciprofloxacin used in this study. The presence of gold in the EDX spectrum was determined to be due to the samples being sputter coated prior to SEM analysis. It is believed that ciprofloxacin particles have been detached from the BC network during the purification of the BC–ciprofloxacin composite in hot alkaline solution. Moreover, the antibiotic is believed to be degraded during purification process at hot temperature. Further investigation on the purification method of BC–ciprofloxacin composite should be carried out to retain the ciprofloxacin on BC network whilst removing the *A. xylinum* cells. Butchosa et al. [6] treated BC pellicles with 1% sodium dodecyl sulphate after in situ biosynthesis to remove the *Acetobacter* cells to avoid further deacetylation of deacetylated chitin nanocrystals (D-ChNC). Furthermore, a suitable binding agent shall also be considered to enhance the attachment of antibiotics to the BC networks during in situ incorporation.

3.3. FTIR spectroscopy

Fig. 5 illustrates the FTIR spectra of native BC, ciprofloxacin, and purified and unpurified BC–ciprofloxacin composites. There is one prominent characteristic peak found between 3100 and 3000 cm^{-1}, representing alkene and aromatic C–H stretching, mainly υ=C–H for pure sample of ciprofloxacin. Another band, between 2900 and 2800 cm^{-1}, corresponded to alkane stretching. The bands between 1750 and 1700 cm^{-1} were assigned to carbonyl C = O stretching, namely, υC═O, and the peak between 1650 and 1600 cm^{-1} to quinolones. The bands between 1450 and 1400 cm^{-1} corresponded to υC—O (S. [29]), whereas the strong band between 1400 and 1000 cm^{-1} indicated fluoro compound C–F stretching [8].

Native BC presented the typical FTIR spectra of cellulosic substrates with strong bands at around 3342, 2918, 1651 and 1054 cm^{-1}. The

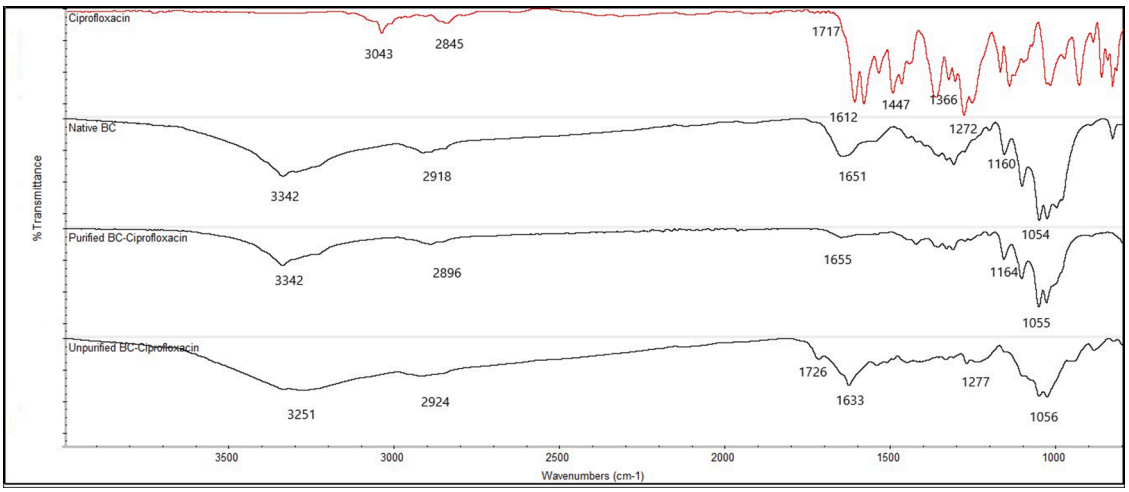

Fig. 5. Fourier transform infrared (FTIR) spectra of ciprofloxacin, native BC, purified BC–ciprofloxacin and unpurified BC–ciprofloxacin.

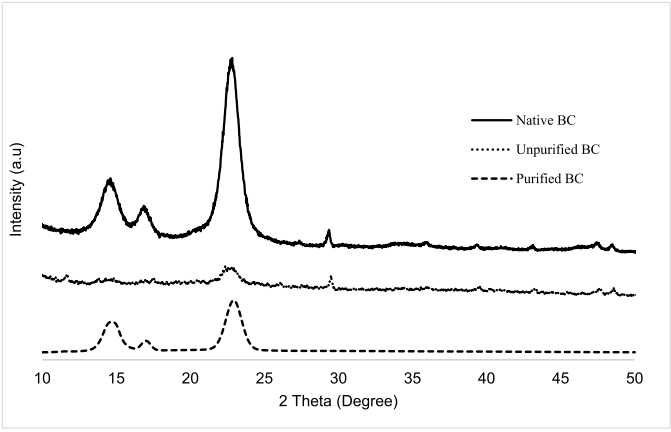

Fig. 6. X-ray diffraction (XRD) patterns of native BC, purified BC–ciprofloxacin and unpurified BC–ciprofloxacin.

strong band at 3342 cm^{-1} arose from the stretching of hydroxyl groups. The bands at 2918 and 1651 cm^{-1} originated from the C–H stretching and the H–O–H bending of the absorbed water, respectively [26]. A strong band at 1054 cm^{-1} was due to the C–O–C pyranose ring skeletal vibration [39]. In addition, there is high peak at 1160 cm^{-1}, indicating the presence of stretching aliphatic ether. Overall, the characteristic peaks observed on the structure of native BC are consistent with previous reports [9, 14].

Interaction between ciprofloxacin and BC was determined by comparing the FTIR spectra of native BC, ciprofloxacin, purified and unpurified BC–ciprofloxacin composites. When compared to the spectrum of pure ciprofloxacin, the absorption peaks at 1717 cm^{-1} (CO group), 1612 cm^{-1} (quinolines S–N–H bending) and 1272 cm^{-1} (C–F group) in the spectrum of ciprofloxacin were shifted to 1726 cm^{-1}, 1633 cm^{-1} and 1277 cm^{-1}, respectively, in the spectrum of unpurified BC–ciprofloxacin composite. This leads to the fact that there was an interaction between BC and ciprofloxacin in unpurified BC–ciprofloxacin composite, resulting in the formation of chemical bonding between the hydroxyl residues of BC and the groups of ciprofloxacin. However, these peaks were not observed in the purified BC–ciprofloxacin composite. This finding is in agreement with EDX analysis which confirmed the detachment of ciprofloxacin from the BC networks as a result of alkaline purification step. Moreover, it was clearly seen that the FTIR spectrum of both native BC and purified BC–ciprofloxacin is similar to each other, indicating no ciprofloxacin interaction in the purified BC–ciprofloxacin composite.

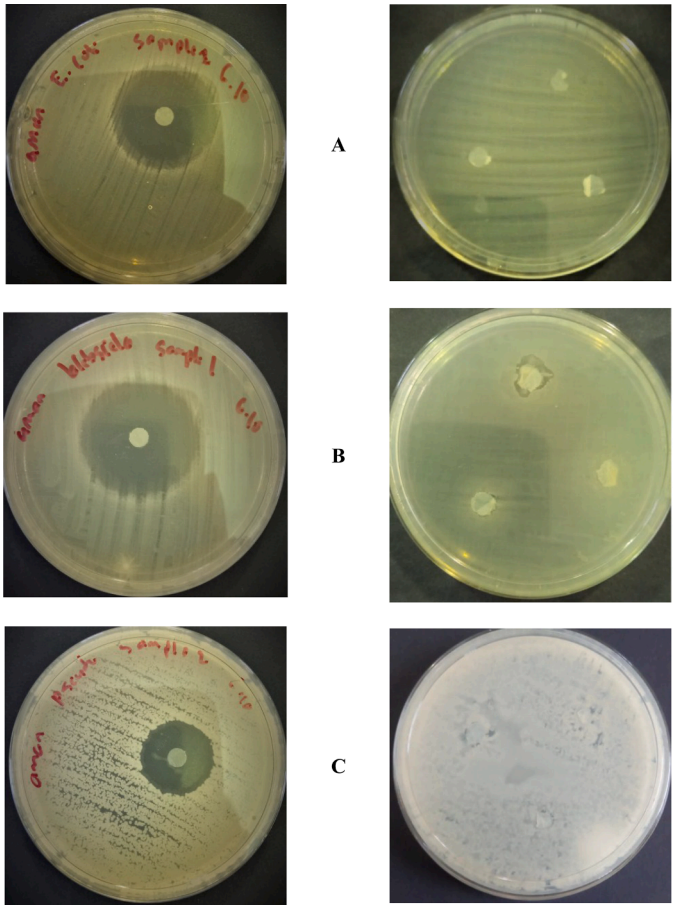

Fig. 7. Representative photos of the inhibition zone of unpurified BC–ciprofloxacin composites (left), purified BC–ciprofloxacin composites and native BC (right) against (A) *Escherichia coli*, (B) *Klebsilla sp.* and (C) *Pseudomonas aeruginosa.*

3.4. XRD

The XRD spectra of native BC, purified and unpurified BC–ciprofloxacin composites are shown in Fig. 6. Native BC presented three typical diffraction pattern peaks at 2θ = 14.60°, 16.90° and 22.77°,

which emphasize the crystalline structure. This findings are similar to the recent studies of atomic planes of cellulose structure [2, 14, 19, 30]. Three prominent peaks of pure ciprofloxacin are normally found at $2\theta =$ 19.22°, 26.39° and 29.16° (Subhashree [27]a). Both purified and unpurified BC–ciprofloxacin composites demonstrate similar XRD pattern to native BC. However, it was clearly shown that the relative intensities of the peaks have decreased in the BC–ciprofloxacin composites, reflecting a lower crystallinity as compared to native BC. The decrease in relative intensities of these peaks appears to be due to the change in atomic densities in that particular plane of crystal lattice (Subhashree [29]a). BC had a higher crystalline index than purified and unpurified BC–ciprofloxacin, with values of 74.76%, 40.6% and 49.35%, respectively. It is possible that a slight shift in the orientation of the crystal lattice occurs as a result of the incorporation of some additional atoms. This behaviour could be explained by an interaction between ciprofloxacin and the BC during the synthesis of the BC–ciprofloxacin composite, resulting in a decreased degree of crystallinity.

3.5. Antimicrobial activity

As illustrated in Fig. 7, unpurified BC–ciprofloxacin composite showed a substantial inhibition zone against *E. coli, Klebsiella* sp. and *Pseudomonas aeruginosa*. In accordance with Masadeh et al. (2015), the findings of this study confirm the antibacterial activity of ciprofloxacin against the bacteria tested in the previous research. These bacteria strains are gram negative and commonly found in diabetic foot ulcer [32]. These isolates are known to cause wound healing to be delayed when they are present. Therefore, it is vital to ensure that the BC–ciprofloxacin composite is effective in inhibiting gram-negative bacteria, which have been identified as the most common cause of delayed wound healing and the most significant limitation of standard wound care protocols. However, due to detachment and degradation of the antibiotic that occurred during the purification process of the BC-ciprofloxacin composite, the purified BC–ciprofloxacin failed to inhibit any of the bacteria tested.

The BC producer, *A. xylinum* (also known as *Gluconacetobacter xylinus* and *Komagataeibacter xylinus*) is a member of acetic acid bacteria. Few risk assessments that have been conducted on the species have shown that the species is safe. There is no evidence in the literature that the species is capable of producing toxins that are toxic to humans or animals, nor is there evidence that the species is capable of infecting humans or animals. Additionally, a recent study identified *Komagataeibacter xylinus* as a novel probiotic candidate. Despite the fact that rats were fed a high dose of bacteria, no clinical signs were observed. Histopathological experiments confirmed the findings with no presence of bacteria was observed in the organs. Additionally, histopathological examinations revealed no evidence of inflammation, degeneration, or necrosis in the cells [16]. Besides, in vitro studies revealed the minimum cytotoxicity of BC- Ciprofloxacin films in human fibroblasts with 95.0% cell viability [7]. Although no genotoxicity or cytotoxicity studies on the unpurified BC-ciprofloxacin composite have been done to date, past risk assessments indicate that the strain employed is safe for humans.

4. Conclusion

Ciprofloxacin was successfully incorporated on the BC network during the synthesis process. The interaction of BC and ciprofloxacin was validated by SEM EDX, FTIR spectroscopy, XRD and antimicrobial study. Moreover, BC–ciprofloxacin significantly prevented the growth of bacteria associated with diabetic foot ulcers such as *E. coli, K. pneumoniae* and *P. aeruginosa*. Nonetheless, improvements in the particle size of ciprofloxacin should be explored in order to effectively incorporate this antibiotic into BC networks. Additionally, it is necessary to investigate the purification process of the BC–ciprofloxacin composite and the application of an appropriate binding agent in order to increase the composite's efficacy as a wound dressing substitute.

Declaration of Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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