Journal of Applied Biosciences 162: 16675 - 16693
ISSN 1997-5902

Bacterial Cellulose/Chitosan Hydrogels Synthesized *In situ* for Biomedical Application

Joachim Emeka Arikibe[a,b*], Roselyn Lata[b], David Rohindra[b]
[a]Department of Chemistry, Faculty of Mathematics and Natural Science, University of Bergen, 5007, Bergen, Norway.
[b]School of Agriculture, Geography, Earth Science, Oceans and Natural Sciences, The University of the South Pacific, Laucala, Suva Fiji.
*Corresponding author. E-mail address: jar022@uib.no , emeka.ariks@gmail.com , Tel.: +4747749097

Submitted on 7th April 2021. Published online at www.m.elewa.org/journals/ on 30th June 2021
https://doi.org/10.35759/JABs.162.1

ABSTRACT

Objective: Bacterial cellulose (BC) is a biopolymer whose application has been limited due to the difficulty to introduce modifiable functional groups onto its network. The present study introduces new functional groups from chitosan (Chs) by *in-situ* method and further modified by crosslinking with genipin (Gp), a non-toxic agent.

Methodology and Results: Bacterial cellulose-chitosan (BC-Chs) hydrogels were synthesized *in-situ*, dried, immersed in genipin (Gp) solution to yield BC-Chs crosslinked hydrogel (BC-Chs-Gp) and characterized. The presence of amide 1 and II and crosslinking with genipin was revealed by FT-IR and SEM showed BC-Chs-Gp had a compact fibril network. Low tensile strength, less swelling ratio and moisture were exhibited by BC-Chs-Gp due to crosslinking. Hydrogels were active against *E. coli* and *S. aureus. In vitro* drug release studies of hydrogels using quetiapine fumarate followed the Higuchi model with a super case II transport mechanism and non-Fickian for BC-Chs-Gp.

Conclusion and Application of findings. The present study utilized non-pathogenic bacteria, *G. Xylinus* and a natural resource (coconut) to obtain non-toxic and biocompatible hydrogels. BC-Chs hydrogels were prepared by simple and direct *in-situ* method by the introduction of Chs onto the growing network of BC and subsequently crosslinked using genipin by *ex-situ* modification method. The properties of the hydrogels in term of swelling ratio, moisture content, tensile strength, antibacterial activity, and their controlled drug release ability make them suitable for potential application in biomedicine, especially in transdermal patches as wound dressing and wound healing agents.

Keywords: Bacterial cellulose; Chitosan; Genipin; Biocompatibility; mechanical properties.

INTRODUCTION

Hydrogels are three-dimensional networks of hydrophilic polymers and are used in several medical applications like drug delivery, tissue engineering and wound dressing Controlled and localized delivery of drugs from hydrogels offers many benefits. The interest in using biodegradable polymers in such applications has grown over the past decade because of several advantages like degradation of the hydrogels into non-toxic components. One promising biopolymer engaged in the development of hydrogels is bacterial cellulose (BC) (Silva *et al.,* 2014; Esuendale and Gabriel, 2016). BC possesses exceptional properties such as high mechanical strength, high water absorbing capacity and highly pure nanofibril network structure (Liu *et al.,* 2020). The synthesis and modification of BC hydrogels for biomedical applications have gained momentum in recent times. Diverse approaches to modify BC, either through chemical or physical modification to alter the porosities, crystallinities and fibre densities have been employed to improve the properties of BC for different applications. These two techniques of modification utilize two major implementation strategies viz; *in situ* and *ex situ* methods (Stumpf *et al.,* 2018; Dreiss, 2020; Omidi *et al.,* 2020). *In situ* modification of BC is performed by varying the conditions of the cell culture and introducing additives or reinforcement materials. The added materials are incorporated onto the growing network of BC fibrils during synthesis. This method of modification has been reported to alter the mechanical, physichochemical and morphological properties of BC (Saska *et al.,* 2012; Romanov *et al.,* 2014; *et al.,* Stumpf *et al.,* 2018). BC has been modified using laporite, (Perotti *et al.,* 2011) *A. xylinum* JCM 9730, (Hu and Catchmark, 2010) hydroxypropylmethyl cellulose (HPMC) (Huang *et al.,* 2010) and *aloe vera* (Saibuatong and Phisalaphong, 2010) to improve swelling and drug delivery. On the other hand, *ex situ* modification has also been reported (Chang *et al.,* 2012; Feng *et al.,* 2012; Tsalagkas *et al.,* 2016) where BC matrix have been impregnated with different materials either through chemical or physical means (Stumpf *et al.,* 2018). The *ex-situ* modifications are mostly performed through absorption resulting in strong hydrogen bonding between BC and the absorbed molecules (S.-P. Lin *et al.,* 2013). The modification of BC with silk-fibroin solutions with non-cytotoxicity and non-genetoxicity (Oliveira Barud *et al.,* 2015) silver alginate and silver sulfadiazine with antibacterial property against *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans* (Shao *et al.,* 2015) polyvinyl alcohol (PVA) with potassium sorbate (Mihaela Jipa *et al.,* 2012) benzakonium chloride (Wei *et al.,* 2011) with antimicrobial ability have been reported. Chitosan (Chs) [Poly (1, 4-β-D-glucopyranosamine)] is the *N*-deacetylated polysaccharide form of chitin which possesses important properties for biomedical applications (Hamdi *et al.,* 2020). As a biopolymer, chitosan has the same β-(1→4)-D-glucopyranose units backbone as found in cellulose, except that the 2-hydroxy is replaced by an acetamide group (Wu *et al.,* 2004). It has been well documented that chitosan accelerate wound healing in humans and possess excellent antimicrobial properties against a broad spectrum of bacteria. The antimicrobial property has been found to be maintained when added to other polymers (Pal *et al.,* 2013; El Knidri *et al.,* 2018; Hamedi *et al.,* 2018; W.-C. Lin *et al.,* 2013; Mittal *et al.,* 2018; Arikibe *et al.,* 2019, Shariatinia, 2019). In acidic medium, the poor structural stability of chitosan has been improved by crosslinking with glutaraldehyde (a toxic crosslinker). On the contrary, genipin, a plant extract, used as herbal remedy by the Asians with low cytotoxicity and reduced inflammatory (De Azeredo *et al.,* 2014) responses have been used as a crosslinker for chitosan (Khurma *et al.,*

2005). In addition, wound healing has been shown to be enhanced when treated with genipin (Aramwit *et al.*, 2013; Pal *et al.,* 2013). There are several reports on the modification of BC with Chs (Aramwit *et al.*, 2013; Pal *et al.,* 2013; W.-C. Lin *et al.,* 2013; De Azeredo *et al.*, 2014; *et al.,* El Knidri *et al.*, 2018; Hamedi *et al.,* 2018; Mittal *et al.*, 2018; Arikibe *et al.*, 2019; Carvalho *et al.*, 2019; Portela *et al.*, 2019; Shariatinia, 2019;) where the synthesized BC was immersed in the acidified solution of chitosan and allowed to be absorbed and later crosslinked with citric acid (Lin *et al.,* 2013) or freeze thawed (Arikibe *et al.,* 2019; Carvalho *et al.*, 2019; Portela *et al.*, 2019) and other methods involving combining different weight ratios of BC and Chs (Carvalho *et al.*, 2019; Portela *et al.*, 2019). Despite various approaches undertaken in processing BC for different applications, several bottlenecks still exist such as the introduction of functional groups to BC matrix for distinct applications (Czaja *et al.*, 2007). These limitations have restricted the utilization of BC in biomedical applications, especially in tissue engineering and wound dressing. Development of BC membranes modified with chitosan and investigating the drug release mechanisms is an area of interest. The modification of BC with chitosan is an effective and convenient way to improve the physical properties of BC for practical utilization. Based on literature survey, BC-Chs hydrogels (Lin *et al.,* 2013) have been developed by dipping the swollen BC films in the Chs solution and crosslinked. The Chs encapsulates the BC chains and therefore the swelling and release behaviour from such hydrogel is dependent mostly on the crosslinked Chs surface. A new approach undertaken in this study was to synthesize BC-Chs membrane via *in situ* technique. To introduce the crosslinked structure throughout the BC-Chs matrix, the Chs solution was added to the *G. xylinum* culture medium. Immersing this membrane in genipin (Gp) solution, only Chs was crosslinked throughout the matrix resulting in a semi-interpenetrating hydrogel. This study reports the technique for the first time based on literature survey. Although BC-Chs has physical, crosslinking through hydrogen bonds, for simplicity in the text, BC-Chs is referred to as membranes while BC-Chs-Gps is referred to as hydrogels.

MATERIALS AND METHOD

Materials: *Gluconacetobacter xylinus* (*G. xylinus*) BCRC 14182 was purchased in freeze-dried form from the Bioresource Collection and Research Centre, Hsinchu, Taiwan. Hestrin and Schraum (HS) medium was used to activate and subculture the bacteria. The bacteria culture was allowed to grow in the medium incubated at 28^{o}C for 3 days and stored at 4^{o}C for further use (Arikibe *et al.,* 2019). Chitosan (Chs) from crab shells was purchased from Sigma-Aldrich (product number C3646, 85% deacetylated). Genipin (Gp) was obtained from Linchuan Zhixin Bio-Technology Co., Ltd China. Fresh coconuts from the palm trees in Fiji were handpicked. Yeast extract, potassium dihydrogen phosphate, sucrose, magnesium sulfate and bactopeptone were purchased from Sigma Aldrich. All chemicals used were of analytical grade.

Production of BC starter medium*:* The bacteria starter medium was prepared by a modification of the method reported by Pa'e *et al.*, 2007. Into a 500 mL beaker, 300 mL coconut water from tender green nuts was measured, sieved and heated to boiling on a hot plate for 20 min. Accurately weighed 2.5 g of yeast extract and 0.6 g of ammonium sulfate $(NH_4)_2SO_4$, 0.5g KH_2PO_4, 0.2g $MgSO_4$ were added into the coconut water with continuous stirring and the medium was cooled to 28 ^{o}C. The pH of the medium was adjusted to pH 5.0 using 0.1 M, NaOH. The medium was transferred into a conical flask, covered with

cotton wool, sealed with aluminium foil, and autoclaved at 121 ^{o}C for 15 min. After autoclaving, the medium was cooled to room temperature and 30 mL of *G. xylinus* was added to the medium using aseptic technique under laminar flow. The solution was homogenized by slowly swirling the flask. Using a fresh set of cotton wool and aluminium foil, the flask was closed and sealed again and placed in the incubator at a temperature of 28 ^{o}C for 7 days. The BC membrane formed was harvested, washed with both distilled water and 0.1 M NaOH solution and rinsed a final time with distilled water.

***In situ* modification of BC/ preparation BC-Chs composite membrane:** To modify BC, 5 mL of chitosan (2 % w/v) dissolved in 2 % acetic acid was aseptically transferred into the culture medium on the second day and another 5 mL on the third day of the fermentation process and incubated for 7 days. The BC-Chs membrane formed (brownish in colour) was removed from the medium and washed with distilled water followed by soaking in 2% acetic acid solution for 20 min to remove any adhering chitosan on the surface of the synthesized membrane that was not part of the network. The surface was neutralized by washing with dilute NaOH solution and subsequently with distilled water. The BC and BC-Chs membranes were freeze-dried and used for further studies.

Modification of BC-Chs composite membrane with genipin: To crosslink BC-Chs membrane, genipin (1% w/v) was prepared in phosphate buffer saline (PBS) at room temperature. The BC-Chs membrane produced previously was immersed in the genipin solution for 24 h, yielding a bluish-green BC-Chs-Gp hydrogel, which was freeze-dried for further studies.

Characterization of BC, BC-Chs membranes and BC-Chs-Gp hydrogel: The BC, BC-Chs membranes and BC-Chs-Gp hydrogel were characterized using Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM), swelling behaviour, drug release, water retention, water vapour transmission, mechanical properties, and antibacterial action.

FTIR Analysis: The chemical structures of the membrane and the hydrogel were studied using FTIR (Perkin Elmer Spectrum Two FTIR Spectrometer, USA) with an attenuated total reflectance (ATR) accessory and zinc selenide (ZnSe) crystal for internal reflection. Data collection was done over 20 scans at 0.5cm^{-1} resolution and analysis of spectra was performed using the Spectrum 10^{TM} software.

Scanning Electron Microscopy (SEM): The morphology of the membrane and the hydrogel were acquired using SEM (JSM-5310LV, Joel, Tokyo, Japan). The samples were broken in liquid nitrogen to view the internal morphology. To avoid accumulation of charges under the electron beam, the surface was sputter coated with platinum/palladium under argon gas prior to scanning.

Swelling Behaviour: The gravimetric method (Lin *et al.*, 2013) was used to determine the swelling behaviour of the samples. Membranes and hydrogel were cut into 3 cm × 3 cm pieces and weighed accurately to obtain their dry weight (W_{dry}). Each sample was put in a vial containing 30 mL deionized water and this was incubated at 25 ^{o}C and allowed to swell. At certain time intervals, the swollen samples were withdrawn from the water and their wet weight (W_{wet}) were accurately taken after removing excess water from the surface by blotting gently with a filter paper. Equations 1 and 2 defined the water swelling behaviour and moisture content of the membranes, respectively. The average of three trials was taken.

$$\text{Swelling ratio}(\%) = \frac{\text{Wwet} - \text{Wdry}}{\text{Wdry}} \times 100 \quad (1)$$

$$\text{Moisture content}(\%) = \frac{\text{Wwet} - \text{Wdry}}{\text{Wwet}} \times 100 \quad (2)$$

Water Vapour Transmission: The ASTM E96 standard method was employed to determine the water vapour transmission (WVT). The mouth of a cylindrical cup (2.75 cm in diameter) containing 30 mL deionized water was sealed with each membrane and hydrogel samples. These samples were tightly fastened by barrier tapes to prevent any loss of moisture. The membrane-cup assembly was placed in an incubator at 37 ^{o}C. Determination of evaporation of water via the sample specimen was done by periodic weighing. The decrease in weight showed water loss. The profile of weight loss versus time was plotted for each sample. Calculation of water vapour transmission (WVT) was done by dividing the weight loss of water with the area of the cup opening. The measurement was repeated four times for each sample and the average was taken.

Water retention property: The water retention capacity of the membranes and hydrogels was evaluated using the water retention ratio test as defined in Equation 3. After measuring the initial dry weight (W_{dry}), the samples were immersed in deionized water for 24 hr. The swollen samples were then wiped with a filter paper to remove excess surface water and then placed in an open mouth dish at room temperature. At preset time, the samples were taken out and weighed (W_{wet}). The test was conducted in triplicates.

$$\text{Water retention ratio}(\%) = \frac{\text{Wwet} - \text{Wdry}}{\text{Wdry}} \times 100 \quad (3)$$

Determination of gel content: The hydrogel was dried for 6 h at 50 ^{o}C under vacuum (W_o). Next, it was soaked in distilled water for 24 hrs with constant swirling to remove the soluble parts up to a constant weight. The gel was then dried again at 50 ^{o}C under vacuum (W_e). The gel fraction percentage was calculated by Equation 4 (Sung *et al.*, 2010)

$$\text{Gel fraction}\% = \left(\frac{W_e}{W_o}\right) \times 100 \quad (4)$$

Where w_o is the weight of the hydrogel sample dried for 6 h at 50 ^{o}C and w_e after soaking. The average of four trials was taken and the standard deviation (S.D) was calculated.

Determination of mechanical properties: The mechanical properties of the dried membrane and hydrogel samples were determined using E Z Test machine (SM-50N-168, Shimadzu) which was set in tensile mode. The samples were cut into 5 cm x 1 cm size and stretched at a crosshead speed of 50 mm/min. Average values obtained from four measurements were used to calculate the tensile strength and Young's modulus from the stress-strain data.

Drug absorption and release studies: Dried samples of each hydrogel (about 4 x 4 cm) was immersed into Quetiapine fumarate solution of concentration of 2 mg/mL for 24 hrs to allow for maximum absorption of the drug into the hydrogel matrix. The amount of drug absorbed was calculated from the difference in the concentration of the Quetiapine fumarate solution before and after absorption. The

swollen hydrogels containing the drug was dried under vacuum at 40 ^{o}C until constant weight was achieved. The dried hydrogels were placed in distilled water and at 10 min intervals; the drug release was monitored by recording the absorbance at 298 nm using the Perkin Elmer Lambda 25 UV spectrophotometer. Average from three trials was considered. Data obtained were fitted into zero order (5), first order (6) and Higuchi (7) kinetic models as well as the Korsmeyer-Peppas power law (8) to ascertain the drug release kinetics and transport mechanisms (n), respectively.

$$Mt = K_1 t + b \quad (5)$$

$$ln\,(100 - Mt) = K_2 t + b \quad (6)$$

$$Mt = K_3 t^{1/2} + b \quad (7)$$

$$\ln Mt = K_5 lnt + b \quad (8)$$

Where:

Mt = Cumulative amount of drug release at time "t"
b = Intercept from the plot
t = time (min)
K_1 = zero order constant
K_2 = first order constant
K_3 = Higuchi constant
K_5 = Korsmeyer-Peppas constant

The constants K_1-K_5 incorporate the structural and geometric characteristics of the hydrogels.

Antibacterial evaluation: The extent to which the membranes and hydrogel samples exhibited antibacterial action was assessed in line with the Japanese Industrial Standard JIS Z 2801:2000. The strains of bacteria utilized for the current study were *Staphylococcus aureus* and *Escherichia coli*. The cultivation of the bacteria was done in nutrient broth with composition of 3 g beef extract, 10 g peptone and 5 g sodium chloride in 1000 mL of water. Samples of equal circumference were first immersed in sterile water for swelling and then placed in petri dishes, which were inoculated with 400 µL of the cell suspension of each bacteria (2.5 x 10^5 to 1 x 10^6 CFU/mL). Incubation of the dishes were done at 35 ^{o}C and 90 % relative humidity for 24 hrs for the samples to fully establish contact with the bacteria, after which the residual bacteria were washed out and diluted serially for counting of the colony. 100 µL aliquot of diluted bacteria solution were spread on agar plates and incubated at 37 ^{o}C for 24 h. Colonies on the agar were visually counted as CFU per sample. The inhibition of bacterial growth was calculated using Equation 9.

$$\text{Antibacterial efficiency} = (N_0\text{-}N)/N_0 \quad (9)$$

Where N_o and N represents the number of bacteria of control and the experimental group, respectively. The average of six trials was taken.

Statistical Data Analysis: Data obtained were analysed using one-way ANOVA followed by t-Test to evaluate if there were any significant difference in alpha value, $p < 0.05$. Analysis was conducted using Microsoft Excel 2013 (Microsoft Corporation, Redmond, WA).

RESULTS AND DISCUSSION

Physical appearances of membranes: Figure 1A – D shows respectively, the *in-situ* fermentation media of BC, synthesized BC membrane, BC-Chs membrane and BC-Chs-Gp hydrogel. The membranes and hydrogels were observed to have different colours: white for BC, gray for BC-Chs and bluish green for BC-Chs-Gp. The colour of BC-Chs is due to the presence of chitosan in the cell culture during the time of *in situ* biosynthesis and modification. However, with the crosslinking process involving genipin, the colour of BC-Chs-Gp hydrogel changed to bluish green. This colour is due to the oxygen-induced polymerization of genipin as well as the reaction of genipin with the nucleophilic amino (NH_2) groups of chitosan (Arikibe *et al.*, 2019; Sung *et al.*, 2010)

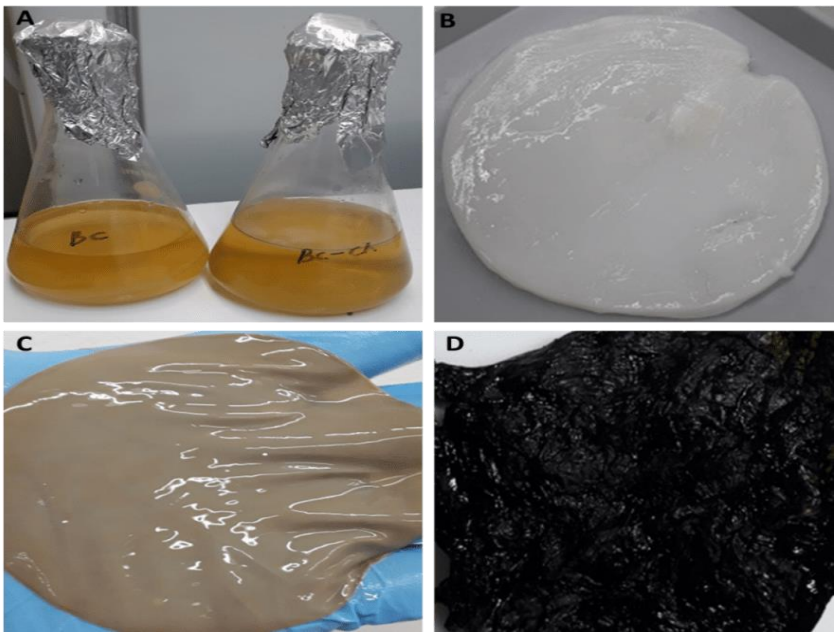


Figure 1: (A) *In situ* fermentation media of BC, (B) BC membrane, (C) BC-Chs membrane and (D) BC-Chs-Gp hydrogel.

FTIR Analysis: The normalized FTIR spectra for BC, Chs, BC-Chs and BC-Chs-Gp are shown in Figure 2. A broad band observed between 3700 and 3100 cm^{-1} is assigned to the stretching of O-H and the inter and intramolecular hydrogen bonds which also involves the NH_2 functional group in the chitosan containing samples. The vibrational bands between 2970 and 2896 cm^{-1} refers to the stretching of aliphatic C-H. Bands associated with the NH_2 groups at 1632 cm^{-1} (Amide I) and 1536 cm^{-1} (Amide II) present in chitosan was also observed in the BC-Chs and BC-Chs-Gp spectra. These bands shifted to 1627 cm^{-1} and 1533 cm^{-1} respectively for BC-Chs-Gp. The presence of the amide I and II bands in both BC-Chs and BC-Chs-Gp indicated that chitosan was incorporated into the BC fibril networks during the *in-situ* modification. The shifts observed in the amide groups for BC-Chs-Gp is attributed to the effect of crosslinking reaction between genipin and the amine group (NH_2) of chitosan within the network of the hydrogel.

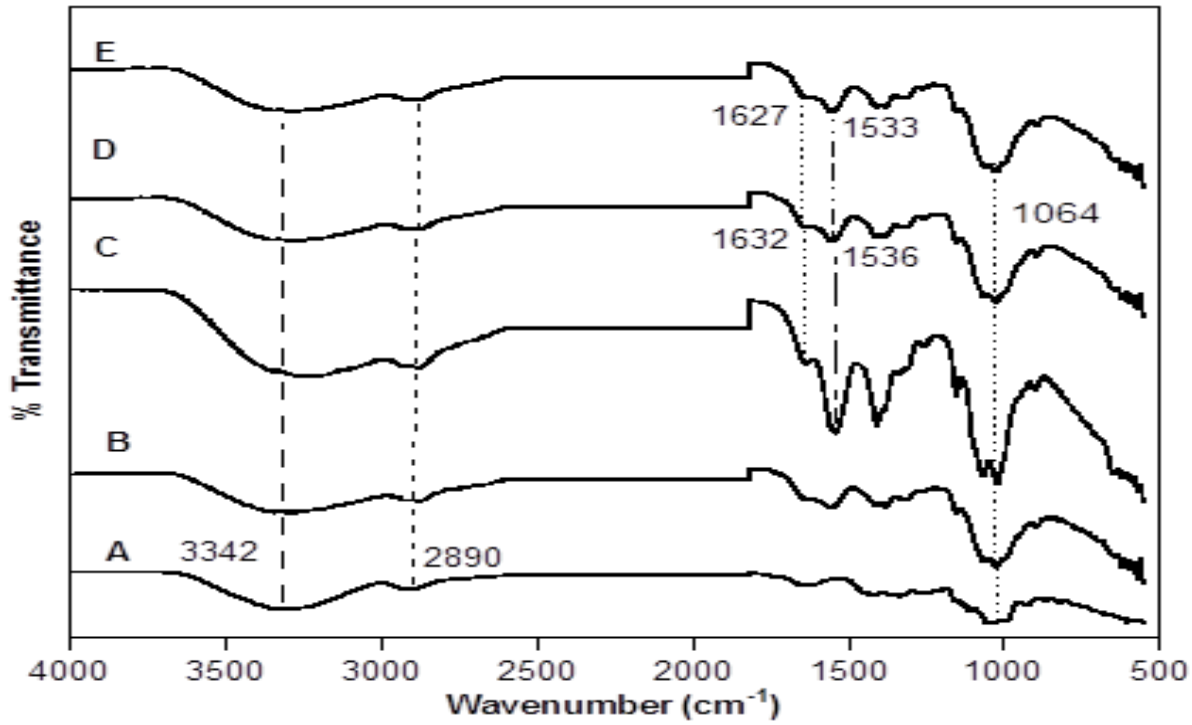


Figure 2: Normalized FTIR spectra of (A) BC (B) Chs-Gp (C) Chs (D) BC-Chs (E) BC-Chs-Gp

Scanning Electron Microscopy (SEM): The SEM images of BC, Chs, BC-Chs and BC-Chs-Gp are presented in Figure 3A – D, respectively. BC was observed to possess a well-arranged 3-D network of fibrils and some empty spaces in-between. The BC structure observed here agrees with that reported in literature. (Muzzarelli, 2009; Arikibe *et al.*, 2020) BC-Chs appeared to be compact, attributed to the incorporation of chitosan within the BC network and this compactness was observed to be more pronounced in the BC-Chs-Gp, because of the crosslinking reaction between chitosan and genipin. Since chitosan is incorporated into the BC, network and interacts with the microfibrils of BC during the biosynthesis process, thereby altering the porosity and physico-chemical properties of BC. The reduction in porosity and formation of a denser network structure in BC-Chs and BC-Chs-Gp compared to BC is attributed to the incorporation of chitosan within the BC microfibril. When compared to the internal morphology of the hydrogel system prepared in our previous study (Arikibe *et al.*, 2019), the variations in the porosities and compactness of hydrogel are less indicating minimum differences in the two methods of preparation. The current method is preferable due to fewer chemicals used.

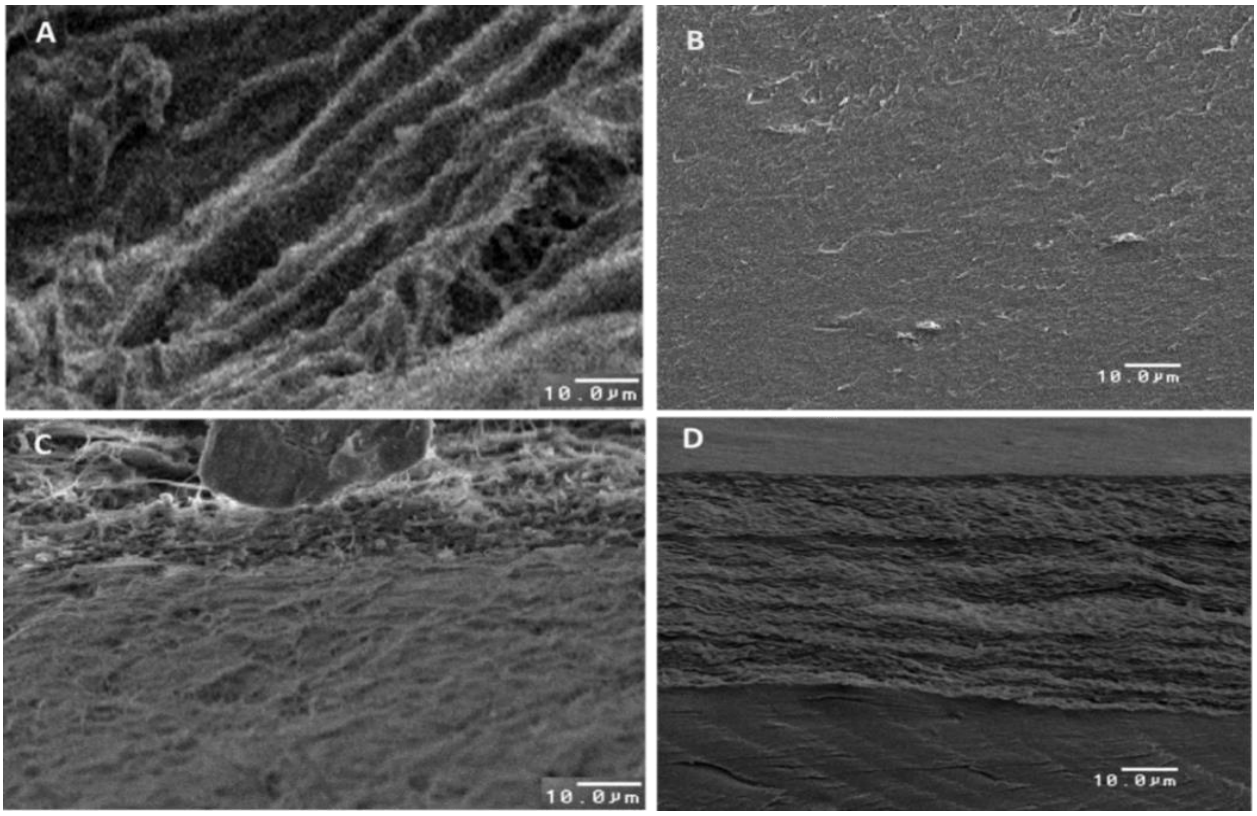


Figure 3: SEM image of (A) BC, (B) Chs, (C) BC-Chs and (D) BC-Chs-Gp

Gel fraction analysis: To evaluate the extent of crosslinking, the gel content of the hydrogel was evaluated. The synthesis of BC-Chs membrane by *G. xylinus* using *in situ* technique resulted in interpenetrating network where BC and Chs chains are held together through entanglements or physical crosslinking due to intermolecular hydrogen bonding. The chemical crosslinking using genipin involved only Chs (where the NH_2 groups of chitosan reacted with the genipin via a ring opening process) resulting in a semi-interpenetrating hydrogel. The percentage gel fraction gives an insight into the extent to which Chs was chemically crosslinked. The percent gel fraction obtained were 91.22-± 0.03% and 97.39 ± 0.26 % for BC-Chs and BC-Chs-Gp, respectively, which were significantly different ($p < 0.05$). The results represent relatively high percentage gel fraction in both samples. In BC-Chs due to intermolecular hydrogen bonding and in BC-Chs-Gp combined with chemical crosslinking, very little amount of unreacted species were present. BC-Chs-Gp therefore, recorded a higher gel fraction. However, BC-Chs, which showed a little lower value, may be due to the leaching of the polymer chains, which may have formed stronger hydrogen bonds with water than the intermolecular hydrogen bonding among the polymer chains.

Swelling Studies and Moisture content: Active substances penetration, defence of wounds against invasion by bacteria and the provision of painless removal from the surface of wound after the recovery process can be enhanced and promoted by a moist environment (Shezad *et al.,* 2010). The ability to absorb water is an important parameter to assess the suitability of membranes and hydrogel for use in wound dressing. Swelling profile and moisture contents of the samples

are presented in Figure 4 A and B. Samples reached equilibrium swelling in 24 hrs. The highest swelling ratio was shown by BC, 30 times its dry weight. The addition of chitosan during the *in-situ* modification process decreased the swelling ratio for BC-Chs membrane by half of that of BC. Furthermore, the modification of BC-Chs membrane by crosslinking with genipin decreased the swelling of BC-Chs-Gp to about one-third compared to the swelling of BC-Chs. The moisture content ratio of BC was about 97 % and differed significantly from the others ($p < 0.05$), while BC-Chs and BC-Chs-Gp recorded about 94 % and 93 %, respectively without any significant difference ($p > 0.05$). The high moisture content capacity of BC compared to BC-Chs and BC-Chs-Gp may be explained in terms of the porosities in their respective structures. It has been reported that water molecules are physically trapped by delicate hydrophilic network structure of BC (Ul-Islam *et al.*, 2012). The introduction/or addition of chitosan onto the network of BC may result in the BC-Chs becoming more compact due to reduced porosity, which gave rise to a decrease in the amount of water uptake (Lin *et al.*, 2013). However, an even more compact structure is expected after the chemical crosslinking reaction using genipin that further reduced the amount of water absorbed by the BC-Chs-Gs hydrogel.

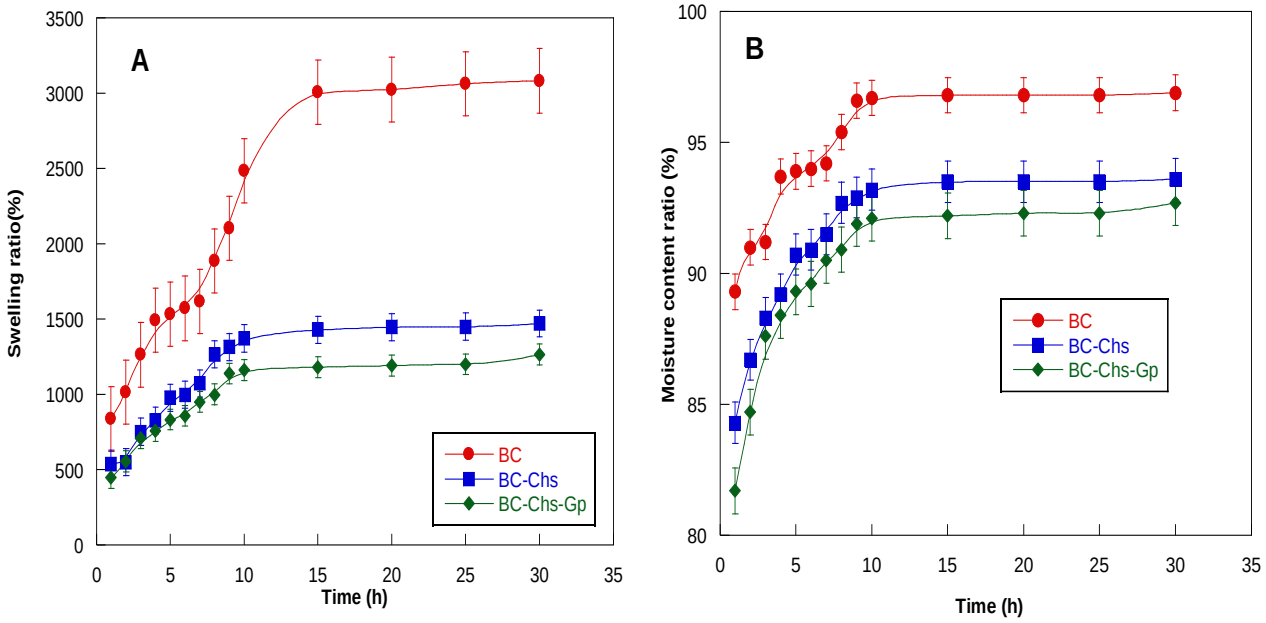
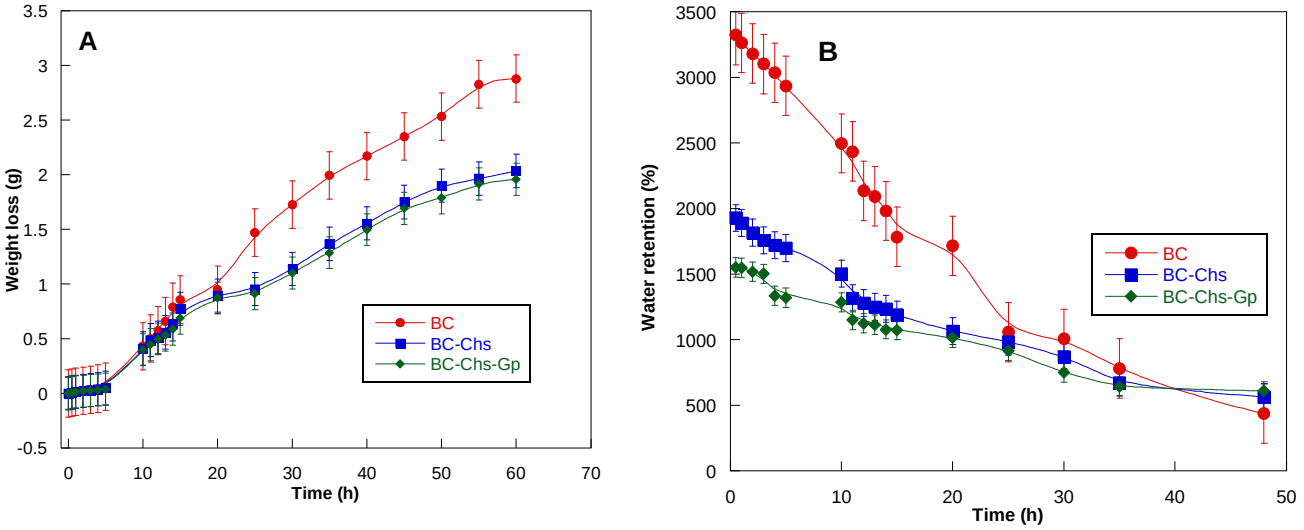


Figure 4: Physico-chemical parameters of BC, BC-Chs membranes and BC-Chs-Gp hydrogel (A) Swelling ratio (B) Moisture content ratio.

Water Retention and Water Vapour Transmission (WVTR): Figure 5 A and B show the water retention and water loss profiles respectively of BC, BC-Chs and BC-Chs-Gp over 48 hr period. The results indicate that the different samples retained water in similar pattern without much disparity and showed no significant difference ($p > 0.05$). The rates of evaporation of water were observed to be rapid in the beginning for the samples possibly because of surface water escaping. The ratio of water retention for BC-Chs membrane and BC-Chs-Gp hydrogel seemed to be constant after the initial

evaporation and the process took approximately 60 hr for the samples to evaporate to near dryness to yield dry weight. The calculated WVTR of BC, BC-Chs and BC-Chs-Gp were 843, 867 and 663 g/m^2/day respectively, which were significantly different ($p < 0.05$) from each other. The typical WVRT of a normal skin is 204 g/m^2/day. The WVRT of injured skin varies in broad range of 279 g/m^2/day – 5138 g/m^2/day for first-degree burn and granulated wound, respectively (Lin *et al.*, 2013; Wu *et al.*, 2004). Wu *et al.* 2004 reported that the WVRT of chitosan film was 1063 g/m^2/day while that of cellulose was 880 g/m^2/day. Their study showed that as cellulose and chitosan were combined in different ratios, the blend membranes approached the transpiration of original cellulose (816.864 g/m^2/day). In the present study, the presence of chitosan in the hydrogel led to the observed increase from 843 to 867 g/m^2/day for BC and BC-Chs respectively due to stronger affinity of chitosan to water and due to the larger Van der Waals volume of –NH_2 side group (19 cm^3/mol) when compared to BC with OH side group (13cm^3/mol) (Shieh and Huang, 1998). However, crosslinking with genipin lowered the WVRT to 663 g/m^2/day for BC-Chs-Gp. The WVTR in the present study indicates some level of segregation between chitosan and BC. This is because BC holds its intra-molecular and intra-strand hydrogen bonding to form compact structure in the membrane, which dominated the WVTR. The WVRT values in the present study shows that samples could maintain and retain adequate environment of moisture for low range exudative wounds without leading to excessive dehydration. Since an ideal wound dressing material should prevent both excessive dehydration as well as build-up of exudates, these values are within the range 279-5138 g/m^2/day which could provide an adequate level of moisture without risking wound dehydration (Wu *et al.*, 2004) for low-exudative wounds. This behaviour can be ascribed to the microfibrial nature of these membranes and hydrogel, which aided to retain water efficiently via the strong intermolecular forces of hydrogen bonding.



Figures 5: Physico-chemical properties of BC, BC-Chs membranes and BC-Ch-GP hydrogel (A) water retention capacity (B) water vapour transmission.

Mechanical Properties*:* In furtherance to maintaining adequate moisture environment, potential wound dressing material also, is required to maintain its shape at the time of application. The mechanical properties of the membrane and the hydrogel are shown in Figure 6. The tensile strength for BC was about 64 MPa while its Young's modulus was 39 MPa. A similar study (Lin *et al.*, 2013) reported a tensile strength of 33 MPa for BC which is lower than that found in this study while another study reported tensile strength of 100-260 MPa (Yano *et al.*, 2008) which is higher than that recorded in the present study. Variations in the results from this study and those from reported studies are due to disparities in culture time, supplement used in the medium or post treatment (Lin *et al.*, 2013; Zhijiang *et al.*, 2011). For BC-Chs, the tensile strength and Young's Modulus were about 21 MPa and 53 MPa respectively, while BC-Chs-Gp had tensile strength of about 9 MPa and Young's modulus of about 89 MPa, respectively. The decrease in the tensile strength of BC-Chs-Gp may have occurred due to the crosslinking of NH_2 group in chitosan by genipin. Ideally, when polymer pairs exist in two different phases, the mechanical properties of the composite material are governed by the distribution of the respective polymers within the composite i.e., the properties may be dominated by the higher volume polymer phase. Polymer-rich phase usually forms a continuous matrix, whereas the secondary phase plays the role of reinforcing the matrix by stress transfer between interfaces (Arikibe *et al.*, 2019; Sung *et al.*, 2010). The higher Young's modulus of BC-Chs-Gp hydrogel was attributed to the addition of chitosan and subsequent crosslinking with genipin.

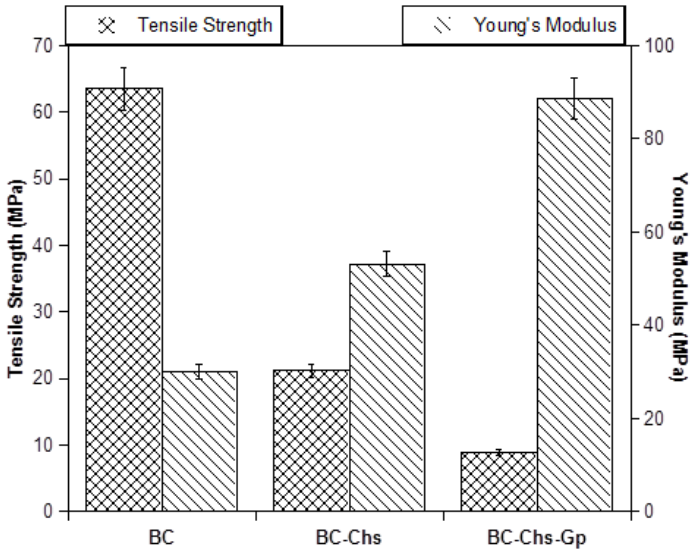


Figure 6: Mechanical properties of membranes: Tensile strength and Young's modulus.

Drug release studies: The drug release behaviour studied for 60 min from the hydrogel shown in Figure 7 A. BC membrane showed burst release within 20 min (data not presented). However, the highest release was observed for BC-Chs (65.12 %) followed by Chs (47.61 %) and then BC-Chs-Gp (41.29 %). There was no significant difference observed in the release behaviour between Chs and BC-Chs and Chs and BC-Chs-Gp ($p > 0.05$). However, BC-Chs and BC-Chs-Gp were significantly different ($p < 0.05$) from each other. The observed percentage of drug released can be attributed to the porosity on

each membrane structure and the extent to which water molecules can penetrate. Evaluation of the drug release kinetics revealed that Chs and BC-Chs-Gp followed the Higuchi model since the r^2 value of the plots were found to be closer to 1 than those of zero order and first order, respectively (Figure 7 B). This suggests that the release was diffusion controlled. However, release from BC-Chs hydrogels followed the first order kinetic implying that release of the drug from the matrix was dependent on concentration. It could be inferred that in BC-Chs-Gp, the presence of Chs and subsequent crosslinking reaction involving genipin, resulted in a more compact structure with less pore sizes, which may be responsible for the observed slow and controlled release. The present study demonstrates that membranes possess the ability to absorb and release drugs as required for application in transdermal patches. The Korsmeyer-Peppas plot showed that the transport mechanism of drug release from the hydrogel matrixes was super case II as the release exponent, n > 1 for Chs (n = 1.08), BC-Chs (n = 1.1) and anomalous or non-Fickian for BC-Chs-Gp (n = 0.90). Interestingly, the drug release behaviour was found to be like that of our previous study[31], even though the preparation method was different; freeze-thaw method. At pH 7, the membranes and the hydrogels followed the Higuchi model. The release exponent and transport mechanism were also in close agreement. This result suggests that the method employed in this study is simple and cost effective to achieve similar results to that of the freeze thaw method.

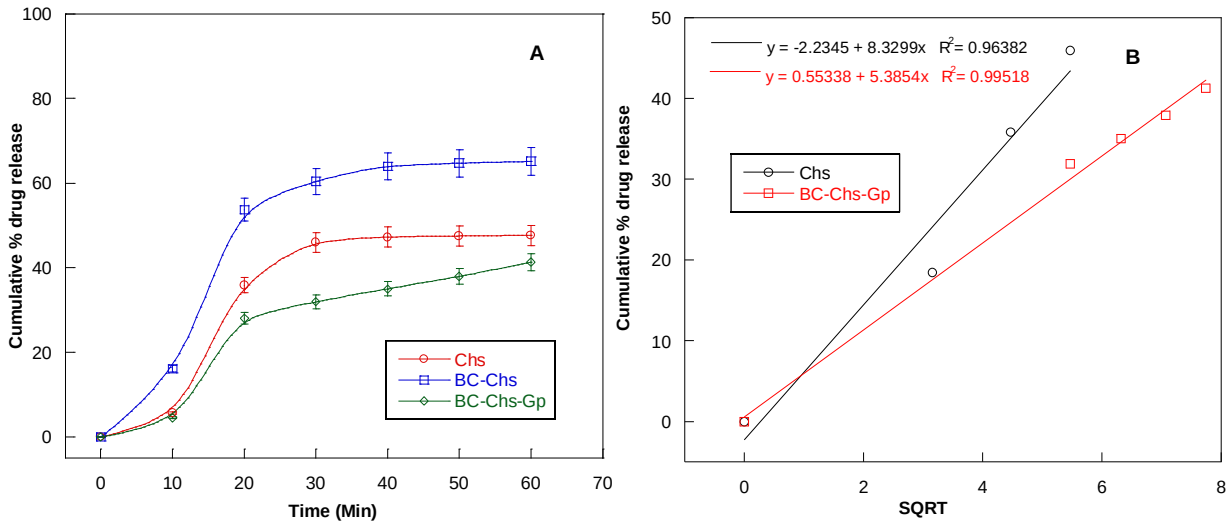


Figure 7: Profiles showing (A) drug release and (B) Higuchi kinetic model for hydrogel and membranes.

Antimicrobial Evaluation: Infections caused by bacteria can have severe adverse effect in wound dressing and the healing process. The intensive use of antibiotics is showing signs of resistance by some bacteria. Quest for new sources of natural materials possessing antimicrobial properties is an emerging area of interest. Chs has been reported to possess antibacterial properties (Hamedi *et al.*, 2018); therefore, modification of BC with Chs will decrease bacterial growth and activity on BC-Chs membrane and BC-Chs-Gp hydrogel. In the present study, the number of colony of bacteria obtained is presented on a scale of logarithm (Figure 8 A and B). It was observed that BC, BC-Chs and BC-Chs-Gp recorded a

decline in viable *E. coli* of 39%, 95% and 93%, respectively. In the case of *S. aureus*, 26%, 96% and 92% of bacterial growth inhibition was recorded on BC, BC-Chs and BC-Chs-Gp, respectively. BC-Chs and BC-Chs-Gp showed significant action of inhibition against *E. coli* and *S. aureus*, which are Gram-negative and Gram-positive bacteria, respectively. BC-Chs membrane showed a higher antibacterial activity than BC-Chs-Gp hydrogel. This may be ascribed to the effect of crosslinking Chs with genipin, which reduced the amount of amino group (NH_2) on Chs. There are two proposed mechanisms under which the antibacterial action of Chs falls and are related to the amount of active amino groups. (Goy *et al.,* 2009) The first mechanisms suggests that, there would be an interaction between the positively charged Chs and negatively charged surface of the bacteria, resulting to permeability of the bacterial membrane been raised and inhibition in cell growth. In the other mechanism, it suggests that there may be suppression of the mRNA generation of bacteria due to the Chs binding with the DNA of the bacteria. Furthermore, the decline in viable counts for *E. coli* and *S. aureus* for BC (38.6% and 26.3) may be due to the roughness of the surface, which made it difficult for the bacteria to adhere properly. The antibacterial activity recorded in this study is lower than that reported in a similar study, (W.-C. Lin *et al.,* 2013) where BC and BC-Chs recorded 49.2% and 99.9% reduction on *E. coli* and 30.4% and 99.9% *S. aureus* reduction, respectively. The attribution could be due to the difference in the method of preparation and the amount of incorporated chitosan onto the BC fibril network (Arikibe *et al.,* 2019).

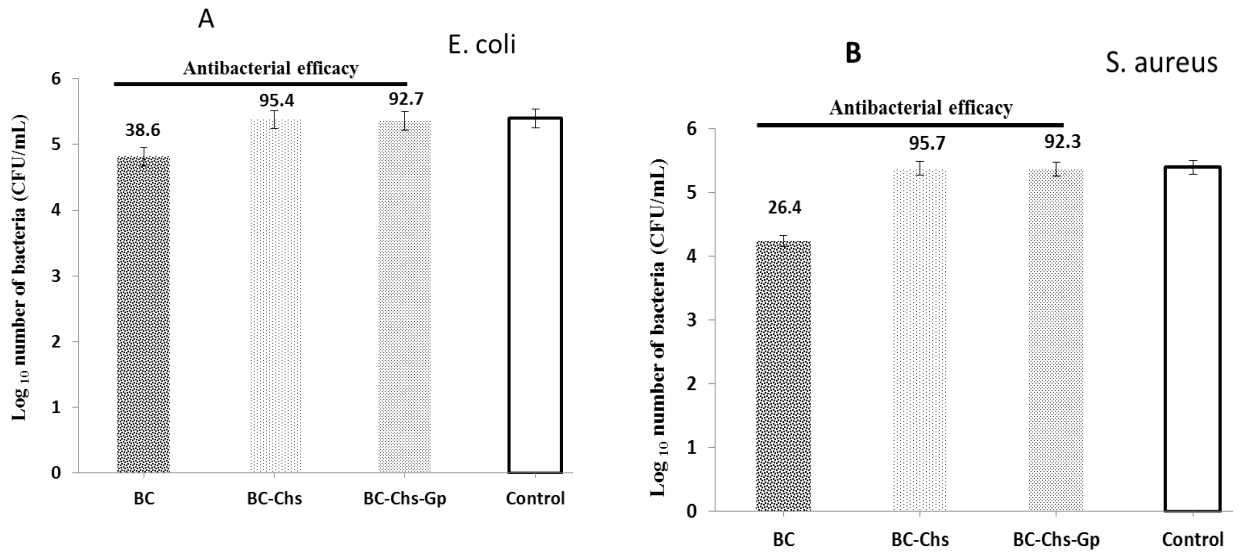


Figure 8. Antibacterial action of BC, BC-Chs and BC-Ch-GP membranes against (A) *E. coli* and (B) *S. aureus.*

The diffusion disc method employed showed slight inhibition zones in the BC-Chs samples except for the control. Since the BC-Chs membrane obtained after the synthesis, washed repeatedly with acetic acid, allowed all free Chs adhering to the surface of the membrane removed. Only the chitosan being deeply incorporated into the BC fibrils during *in situ* modification was present in the membrane. The amount of amino group on Chs was further decreased through crosslinking. Thus, no significant inhibition zone on the agar plate

was observed. Interestingly, synthesis of BC-Chs was achieved without any inhibition action by Chs on *G. xylinus.* This was expected because *G. xylinus*, is an acetic acid bacteria and the resultant effect was that its activity/viability was enhanced in the cell culture. Thus, the dissolution of Chs in acetic acid (pH 4.5-5) resulted in Chs having insignificant activity/or viability against the *G. xylinus* in the cell culture.

CONCLUSION AND APPLICATION OF RESULTS

Interpenetrating BC-Chs membrane was successfully produced *in situ* during synthesis of BC using *G. xylinus*. BC-Chs membrane was further modified *ex situ* by crosslinking the imbedded Chs in the membrane matrix with genipin to obtain semi-interpenetrating hydrogel. The incorporation of Chs into the synthesized BC fibrils was confirmed by FTIR on the emergence of amide I and II peaks. Swelling ratio decreased for the BC-Chs-Gp hydrogel compared to BC-Chs membranes because of decreased porosity and increased compactness in the structure due to crosslinking of the Chs chains. Water retention and water vapour transmission of the hydrogel showed promising potential for application in wound dressing for low exudative wounds. The tensile strength of the membrane and the hydrogel were lower than their Young's modulus. The hydrogel showed antibacterial activity against *E. coli* and *S. aureus. In vitro* drug release studies of quetiapine fumarate in medium of pH 7 over 24 hrs followed the Higuchi model. The Korsmeyer-Peppas plot showed that the transport mechanism of drug release was super case II as the release exponent, n, was greater than 1 for Chs and BC-Chs while anomalous or non-Fickian for BC-Chs-Gp. The BC-Ch-Gp hydrogel showed promising results for biomedical applications.

ACKNOWLEDGEMENT

The authors are grateful to the University of the South Pacific (USP), Suva, Fiji providing resources to conduct this research.

Conflict of interest

Authors declare no conflict of interest.

REFERENCES

Ajji, Z., Othman, I., Rosiak, J.M., 2005. Production of hydrogel wound dressings using gamma radiation. Nucl. Instrum. Methods Phys. Res. Sect. B Beam Interact. Mater. At. 229, 375–380. https://doi.org/10.1016/j.nimb.2004.12.135

Aramwit, P., Siritienthong, T., Srichana, T., Ratanavaraporn, J., 2013. Accelerated Healing of Full-Thickness Wounds by Genipin-Crosslinked Silk Sericin/PVA Scaffolds. Cells Tissues Organs 197, 224–238. https://doi.org/10.1159/000345600

Arikibe, J.E., Lata, R., Rohindra, D., 2020. Synthesis, Preparation and Characterization of Amine-Induced Bacterial Cellulose-Poly(Vinyl Alcohol) Semi-Interpenetrating Network Hydrogel. Key Eng. Mater. 841, 238–242. https://doi.org/10.4028/www.scientific.net/KEM.841.238

Arikibe, J.E., Lata, R., Kuboyama, K., Ougizawa, T., Rohindra, D., 2019. pH-Responsive Studies of Bacterial Cellulose /Chitosan Hydrogels Crosslinked with Genipin: Swelling and Drug Release Behaviour.

Chemistry Select 4, 9915–9926. https://doi.org/10.1002/slct.201902290

Carvalho, T., Guedes, G., Sousa, F.L., Freire, C.S.R., Santos, H.A., 2019. Latest Advances on Bacterial Cellulose-Based Materials for Wound Healing, Delivery Systems, and Tissue Engineering. Biotechnol. J. 1900059. https://doi.org/10.1002/biot.201900059

Chang, S.-T., Chen, L.-C., Lin, S.-B., Chen, H.-H., 2012. Nano-biomaterials application: Morphology and physical properties of bacterial cellulose/gelatin composites via crosslinking. Food Hydrocoll. 27, 137–144. https://doi.org/10.1016/j.foodhyd.2011.08.004

Czaja, W.K., Young, D.J., Kawecki, M., Brown, R.M., 2007. The Future Prospects of Microbial Cellulose in Biomedical Applications. Biomacromolecules 8, 1–12. https://doi.org/10.1021/bm060620d

De Azeredo, H.M.C., Rosa, M.F., De Sá, M., Souza Filho, M., Waldron, K.W., 2014. The use of biomass for packaging films and coatings, in: Advances in Biorefineries. Elsevier, pp. 819–874. https://doi.org/10.1533/9780857097385.2.819

Dreiss, C.A., 2020. Hydrogel design strategies for drug delivery. Curr. Opin. Colloid Interface Sci. 48, 1–17. https://doi.org/10.1016/j.cocis.2020.02.001

El Knidri, H., Belaabed, R., Addaou, A., Laajeb, A., Lahsini, A., 2018. Extraction, chemical modification and characterization of chitin and chitosan. Int. J. Biol. Macromol. 120, 1181–1189. https://doi.org/10.1016/j.ijbiomac.2018.08.139

Esuendale, D., Gabriel, T., 2016. Cellulosic on Transdermal Drug Delivery System: A Review. J. Drug Deliv. Ther. 6, 57–64. https://doi.org/10.22270/jddt.v6i5.1275

Feng, Y., Zhang, X., Shen, Y., Yoshino, K., Feng, W., 2012. A mechanically strong, flexible and conductive film based on bacterial cellulose/graphene nanocomposite. Carbohydr. Polym. 87, 644–649. https://doi.org/10.1016/j.carbpol.2011.08.039

Goy, R.C., Britto, D. de, Assis, O.B.G., 2009. A review of the antimicrobial activity of chitosan. Polímeros 19, 241–247. https://doi.org/10.1590/S0104-14282009000300013

Hamdi, M., Nasri, R., Li, S., Nasri, M., 2020. Bioinspired pH-sensitive riboflavin controlled-release alkaline hydrogels based on blue crab chitosan: Study of the effect of polymer characteristics. Int. J. Biol. Macromol. 152, 1252–1264. https://doi.org/10.1016/j.ijbiomac.2019.10.222

Hamedi, H., Moradi, S., Hudson, S.M., Tonelli, A.E., 2018. Chitosan based hydrogels and their applications for drug delivery in wound dressings: A review. Carbohydr. Polym. 199, 445–460. https://doi.org/10.1016/j.carbpol.2018.06.114

Hu, Y., Catchmark, J.M., 2010. Formation and Characterization of Spherelike Bacterial Cellulose Particles Produced by Acetobacter xylinum JCM 9730 Strain. Biomacromolecules 11, 1727–1734. https://doi.org/10.1021/bm100060v

Huang, H.-C., Chen, L.-C., Lin, S.-B., Hsu, C.-P., Chen, H.-H., 2010. In situ modification of bacterial cellulose network structure by adding interfering substances during fermentation. Bioresour. Technol. 101, 6084–6091.

https://doi.org/10.1016/j.biortech.2010.03.031

Khurma, J.R., Rohindra, D.R., Nand, A.V., 2005. Swelling and Thermal Characteristics of Genipin Crosslinked Chitosan and Poly (vinyl pyrrolidone) Hydrogels. Polym. Bull. 54, 195–204. https://doi.org/10.1007/s00289-005-0375-4

Lin, S.-P., Loira Calvar, I., Catchmark, J.M., Liu, J.-R., Demirci, A., Cheng, K.-C., 2013. Biosynthesis, production and applications of bacterial cellulose. Cellulose 20, 2191–2219. https://doi.org/10.1007/s10570-013-9994-3

Lin, W.-C., Lien, C.-C., Yeh, H.-J., Yu, C.-M., Hsu, S., 2013. Bacterial cellulose and bacterial cellulose–chitosan membranes for wound dressing applications. Carbohydr. Polym. 94, 603–611. https://doi.org/10.1016/j.carbpol.2013.01.076

Liu, W., Du, H., Zhang, M., Liu, K., Liu, H., Xie, H., Zhang, X., Si, C., 2020. Bacterial Cellulose-Based Composite Scaffolds for Biomedical Applications: A Review. ACS Sustain. Chem. Eng. 8, 7536–7562. https://doi.org/10.1021/acssuschemeng.0c00125

Mihaela Jipa, I., Dobre, L., Stroescu, M., Stoica-Guzun, A., Jinga, S., Dobre, T., 2012. Preparation and characterization of bacterial cellulose-poly (vinyl alcohol) films with antimicrobial properties. Mater. Lett. 66, 125–127. https://doi.org/10.1016/j.matlet.2011.08.047

Mittal, H., Ray, S.S., Kaith, B.S., Bhatia, J.K., Sukriti, Sharma, J., Alhassan, S.M., 2018. Recent progress in the structural modification of chitosan for applications in diversified biomedical fields. Eur. Polym. J. 109, 402–434. https://doi.org/10.1016/j.eurpolymj.2018.10.013

Muzzarelli, R.A.A., 2009. Genipin-crosslinked chitosan hydrogels as biomedical and pharmaceutical aids. Carbohydr. Polym. 77, 1–9. https://doi.org/10.1016/j.carbpol.2009.01.016

Oliveira Barud, H.G., Barud, H. da S., Cavicchioli, M., do Amaral, T.S., Junior, O.B. de O., Santos, D.M., Petersen, A.L. de O.A., Celes, F., Borges, V.M., de Oliveira, C.I., de Oliveira, P.F., Furtado, R.A., Tavares, D.C., Ribeiro, S.J.L., 2015. Preparation and characterization of a bacterial cellulose/silk fibroin sponge scaffold for tissue regeneration. Carbohydr. Polym. 128, 41–51. https://doi.org/10.1016/j.carbpol.2015.04.007

Omidi, S., Pirhayati, M., Kakanejadifard, A., 2020. Co-delivery of doxorubicin and curcumin by a pH-sensitive, injectable, and in situ hydrogel composed of chitosan, graphene, and cellulose nanowhisker. Carbohydr. Polym. 231, 115745. https://doi.org/10.1016/j.carbpol.2019.115745

Pa'e, N., Hui, C.C., Muhamad, I.I., 2007. Shaken culture fermentation for production of microbial cellulose from pineapple waste, in: International Conference on Waste to Wealth. Presented at the International Conference on Waste to Wealth, Putra World Trade Centre (PWTC) Kaula Lumpur, Malaysia, pp. 1–10.

Pal, K., Paulson, A.T., Rousseau, D., 2013. Biopolymers in Controlled-Release Delivery Systems, in: Handbook of Biopolymers and Biodegradable Plastics. Elsevier, pp. 329–363. https://doi.org/10.1016/B978-1-4557-2834-3.00014-8

Perotti G.F., Barud, H.S., Messaddeq, Y., Ribeiro, S.J.L., Constantino, V.R.L., 2011. Bacterial cellulose–laponite clay nanocomposites. Polymer 52, 157–163. https://doi.org/10.1016/j.polymer.2010.10.062

Portela, R., Leal, C.R., Almeida, P.L., Sobral, R.G., 2019. Bacterial cellulose: a versatile biopolymer for wound dressing applications. Microb. Biotechnol. 12, 586–610. https://doi.org/10.1111/1751-7915.13392

Romanov, D.P., Khripunov, A.K., Baklagina, Yu.G., Severin, A.V., Lukasheva, N.V., Tolmachev, D.A., Lavrent'ev, V.K., Tkachenko, A.A., Arkharova, N.A., Klechkovskaya, V.V., 2014. Nanotextures of composites based on the interaction between hydroxyapatite and cellulose Gluconacetobacter xylinus. Glass Phys. Chem. 40, 367–374. https://doi.org/10.1134/s1087659614030183

Saibuatong, O., Phisalaphong, M., 2010. Novo aloe vera–bacterial cellulose composite film from biosynthesis. Carbohydr. Polym. 79, 455–460. https://doi.org/10.1016/j.carbpol.2009.08.039

Saska, S., Teixeira, L.N., Tambasco de Oliveira, P., Minarelli Gaspar, A.M., Lima Ribeiro, S.J., Messaddeq, Y., Marchetto, R., 2012. Bacterial cellulose-collagen nanocomposite for bone tissue engineering. J. Mater. Chem. 22, 22102. https://doi.org/10.1039/c2jm33762b

Shao, W., Liu, H., Liu, X., Wang, S., Wu, J., Zhang, R., Min, H., Huang, M., 2015. Development of silver sulfadiazine loaded bacterial cellulose/sodium alginate composite films with enhanced antibacterial property. Carbohydr. Polym. 132, 351–358. https://doi.org/10.1016/j.carbpol.2015.06.057

Shariatinia, Z., 2019. Pharmaceutical applications of chitosan. Adv. Colloid Interface Sci. 263, 131–194. https://doi.org/10.1016/j.cis.2018.11.008

Shezad, O., Khan, S., Khan, T., Park, J.K., 2010. Physicochemical and mechanical characterization of bacterial cellulose produced with an excellent productivity in static conditions using a simple fed-batch cultivation strategy. Carbohydr. Polym. 82, 173–180. https://doi.org/10.1016/j.carbpol.2010.04.052

Shieh, J.-J., Huang, R.Y.M., 1998. Chitosan/N-methylol nylon 6 blend membranes for the pervaporation separation of ethanol–water mixtures. J. Membr. Sci. 148, 243–255. https://doi.org/10.1016/S0376-7388(98)00165-3

Silva, N.H.C.S., Rodrigues, A.F., Almeida, I.F., Costa, P.C., Rosado, C., Neto, C.P., Silvestre, A.J.D., Freire, C.S.R., 2014. Bacterial cellulose membranes as transdermal delivery systems for diclofenac: In vitro dissolution and permeation studies. Carbohydr. Polym. 106, 264–269. https://doi.org/10.1016/j.carbpol.2014.02.014

Stumpf, T.R., Yang, X., Zhang, J., Cao, X., 2018. In situ and ex situ modifications of bacterial cellulose for applications in tissue engineering. Mater. Sci. Eng. C 82, 372–383. https://doi.org/10.1016/j.msec.2016.11.121

Sung, J.H., Hwang, M.-R., Kim, J.O., Lee, J.H., Kim, Y.I., Kim, J.H., Chang, S.W., Jin, S.G., Kim, J.A., Lyoo, W.S., 2010. Gel characterisation and in vivo evaluation of minocycline-loaded

wound dressing with enhanced wound healing using polyvinyl alcohol and chitosan. Int. J. Pharm. 392, 232–240. https://doi.org/10.1016/j.ijpharm.2010.03.024

Tsalagkas, D., Lagaňa, R., Poljanšek, I., Oven, P., Csoka, L., 2016. Fabrication of bacterial cellulose thin films self-assembled from sonochemically prepared nanofibrils and its characterization. Ultrason. Sonochem. 28, 136–143. https://doi.org/10.1016/j.ultsonch.2015.07.010

Ul-Islam, M., Khan, T., Park, J.K., 2012. Water holding and release properties of bacterial cellulose obtained by in situ and ex situ modification. Carbohydr. Polym. 88, 596–603. https://doi.org/10.1016/j.carbpol.2012.01.006

Wei, B., Yang, G., Hong, F., 2011. Preparation and evaluation of a kind of bacterial cellulose dry films with antibacterial properties. Carbohydr. Polym. 84, 533–538. https://doi.org/10.1016/j.carbpol.2010.12.017

Wu, Y.-B., Yu, S.-H., Mi, F.-L., Wu, C.-W., Shyu, S.-S., Peng, C.-K., Chao, A.-C., 2004. Preparation and characterization on mechanical and antibacterial properties of chitsoan/cellulose blends. Carbohydr. Polym. 57, 435–440. https://doi.org/10.1016/j.carbpol.2004.05.013

Yano, S., Maeda, H., Nakajima, M., Hagiwara, T., Sawaguchi, T., 2008. Preparation and mechanical properties of bacterial cellulose nanocomposites loaded with silica nanoparticles. Cellulose 15, 111–120. https://doi.org/10.1007/s10570-007-9152-x

Zhijiang, C., Chengwei, H., Guang, Y., 2011. Retracted: Preparation and characterization of a bacterial cellulose/chitosan composite for potential biomedical application. J. Appl. Polym. Sci. 121, 1488–1494. https://doi.org/10.1002/app.33661