JOURNAL OF SUSTAINABLE MATERIALS PROCESSING AND MANAGEMENT
e-ISSN: 2821-2843

JSMPM

Vol. 4 No. 2 (2024) 77-85
https://publisher.uthm.edu.my/ojs/index.php/jsmpm

Production of Biocompatible Bacterial Cellulose-Chitosan Composite from Nata de Coco (*Acetobacter xylinum*) and Squid Gladius (*Sepioteuthis lessoniana*)

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DOI: https://doi.org/10.30880/jsmpm.2024.04.02.008

Article Info

Received: 12 October 2024
Accepted: 24 October 2024
Available online: 17 October 2024

Keywords

Bacterial cellulose, chitosan, simulated body fluid, hydroxyapatite crystals

Abstract

The increasing demand of sustainable biocompatible composites of bacterial cellulose (BC) and chitosan (C) has grown much attention in the past years. In this study, a biocompatible bacterial cellulose–chitosan composite (BCC) was produced from Nata de Coco (NC) and Squid Gladius (SG) as the source of BC and C respectively. The effects of varying chitosan concentrations (2%, 5%, 8% w/v) on the composite's physicochemical properties—including morphology, water holding capacity, and tensile strength—along with its biocompatibility in Simulated Body Fluid (SBF), were investigated. Scanning Electron Microscopy (SEM) revealed that the BCC composite exhibited a denser and more homogenized structure compared to the native BC pellicle. Fourier Transform Infrared Spectroscopy (FTIR) analysis showed characteristic spectra for the pellicles between 2800 and 1200 cm^{-1}, with distinct bands for amide groups observed at 1613, 1550, and 1337 cm^{-1}, indicating the presence of chitosan. Among the composites, the BCC containing 8% w/v chitosan demonstrated the highest water holding capacity (581%), while the composite with 5% w/v chitosan exhibited superior tensile strength. SEM images of BCC samples subjected to the SBF process showed gradual hydroxyapatite (HAp) crystal formation, with a Ca-P ratio approaching 1.67 by the third day, as confirmed by X-ray Fluorescence (XRF), indicating promising biocompatibility. FTIR analysis also revealed typical absorption bands of (HAp), including peaks at 3555 and 622 cm^{-1} corresponding to the stretching and liberation modes of hydrogen-bonded ions, respectively. The findings of this study highlight the potential of producing biopolymers from biomass sources, paving the way for their application in wound dressing materials and other biomedical uses.

1. Introduction

Cellulose is widely known to be the most copious biopolymer present in nature. Originally, cellulose is extracted from plants and their wastes. Although plants are the major provider of cellulose, bacteria, fungi and algae can also be considered as substitutes in cellulose production. But among these, cellulose synthesized by *Acetobacter Xylinum* bacteria or bacterial cellulose has acquired the attention of many scientists due to its distinctive physical and mechanical properties and has been extensively studied for the past decades. *Acetobacter Xylinum*

is the most efficient BC producer that manages to integrate various sugars and yields high quality cellulose in liquid medium [1].

It was reported that the distinctive nanofibrillar structure of BC can become a perfect matrix as an optimal wound healing environment [2]. Particularly notable is the fact that the size of BC fibrils is about 100 times smaller than that of plant cellulose. Its exceptional morphology results in a large surface area that can store a large amount of water and at the same time displays great elasticity, high wet strength and conformability [3]. The small size of BC fibrils seems to one of the key factors that determines its remarkable performance as a wound healing system. Moreover, its membrane is a highly nanoporous material that allows for the potential transfer of drugs (antibiotics etc.) into the wound while also serving as a proficient barrier against any outside infection.

Although BC has its unique properties there still exists limitations that inhibits its applications such as lack of antibacterial properties, optical transparency and stress bearing capability. To overcome these limitations, BC composites have been introduced. Different ways in improving BC have already been devised in past researches, as such is the preparation of composites with various biomaterials –one of which is the integration of Chitosan to BC [4,5].

Sepioteuthis lessoniana, commonly known as the oval squid, is a species found primarily in tropical and subtropical waters of the Indo-Pacific region. It has been recognized as a viable source of chitosan, a biopolymer derived from the exoskeletons of crustaceans and mollusks. Chitosan had been incorporated to BC in order to improve some of the properties of the original BC structures. The cellulose produced was highly transparent, flexible and displayed better mechanical properties. The morphological analysis performed suggested that chitosan molecules can go through the BC forming a three-dimensional multilayer structure scaffold [6]. Also, chitosan has shown to improve other BC's important properties such as the water holding capacity and the water release rate of BC.

Biocompatibility does not only refer to the quality of not having toxic effects on biological systems, but also to the need of having an appropriate reaction to ensure satisfactory performance on a specific application. Biocompatibility of BC for wound dressings and tissue engineering can then be accounted for its unique physical properties and similarity in structure with the extracellular matrix components such as collagen. The porosity of BC networks can influence the response of cells. In the case of BC its loose fibril arrangement, high surface area per unit mass and hydrophilic nature results in a very high water holding capacity thus accelerating the wound healing pro-cess [7]. As for BCC composite, biocompatibility was evaluated by cell adhesion studies. Cell adhesion and proliferation was achieved and in general, BCC composites showed a much better biocompatibility than pure BC.

Acetobacter xylinum has been proven that it has the capability to replace plant as another source of cellulose through oxidative fermentation under static and agitated conditions. However, the high-cost and low-yield productions have limited its commercialization. According to Gorgieva and Trček [2], the final price of these biomaterials will strongly depend on the efficient strains which do not undergo changes over time, nutrition media that are based on inexpensive sources of carbon and nitrogen, and the efficient large-scale fermentation processes. In addition to that, economical composite sources are also required for further commercialization of these biomaterials. Hence, researchers are now tasked to focus mainly on agricultural wastes and industrial by-products as new cost-effective raw material sources [8].

Recent studies on the synthesis of bacterial cellulose (BC) and chitosan composites have explored various methods to enhance their properties for biomedical and industrial applications. For instance, Zao et al. synthesized via biological self-generation process and in situ reduction of a silver (Ag)-polydopamine (PDA)/bacterial cellulose (BC)-chitosan (CTS) composite film with strong antibacterial properties, high biocompatibility, and structural integrity [9]. In addition, a green approach for production of BCC composite in the presence of carbonic acid under high pressure CO_2 was reported by Novikov et al. highlighting that the chitosan impregnation increased the specific surface area of the material [10]. On the other hand, a chemical approach was done wherein an antimicrobial film were produced by combining a bacterial cellulose (BC) suspension as the base material, chitosan for its natural antimicrobial properties, carboxymethyl cellulose (CMC) to ensure uniform distribution, and glycerol to enhance flexibility [11]. Finally, facile method was used to create a series of zwitterionic hydrogels made from bacterial cellulose (BC) and chitosan, where the hydrogels formed in situ through a Schiff base reaction between partially oxidized BC and chitosan, resulting in materials with good mechanical properties. In this study, a direct slow mixing approach fermented BC and extracted C was employed to ensure mechanically stable composite with antimicrobial property.

2. Materials and Methods

2.1 Extraction of Chitosan

The extraction of chitosan was adapted from the work of Li et al. with modification [12]. The squid gladius (*S. lessoniana*) was boiled with 3% NaOH (analytical grade, ≥97.0%, Sigma-Aldrich, St. Louis, Missouri, USA) for 30 minutes to eliminate proteins. The materials were cooled and washed with deionized water to remove all traces of alkali. The deproteinized shells were transferred to a mild steel vessel lined with fiber glass and were treated with 3 wt% HCl (analytical grade, 98%, Sigma-Aldrich, St. Louis, Missouri, USA) for 30 minutes with occasional stirring to eliminate minerals. The excess acid was decanted and the residue was washed until the pH level is normal. Next, the excess water was removed using a screw press, and chitin was obtained. Lastly, the chitin was heated at 90-95°C for about 90 minutes with 40 wt% NaOH (analytical grade, ≥97.0%, Sigma-Aldrich, St. Louis, Missouri, USA) in a mild steel vessel. Chitosan was obtained after the removal of the water.

2.2 Preparation of Bacterial Cellulose (BC) and BCC Composite (BCC)

The preparation of the BC and C composite was adapted from the work of Ambaye at al. with modification [13]. The A*cetobacter xylinum* was used as the acetic acid bacteria strain for the production of the bacterial cellulose. Freshly collected coconut water was filtered using a clean cheesecloth. It was then added by 550 g of sugar forming a 5L sugar-coconut water solution. The solution was transferred to a clean and heat resistant glass jar covered with clean cheesecloth. It was then pasteurized by heating in a water bath for 15-20 minutes and allowed to cool to room temperature. Then glacial acetic acid was added (8 mL per liter of coconut water) and nata starter (20% of the volume of coconut water solution). The containers were covered with sheets of paper and left undisturbed for 7-10 days to ferment at 30-32°C forming some bacterial cellulose (BC) pellicles. Harvesting was done when the pellicles reached the approximate size of 3-7 mm in thickness, 400mm in length and 250mm in width.

The BC pellicles were collected and rinsed with deionized water several times in order to remove the remaining bacteria and nutrients. The wet BC pellicles obtained were sterilized by an autoclave and compressed to remove excess water by a compressor and freeze dried and stored at room temperature prior to use. Chitosan was dissolved in 1% citric acid aqueous solution. Different amounts of chitosan were used to produce 2%, 5%, 8% concentrations of Chitosan. The BC pellicles were immersed in the different Chitosan solution for 12 hours forming the BCC. The end products were freeze-dried.

2.3 Characterization of BC and BCC Composite

The BC and BCC were characterized according to their morphology depending on the effect of the chitosan contents, water holding capacity, and their tensile. The sur-face morphology of the BC and BCC pellicles were analyzed with a Jeol 5300 scanning electron microscope (SEM). The chemical structure of BC and BCC pellicles were analyzed by Fourier transform infrared spectrophotometer (FTIR). Using a Spectrum 2 FT-IR at the Chemistry Laboratory in Adamson University, FT-IR spectra were recorded from KBr pellets at room temperature. The data were collected over 20 scans at 0.5cm^{-1} resolution and were analyzed over the range of 550-4000 cm^{-1}.

The water retention ratio or degree of swelling is an experimental measure of the capacity of the fiber to hold water where calculated using equation 1. While the moisture content property is determine using equation 2. Finally, Tensile test specimens were prepared by cutting the pellicles to 10 mm wide and 65 mm long strips using a precise cutter. Young's modulus of samples was determined from the tensile test conducted according to ASTM D-882-97 as a standard test method for tensile elastic properties of thin sheeting.

$$Water\ Retention\ Ratio\ (\%) = \frac{W_{wet} - W_{dry}}{W_{dry}} \times 100 \quad (1)$$

$$Moisture\ Content\ Ratio\ (\%) = \left(\frac{W_{Wet} - W_{Dry}}{W_{Wet}}\right) x\ 100 \quad (2)$$

2.4 Biocompatibility Test via Simulated Body Fluid

Simulated body fluid was used to determine the biocompatibility of the BCC composite base on the protocol established by Kokubo et al. [9]. The best concentration of the BCC was soaked in different time variations of 1, 2, and 3 days. After soaking the samples were freeze dried and undergone SEM-EDX and FT-IR for morphological and elemental effects determination.

3. Results and Discussion

3.1 Effect of Chitosan on the Morphology of BCC

SEM images of the BCC at different chitosan content at 5000 magnifications is shown in Fig. 1. It can be observed that increasing the chitosan content from 0 % to 8 % shown a significantly denser and more homogenous structure as the concentrations of Chitosan increases. The chitosan penetrated into the pores of bacterial cellulose and interact with the microfibrils, which affected the physicochemical properties of the pellicles [10]. The BCC pellicles had appeared to be thicker due to the coverage by a thick layer of Chitosan. Also, the porous structure remains while the pore size becomes much bigger [11]. It can also be noted that in the BC and BCC composite has well interconnected pore network structure and large surface area necessary for better cellular attachment.

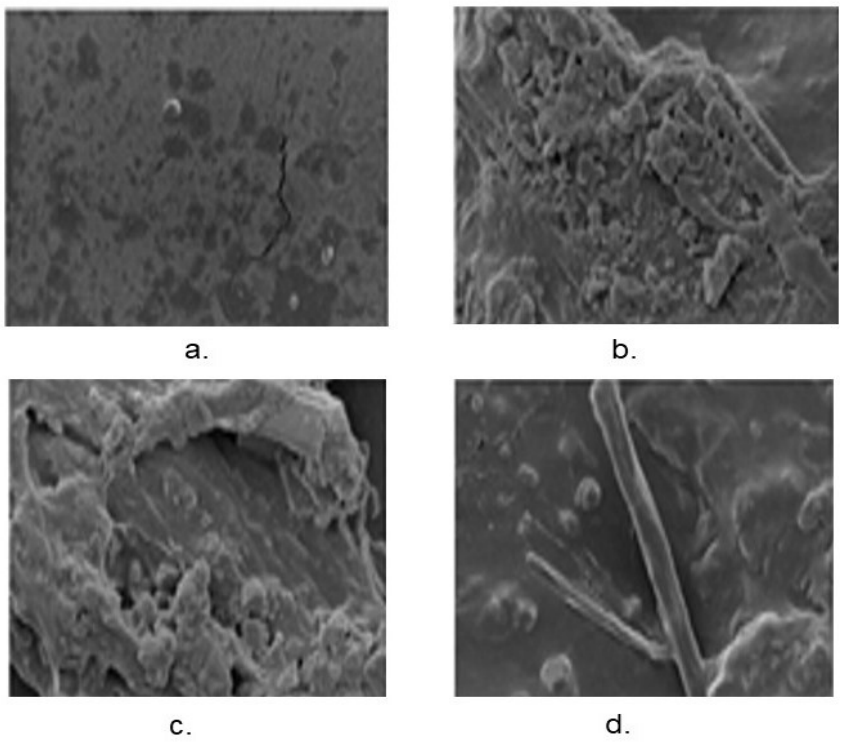

Fig. 1 *SEM images of Surface morphology of the pellicles in 5,000 magnifications, (a) BC –0% Chitosan; (b) BCC-2% Chitosan; (c) BCC -5% Chitosan; (d) BCC-8% Chitosan*

The FTIR (Fourier-transform infrared) spectra of BC (bacterial cellulose) and BCC (bacterial cellulose composite) at varying chitosan concentrations illustrate distinct spectral features attributed to both cellulose and chitosan is presented in Fig. 2. A broad absorption band at 1613 cm^{-1} (amide I), 1550 cm^{-1} (amide II), and 1377 cm^{-1} (amide III) was observed, which corresponds to the characteristic amide groups present in chitosan. These amide groups represent the peptide linkages that form the backbone of chitosan, indicating its presence and interaction within the composite. Additionally, a significant band around 1640 cm^{-1} was assigned to the glucose carbonyl group in cellulose. In particular, for the BCC sample containing 5% chitosan, an intense peak at 1639.1 cm^{-1} was recorded, further confirming the glucose carbonyl group of cellulose. This indicates that the carbonyl functionality in cellulose is well-preserved even in the presence of chitosan.

Moreover, the chitosan-specific absorption bands around 1556 cm^{-1}, which are attributed to the amino groups of chitosan, showed a slight shift with increasing concentrations of chitosan in the composite. With the addition of 2%, 5%, and 8% chitosan, the band shifted from 1556 to 1560 cm^{-1} and 1559.8 cm^{-1}, respectively. This shift suggests an intermolecular hydrogen bonding interaction between the amino groups in chitosan and the hydroxyl groups of cellulose, which likely enhances the overall structural integrity of the composite.

These findings imply that as the concentration of chitosan increases, its interaction with cellulose becomes more pronounced, likely resulting in improved properties of the pellicle formed in the BCC compared to the pure bacterial cellulose sample. The hydrogen bonding between the two components contributes to better composite formation and performance, potentially yielding a more stable material for biomedical applications.

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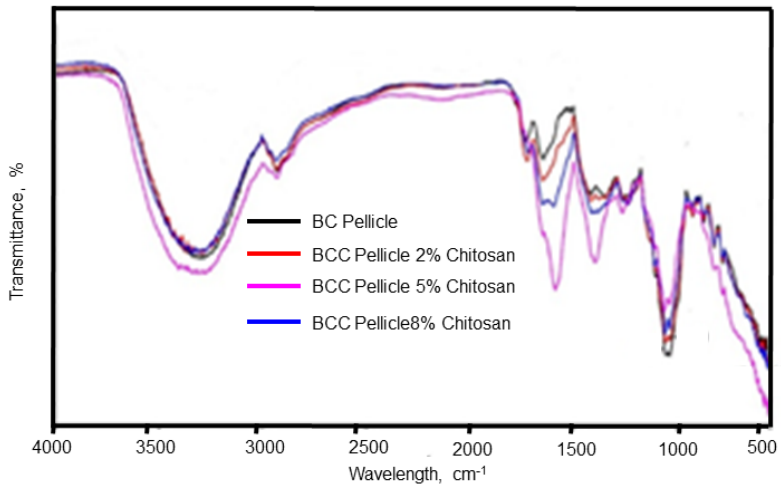

Fig. 2 *FTIR analysis of characteristic bands for BC and BCC composite, (a) BC pellicle; (b) BCC -2% Chitosan; (c) BCC -5% Chitosan; (d) BCC -8% Chitosan*

3.2 Effect of Chitosan on the Moisture Content and Water Retention of BCC

The mean moisture content (MC) and water retention (WR) ratio of BCC at different chitosan concentration as illustrated in Fig. 3. It was observed that a sudden increase of MC (Fig. 3a) was observed when the chitosan content was increased from 0 to 2%, and a steady MC increase as the chitosan concentration was increased to 8 %. This was due to the increasing homogeneity of the BCC in terms of chitosan accumulation in each pellicle resulting to the increase of water molecule in the composite [13]. In addition, all BBC exhibit increasing moisture content as the chitosan concentration increases resulting to increasing trend of water retention (Fig. 3b). The water absorption of the composite is mainly due to the cellulosic material. This increases the rates of water uptake by forming the hydrogen bonding between water and the hydroxyl group from cellulosic cell wall fiber [14].

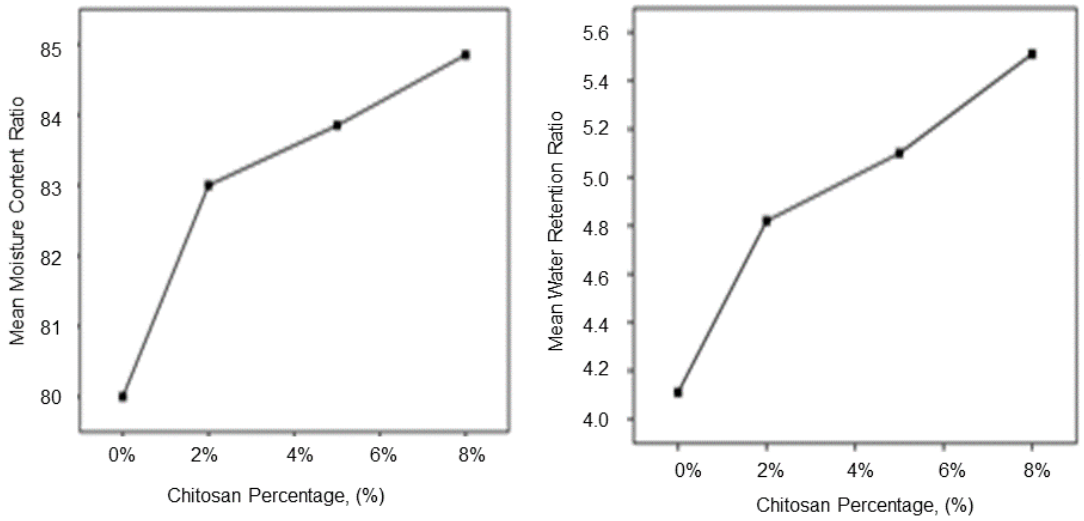

Fig. 3 *Data for (a) moisture content ratio; and (b) water retention of BCC at different chitosan concentration*

3.3 Effect of Chitosan on the Tensile Strength of BCC

It was observed that the tensile strength is directly affected by the amount of chitosan used for the production of composites (Fig. 4). The highest tensile strength recorded was 8.15 MPa with 5% chitosan content. This is because chitosan has excellent film forming capability resulting to stable hydrogen bonding with the cellulose. Also, it was observed that the composites endure more force than the negative cellulose due to their denser and thicker structure.

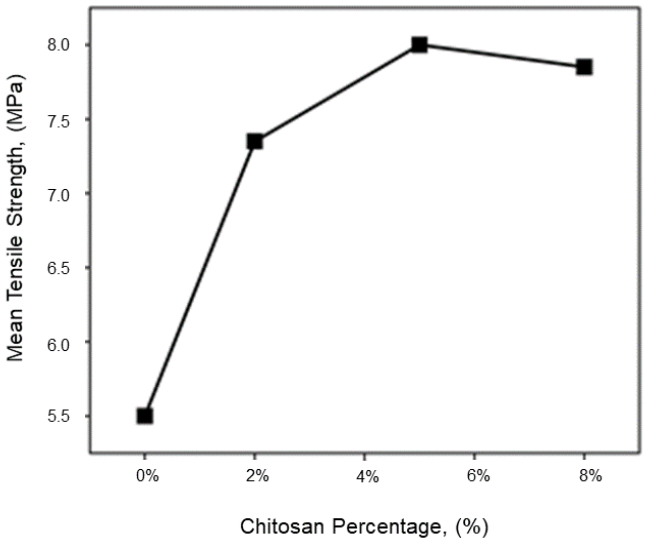

Fig. 4 *Effect of chitosan content on the tensile strength of BCC*

3.4 Characterization of BCC-5% Chitosan Soaked in SBF

The SEM micrographs of the BCC-5% soaked at 1, 2, and 3 days in SBF is presented in Fig. 5. It can be observed the formation of significantly denser structure of hydroxyapatite crystals as the days of soaking was increased from one to three days. The estimated particle size obtained was from 30-80 nm. In addition, more and more crystals were formed for each soaking time performed. This was reported by Lin et al. that the BC nanofibers can mimic collagen nanofibers for Ca-P minerals deposition via biomineralization [15]. In addition, behavior of this Ca-P minerals is platelet-like calcium-deficient hydroxyapatite (HAp), similar to the hydroxyapatite found in natural bone.

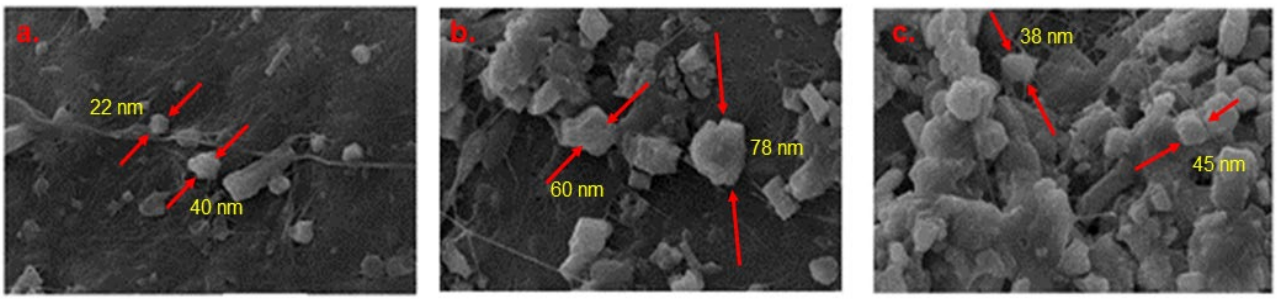

Fig. 5 *SEM images of BCC-5% soaked in SBF for (a) 1 day; (b) 2 days; and (c) 3 days at 5000x magnification*

This was confirmed by the FTIR spectra as shown in Fig. 6. the BCC cellulose immersed in SBF and gives broad peaks and an infrared reflection peak at 1400 cm^{-1}, which is caused by a C0· group. The characteristic bands showed in the sample spectra exhibited two bands observed at 3555 and 622 cm^{-1} due to the stretching mode of hydrogen-bonded OH^- ions and liberational mode of hydrogen bonded OH^- ions, respectively; the band at 1040 cm^{-1} arises from PO_4^{-3} the bands at 603 and 561 cm^{-1} arise from PO_4^{-3}. The FTIR analysis showed all typical absorption characteristics of hydroxyapatite. In addition, some carbonate content also was seen CO_3^{-2} peak around 1600 cm^{-1}, which an indication of the presence of carbonate apatite [16]. This might have originated through the absorption of carbon dioxide from the atmosphere. Therefore, according to these explanations, it is obvious that the synthesized powder is certainly hydroxyapatite.

The EDX analysis of BCC-5% Chitosan soaked in SBF for 1, 2 and 3 days as shown in Fig. 7. The analysis showed a decreasing value of Ca-P ratio from around 15 to as low as 6.7, nearing the ideal 1.67 Ca-P ratio of Hydroxyapatite formation. Although there had been trace elements formed, they have not interfered in the synthesis reaction and are most likely that they would not react during the synthesis, staying in the amorphous phase [17]. As they are in the range of parts per million, they will not alter the overall biocompatibility response

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of the material. From existing literature, the best time span of soaking was that of 24 days to maximize the even formation of the hydroxyapatite crystals throughout the surface of the BCC composite. The images from the Scanning Electron Microscopy clearly showed the formation of hydroxyapatite crystals from the consumption of calcium phosphate from the SBF.

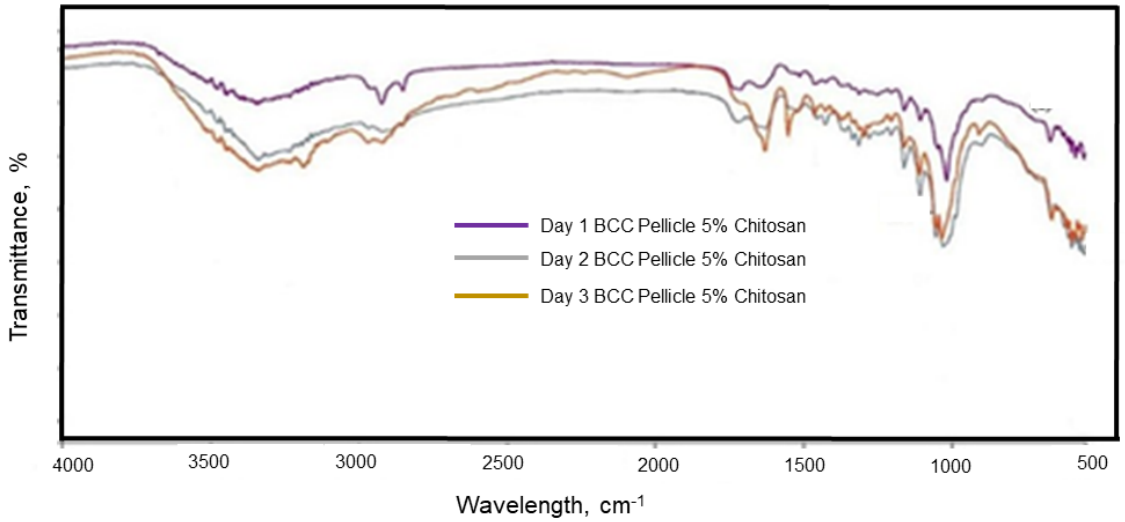

Fig. 6 *FTIR spectra of BCC -5% Chitosan soaked in SBF for (a) 1 day; (b) 2 days; and (c) 3 days*

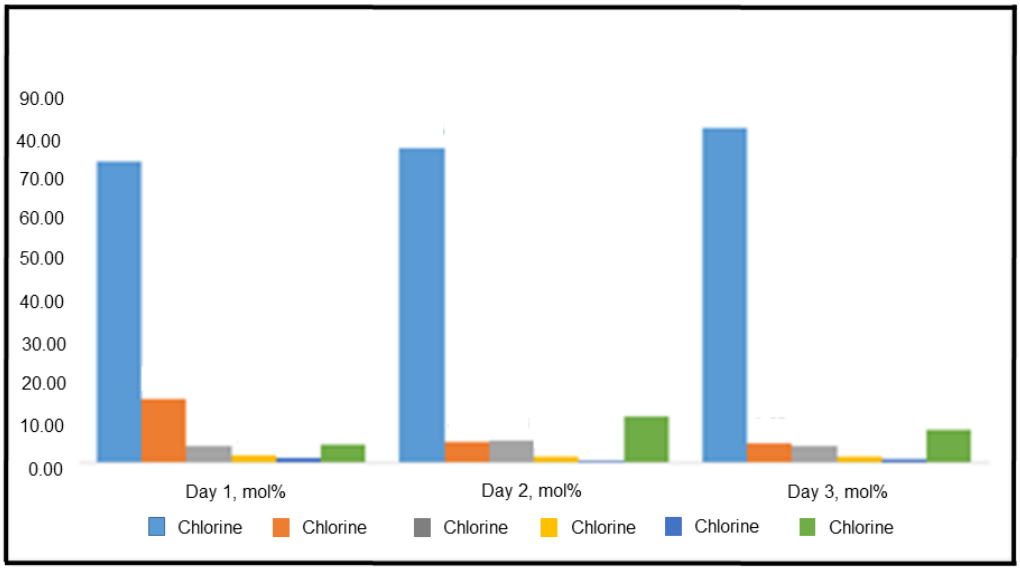

Fig. 7 *EDX analysis of BCC -5% Chitosan soaked in SBF for (a) 1 day; (b) 2 days; and (c) 3 days*

4. Conclusion

The study showed the successful synthesis of dense and porous pellicle biocompatible bacterial cellulose with chitosan as confirmed by the SEM and FTIR results. The formation of a more homogenous BCC composite was obtained with BCC- 5% Chitosan content. In the tensile strength test, the pellicles with 5% and 8% chitosan content yielded almost the same results, although the BC-5% chitosan exhibited a slightly better tensile strength and good water retention and moisture content property. In addition, BCC-5% Chitosan exhibits a biocompatible property with a crystal structure formation of nearing to 1.67 Ca-P ratio as the soaking days is increasing. The results of this study could be beneficial to researches gearing toward biomedical applications of cellulose based biofilm.

Acknowledgement

The researchers would like to extend their gratitude to Engr. Albert C. Evangelista for the guidance during the conduct of experiment in the Chemical Engineering Laboratory of Adamson University.

Conflict of Interest

Authors declare that there is no conflict of interests regarding the publication of the paper.

Author Contribution

The authors confirm contribution to the paper as follows: ***conceptualization and novelty searching:*** *Rubi, R.V.C.,* ***experimentation, data gathering and preparation:*** *Raymundo, M.J., Bucu, J.L.,* ***data analysis and interpretation:*** *Rubi, R.V.C., Lopez, M.A., Dayao, M.J.,* ***manuscript preparation****: Raymundo, M.J.. All authors reviewed the results and approved the final version of the manuscript.*

References

[1] Zhang, Z. Y., Sun, Y., Zheng, Y. D., He, W., Yang, Y. Y., Xie, Y. J., Feng, Z.-X. & Qiao, K. (2020). A biocompatible bacterial cellulose/tannic acid composite with antibacterial and anti-biofilm activities for biomedical applications. Materials Science and Engineering: C, 106, 110249.

[2] Gorgieva, S., & Trček, J. (2019). Bacterial cellulose: Production, modification and perspectives in biomedical applications. Nanomaterials, 9(10), 1352.

[3] Wang, J., Tavakoli, J., & Tang, Y. (2019). Bacterial cellulose production, properties and applications with different culture methods–A review. Carbohydrate polymers, 219, 63-76.

[4] Andriani, D., Apriyana, A. Y., & Karina, M. (2020). The optimization of bacterial cellulose production and its applications: a review. Cellulose, 27(12), 6747-6766.

[5] Blanco Parte, F. G., Santoso, S. P., Chou, C. C., Verma, V., Wang, H. T., Ismadji, S., & Cheng, K. C. (2020). Current progress on the production, modification, and applications of bacterial cellulose. Critical reviews in biotechnology, 40(3), 397-414.

[6] Fernandes, I. D. A. A., Pedro, A. C., Ribeiro, V. R., Bortolini, D. G., Ozaki, M. S. C., Maciel, G. M., & Haminiuk, C. W. I. (2020). Bacterial cellulose: From production optimization to new applications. International Journal of Biological Macromolecules, 164, 2598-2611.

[7] Urbina, L., Corcuera, M. Á., Gabilondo, N., Eceiza, A., & Retegi, A. (2021). A review of bacterial cellulose: sustainable production from agricultural waste and applications in various fields. Cellulose, 28(13), 8229-8253.

[8] Vilando, A. C., Rubi, R. V. D., & Lacsa, F. J. F. (2021). Utilization of low-cost waste materials in wastewater treatments. In Integrated and hybrid process technology for water and wastewater treatment, pp. 99-119. Elsevier.

[9] Zhao, H., Zhang, L., Zheng, S., Chai, S., Wei, J., Zhong, L., He, Y., & Xue, J. (2022). Bacteriostatic activity and cytotoxicity of bacterial cellulose-chitosan film loaded with in-situ synthesized silver nanoparticles. Carbohydrate Polymers, 281, 119017.

[10] Novikov, I. V., Pigaleva, M. A., Naumkin, A. V., Badun, G. A., Levin, E. E., Kharitonova, E. P., Gromovykh, T. I., & Gallyamov, M. O. (2021). Green approach for fabrication of bacterial cellulose-chitosan composites in the solutions of carbonic acid under high pressure CO2. Carbohydrate Polymers, 258, 117614.

[11] Indriyati, Dara, F., Primadona, I., Srikandace, Y., & Karina, M. (2021). Development of bacterial cellulose/chitosan films: structural, physicochemical and antimicrobial properties. Journal of Polymer Research, 28, 1-8.

[12] Li, B., Wu, X., Bao, B., Guo, R., & Wu, W. (2021). Evaluation of α-chitosan from crab shell and β-chitosan from squid gladius based on biochemistry performance. Applied Sciences, 11(7), 3183.

[13] Ambaye, T. G., Vaccari, M., Prasad, S., van Hullebusch, E. D., & Rtimi, S. (2022). Preparation and applications of chitosan and cellulose composite materials. Journal of Environmental Management, 301, 113850.

[14] Kokubo, T., & Takadama, H. (2006). How useful is SBF in predicting in vivo bone bioactivity?. Biomaterials, 27(15), 2907-2915.

[15] Lin, W. C., Lien, C. C., Yeh, H. J., Yu, C. M., & Hsu, S. H. (2013). Bacterial cellulose and bacterial cellulose–chitosan membranes for wound dressing applications. Carbohydrate polymers, 94(1), 603-611.

[16] Cabañas-Romero, L. V., Valls, C., Valenzuela, S. V., Roncero, M. B., Pastor, F. J., Diaz, P., & Martínez, J. (2020). Bacterial cellulose–chitosan paper with antimicrobial and antioxidant activities. Biomacromolecules, 21(4), 1568-1577.

[17] Phisalaphong, M., & Jatupaiboon, N. (2008). Biosynthesis and characterization of bacteria cellulose–chitosan film. Carbohydrate Polymers, 74(3), 482-488.

[18] Jia, Y., Wang, X., Huo, M., Zhai, X., Li, F., & Zhong, C. (2017). Preparation and characterization of a novel bacterial cellulose/chitosan bio-hydrogel. Nanomaterials and Nanotechnology, 7, 1847980417707172.
[19] Yin, N., Du, R., Zhao, F., Han, Y., & Zhou, Z. (2020). Characterization of antibacterial bacterial cellulose composite membranes modified with chitosan or chitooligosaccharide. Carbohydrate polymers, 229, 115520.
[20] Torres, F. G., Commeaux, S., & Troncoso, O. P. (2012). Biocompatibility of bacterial cellulose based biomaterials. Journal of functional biomaterials, 3(4), 864-878.
[21] Na, Y. I. N., Chen, S. Y., Ouyang, Y., Lian, T. A. N. G., Yang, J. X., & Wang, H. P. (2011). Biomimetic mineralization synthesis of hydroxyapatite bacterial cellulose nanocomposites. Progress in Natural Science: Materials International, 21(6), 472-477.
[22] Ahn, S. J., Shin, Y. M., Kim, S. E., Jeong, S. I., Jeong, J. O., Park, J. S., Gwon, H.-J., Seo, D. E., Nho, Y.-C., Kang, S. S., Kim, C.-Y., Huh, J.-B., & Lim, Y. M. (2015). Characterization of hydroxyapatite-coated bacterial cellulose scaffold for bone tissue engineering. Biotechnology and bioprocess engineering, 20, 948-955.