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In Situ Modification of Bacterial Cellulose by γ-Polyglutamic Acid: A Comprehensive Characterization

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Abstract

Modification of bacterial cellulose (BC) with biopolymers enhances its physical and mechanical properties for medical applications, with biocompatibility being critical. γ-PGA, edible, water soluble, and high biocompatible biopolymer, is utilized across various medical fields. The purpose of this research was to investigate how γ-PGA structural alterations of BC and its impact on mechanical, physical, and ultrastructural characteristics of BC. *Gluconacetobacter xylinus* was cultivated in HS medium supplemented with γ-PGA at concentrations of 0.5%, 1.0% and 1.5% (w/v) in static cultivation at 30±2°C for 16 days. Characterization of BC and BC/γ-PGA were carried out by FTIR, XRD, SEM and TGA. Addition of γ-PGA significantly increased dry weight of BC/γ-PGA in comparison to the control by 63.21%, 41.37%, 14.94% when 0.5% and 1.5% of γ-PGA was supplemented in the medium, respectively. The water holding capacity (WHC) of BC/γ-PGA increased to 7.75%,11.49%, and 5.98% by addition of 0.5% and 1.5% γ-PGA, respectively. Conversely, the water absorption rate (WAR) decreased by 28.45%, 29.46%, and 30.21% with 0.5%, 1.0% and 1.5% of γ-PGA supplementation, respectively. Addition of 1.5% γ-PGA resulted in improvement of compressive strength of BC/γ-PGA, increasing it by 451.65% over the control. BC/γ-PGA showed higher crystallinity than native BC and FTIR spectrum confirmed the covalent bonding of γ-PGA to BC resulting to transformation of dendritic structure to strip. BC/γ-PGA had slightly higher thermal stability than BC. Based on WHC and compressive strength, the incorporation of 1.0% and 1.5% γ-PGA appears optimal, respectively. Thus BC/γ-PGA is a potential candidate for medical applications.

Introduction

Bacterial cellulose (BC), exopolysaccharide and water insoluble biopolymer, are made up of 1,4-linked D-glucose units. It is synthesized by different species of bacteria, especially Gram-negative bacteria, including *Gluconacetobacter* (originallygi *Acetobacter*), *Agrobacterium, Azotobacter* and *Rhizobium* (Chawla et al., 2009). BC is highly pure compared to plant-derived cellulose because it lacks lignin, hemicellulose, pectin,

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arabinose, and other components originating from plants (Gu & Catchmark, 2012). BC is a sustainable and eco-friendly nanomaterial. It features a microfibrillar nanostructure, excellent biological compatibility, non-toxicity (Souza et al., 2020), high mechanical strength, high elasticity, thermal stability (Mai et al., 2024; Seddiqi et al., 2021; Dang et al., 2024), and high liquid absorption capacity, biodegradability (Ranjha et al., 2023; Shi et al., 2014), high crystallinity and high degree of polymerization (Phruksaphithak et al., 2019). These properties make BC a highly versatile and valuable material with a wide range of potential applications in various fields (Park et al., 2009). BC can be produced in various shapes and forms, including membranes, films, fibers, and hydrogels, depending on the specific application requirements. Its well-organized 3D network of fibres has been reported to be beneficial in the production of wound dressings, temporary skin replacements, and antibacterial artificial blood vessels (Lee & Park, 2017). BC is attracting interest in the field of wound healing due to its unique properties such as a unique pore structure, non-toxic and biocompatible with human tissue. Additionally, it efficiently collects the fluids produced by the wound, helping to keep the wound moist and conducive to healing. The high swelling behaviour of native BC, despite its poor mechanical characteristics, has an impact on the balance of beneficial behaviours, such as medication transport, water retention, and structural stability. Therefore, modifying BC to enhance its usefulness, mechanical qualities, and biocompatibility has been a fascinating field of study (Tunsound et al., 2023). It is hypothesized that covalent crosslinking largely contributes to the mechanic properties and the swelling behaviour because of its reversibility (Hu et al., 2023). *Ex situ* and *in situ* modifications have both been used to improve a certain property for a specific application (Cazón & Vázquez, 2021).

Several biopolymers have been used for BC modification. For example, chitosan has been reported to enhance the properties of cellulose. Additionally, xylan has been used to modify the properties of cellulose, where xylan can enhance thermal stability and resistant to heat than regular cellulose (Heinonen et al., 2022). Structurally modifying BC with keratin has improved the adhesion properties of cellulose hydrogels and significantly enhanced the amount and distribution of antibacterial agents (Sadeghi et al., 2020). Although prior studies have focused on post modification, *ex situ* modifications have limits despite improvements in both *in situ* and *ex situ* techniques for changing bacterial cellulose (Stumpf et al., 2018). *Ex situ* methods primarily improve the structure of bacterial cellulose by randomizing fiber arrangement, leading to increased porosity, reduced pore size, and overall lessened roughness compared to in situ methods. In contrast, *in situ* methods offer the advantage of enhancing the internal structure of bacterial cellulose. This allows for adjustments in thickness, fiber arrangement, pore size, and changes in both physical and mechanical properties, resulting in improved properties (Ul-Islam et al., 2012). *In situ* modification was developed to maintain the original skeleton and reinforce structural behaviours by reversibly, non-covalently crosslinking in-situ additives with the BC backbone (Sun et al., 2020). When modifying the properties of cellulose for medical applications, it is important to consider materials that originate from living organisms or are hypoallergenic, as these reduce the likelihood of allergic reactions in humans.

γ-Polyglutamic acid (γ-PGA), edible biopolymer produced by certain strains of bacteria, particularly Bacillus subtilis and related species. γ-PGA is anionic biopolymer composed of L-and D-glutamate. It has unique properties such as biodegradability, water solubility, non-toxicity, and biocompatibility (Shih & Van, 2001). γ-PGA has been utilized in conjunction with chitosan through an *in situ* approach, aimed at creating wound dressing materials. It was found that BC/γ-PGA/chitosan exhibits antibacterial properties, biocompatibility with experimental animal cells, and promotes wound healing (Argunşah et al., 2019). BC dispersed in γ-PGA hydrogel matrix shows excellent cytocompatibility for biomaterial applications (Dou et al., 2021). Since the attractive properties of γ-PGA, modification of BC by γ-PGA may improve certain properties of BC or to impart additional functionality to it. Therefore, this research aimed to modify BC produced from *Gluconacetobacter xylinus* by γ-PGA produced from *B. subtilis* NT147 by *in situ* modification method and to characterize BC/γ-PGA composite. In addition, physical and mechanical properties of BC/γ-PGA were evaluated.

Materials and methods

1. Material preparation

1.1 Bacterial strains

Gluconacetobacter xylinus was obtained from

Kasetsart University. *Bacillus subtilis* NT147, a glutamate independent PGA producing strain was isolated from Thai fermented soybean.

1.2 Production of γ-polyglutamic acid (γ-PGA)

γ-PGA was produced by the method described by Ngearnpat et al. (2023) with modification of culture media. Briefly, *B. subtilis* NT147 was cultured in Luria-Bertani broth for 12 hr at 37±2°C with shaking at 150 rpm. The inoculum was added to 100 mL of Sucrose Yeast Extract broth and incubated for 48 hr at 37±2°C with 150 rpm of shaking. Cells were removed by centrifugation at 10,000 rpm for 15 min at 4°C. Cold ethanol was used to precipitate the γ-PGA from supernatant, and it was desalted using dialysis for 24 hr at 4°C. The γ-PGA solution was freeze-dried and stored in a desiccator until used.

1.3 Production of BC and *in situ* modification of BC by γ-PGA

An inoculum was prepared by cultivating *G. xylinus* in coconut water containing 5% sucrose, 0.5% peptone, 0.5% yeast extract, 0.27% Na_2HPO_4, 0.12% citric acid and 0.5% acetic acid. The culture was then incubated for 10 days at 30±2°C, under static condition. For BC production, 10% of inoculum was transferred into a 500 mL beaker containing 100 mL of HS medium (pH 4.0) and incubated for 16 days at 30±2°C, under static conditions. The BC formed on the upper surface of the medium was harvested. BC/γ-PGA biocomposites were prepared by 0.5%, 1.0% and 1.5% (w/v) of PGA into the Hestrin–Schramm (HS) medium. Subsequently, the inoculum was added, and the culture was incubated for 16 days at 30±2°C, under static conditions, in a 500 mL beaker containing 100 mL of HS medium.

1.4 Purification of BC and BC/γ-PGA

To eliminate the entrapped cells, microbial cellulose was treated with 0.1%NaOH solution at 80°C for 1 hr. Following, microbial cellulose was incubated with distilled water overnight to remove impurities. Finally, the purified cellulose was dried at 100°C for 24 hr until reaching constant weight (Sun et al., 2020). Wet weight and dry weight were determined.

2. Investigation of BC and BC/γ-PGA properties

2.1 Water holding capacity (WHC) and water absorption rate (WAR)

WHC was determined by Eq. (1). WAR was calculated by weighing 1 g of sample and then soaking to constant weight at room temperature of BC film using Eq. (2).

$$\text{WHC (\%)} = \{(W0 - W1)/W1\} \times 100\% \quad (1)$$

$$\text{WAR (\%)} = \{(W2 - W1)/W1\} \times 100\% \quad (2)$$

where W0 and W1 are the initial wet weight and oven-dried weight of BC film. W2 is the constant weight after soaking (Liu & Zhu, 2023).

2.2 Mechanical testing

For compression testing, a circular press head with a diameter of 4 mm is placed on a pedestal where the fresh cellulose sheets are positioned, which rests on a cylindrical roller with a diameter of 25 mm. The test measures the maximum compressive force required to cause the cellulose sheets to separate completely.

2.3 Fouirer transform infrared (FTIR)

To analyze the chemical structure and surface properties of microbial cellulose, FT-IR was performed by using a Spectrum Two FT-IR Spectrometer (PerkinElmer Inc., USA). Spectra were collected over the range of 4000 cm^{-1} to 450 cm^{-1} in transmittance mode, with an accumulation of 16 scans.

2.4 X-Ray diffraction (XRD)

XRD patterns of microbial cellulose were analyzed with an Aeris XRD diffractometer (Malvern Panalytical Ltd., UK) using CuKα radiation wavelength (λ = 1.54 Å). Samples were scanned from 0°-60° in a 2θ angle with an increasing size of 0.02°.

2.5 Scanning electron microscopy (SEM)

The microstructure was characterized by Ultra-high resolution SEM (SU8020 model, Hitachi Ltd., Japan). The samples were attached to slab surfaces with double sided adhesive tape and then coated with a layer of carbon. All samples were examined using an accelerating voltage of 2 kV.

2.6 Thermogravimetric analyzes (TGA-DSC)

Thermogravimetric analyses were performed by using TG209 F3 Tarsus model (NETZSCH Ltd., Germany) under N_2 atmosphere with an average weight of samples were approximately 10 mg over a dynamic temperature range of 30-650°C.

3. Statistical analysis

Statistical analysis was carried out using SPSS 16.0 (SPSS Inc., Illinois, USA). Means of different treatments were compared using Duncan's multiple range test at the 0.05 level of probability. BC without γ-PGA modification was used as a control.

Results and discussion

1. Effect of *in-situ* modification on yield, physical and mechanical properties of BC/γ-PGA

Cellulose synthesized by *G. xylinus* in HS-medium supplemented with γ-PGA at concentrations of 0.5%, 1.0%, and 1.5% showed an increasing trend in fresh weight compared to the control group (BC). However, there was no significant difference in fresh weight. Nevertheless, cellulose synthesized from the medium supplemented with γ-PGA (BC/γ-PGA) had significantly higher dry weights than the control group (Table 1). The dry weight increased 63.21%, 41.37%, 14.94% when 0.5%, 1.0% and 1.5% of γ-PGA was supplemented into the culture medium, indicating that γ-PGA may promote BC synthesis. γ-PGA is a biopolymer composed of subunits of glutamic acid, which can potentially serve as a nitrogen source for bacterial growth (Shih & Van, 2001). However, an increase of γ-PGA amount tends to reduce BC yield, suggesting that γ-PGA may interfere with the assembly or alignment of cellulose fibrils by occupying space or altering the microenvironment where cellulose synthesis occurs. Regarding water absorption rate (%WAR) of BC, it was found to decrease. Addition of 0.5%, 1.0% and 1.5% of γ-PGA reduced %WAR by 28.45%, 29.46% and 30.21%, respectively. FTIR analysis results presented in Fig. 1 suggest that γ-PGA can participate in cross-linking reactions with bacterial cellulose. These cross-links can enhance the structural integrity of the cellulose network, making it less porous and thus less able to absorb water. According to SEM examination, cellulose treated with γ-PGA at 0.5%, 1.0%, and 1.5% exhibited larger porosity diameters and a rougher or porous morphology than the control group (Fig. 3). This led to a lower percentage in WAR. Additionally, the cross-linked network can restrict the swelling capacity of the cellulose. When considering water holding capacity (WHC), cellulose synthesized from media supplemented with γ-PGA at 0.5% and 1.0% showed no difference compared to the control group. However, cellulose synthesized from the medium supplemented with γ-PGA at 1.5% exhibited significantly higher WHC compared to the control group. The WHC increased 7.75%, 11.49% and 5.98% when 0.5%, 1.0% and 1.5% γ-PGA was added to the medium. γ-PGA is a hydrophilic polymer that is well-known for having a large capacity for water absorption (Abdelnaby et al., 2023), suggesting that the incorporation of γ-PGA has the potential to considerably boost the water holding in terms of percentage ratio. As shown in the SEM result (Fig. 3), increasing the pore sizes in the BC/γ-PGA composite may enhance its water holding capacity. Furthermore, γ-PGA significantly enhances strength of BC. Supplementation of 0.5, 1.0 and 1.5% of γ-PGA increased compressive strength by 371.14%, 252.06% and 451.65%, respectively (Table 1). The FTIR analysis presented in Fig. 1 suggests that γ-PGA potentially forms crosslinks with cellulose or other matrix components, thereby establishing robust chemical bonds that fortify the material against compression forces. These crosslinking interactions are implicated in bolstering the cohesive strength of bacterial cellulose, thereby augmenting its mechanical resilience.

Table 1 Physical and mechanical properties of BC and BC/γ-PGA

	Wet weight (g)	Dry weight (g)	WAR (%)	WHC (%)	Compression (N)
BC	50.43 ± 12.18^{b}	0.87 ± 0.22^{c}	494.49 ± 83.95^{a}	5065.5 ± 389.4^{b}	2.42 ± 0.11^{c}
BC/0.5% γ-PGA	66.48 ± 0.47^{a}	1.42 ± 0.02^{a}	353.80 ± 32.88^{b}	5458.3 ± 134.1^{ab}	11.45 ± 1.83^{a}
BC/1.0% γ-PGA	67.04 ± 3.22^{a}	1.23 ± 0.03^{ab}	348.80 ± 18.15^{b}	5647.9 ± 112.3^{a}	8.52 ± 1.16^{b}
BC/1.5% γ-PGA	61.16 ± 6.31^{ab}	1.00 ± 0.16^{bc}	345.12 ± 6.74^{b}	5368.7 ± 96.3^{ab}	13.35 ± 0.60^{a}

Remark: Different superscripts in a column differ significantly ($P < 0.05$) according to Duncan's multiple range test

2. Effect of *in-situ* biochemical modification on BC/γ-PGA structure

For applications, not only the BC yield but also the BC structure and function are important, especially for BC applications. In this section, the effect of *in-situ* biochemical modification on the BC/γ-PGA structure was evaluated systematically by FTIR, XRD, SEM, and TGA. The structure of both BC and BC/γ-PGA with various γ-PGA addition was examined.

2.1 FTIR

The chemical properties of cellulose modified with γ-PGA were analyzed using Fourier Transform Infrared (FTIR) spectroscopy in the wavenumber of 4,000–500 cm^{-1} to learn the effect of *in-situ* biochemical modification on the functional groups of BC (Fig. 1).

The characteristic cellulose vibration peaks, which have been reported in prior study (Santoso et al., 2021) could also be found in both BC and BC/γ-PGA. The FTIR spectrum of control cellulose showed peaks at specific wave numbers: 3,344 cm^{-1} (indicative of -OH stretching) and 1,049 cm^{-1} (C-O-C stretching), as shown in Fig. 1(a). This aligns with FTIR analysis of cellulose from bacteria by Dou et al. (2021), where typical peaks for bacterial cellulose were observed at wave numbers such as 3,344-3,345 cm^{-1} (-OH stretching), 1,327-1,400 cm^{-1} (C-H bending), and 1,047 cm^{-1} (C-O stretching) (Su et al., 2016). The FTIR spectra of BC/γ-PGA revealed significant peaks at various wave numbers: 3,200-3,400 cm^{-1} (N-H Stretching), 3,345-3,344 cm^{-1} (-OH stretching), 1,647 cm^{-1} (-C=O stretching), 1,327-1,328 cm^{-1} (C-N stretching), and 1,049-1,047 cm^{-1} (C-O stretching). In the case of γ-PGA 1.0% (Fig. 1(c)), peaks were prominent at 3,344 cm^{-1} (-OH stretching), 1,642 cm^{-1} (C=O stretching), 1,327 cm^{-1} (C-N stretching), and 1,048 cm^{-1} (C-O stretching). For γ-PGA 1.5% (Fig. 1(d)), peaks appeared at 3,284 cm^{-1} (indicative of -H stretching associated with hydroxyl groups), 1,631 cm^{-1} (C=C stretching), 1,376 cm^{-1} (C-N stretching), and 1,051 cm^{-1} (C-O stretching). Comparing the peak signals of cellulose with those of cellulose modified by γ-PGA, an increase in the C=O stretching peak (amide) at wavenumbers 1,650-1,700 cm^{-1} suggests an enhancement of carbonyl groups derived from amides in the γ-PGA structure. Additionally, there was an increase in the C-N stretching peak intensity at wave numbers 1,200-1,300 cm^{-1}, attributed to vibrations of C-N bonds within amide groups in the γ-PGA structure. Changes in peak intensities (%T) of the O-H stretching (3,200-3,400 cm^{-1}) indicated hydrogen bond formation between cellulose and γ-PGA.

2.2 XRD

The analysis of the crystallinity of BC/γ-PGA using X-ray Diffractometer (XRD) revealed that when cellulose was cultured with γ-PGA at concentrations of 0.5%, 1.0%, and 1.5%, distinct patterns emerged in the XRD results shown in Fig. 2. Normally, γ-PGA exhibits major peaks in XRD at significant angles such as 20.00, 21.80, and 28.40 (Wang et al., 2021), while untreated BC typically shows peaks at 14.05, 16.77, and 22.68 (Aditya et al., 2022), which aligns with the crystallinity patterns observed in cellulose cultured with γ-PGA. Additionally, several other peaks were identified: for γ-PGA at 0.5%, peaks were found at 7.85, 35.15, and 65.68; for γ-PGA at 1.0%, peaks were found at 10.05, 33.90, and 36.16; and for γ-PGA at 1.5%, peaks were found at 6.57 and 36.71. The XRD analysis demonstrated the relationship between γ-PGA and the crystallinity of cellulose. Different concentrations of γ-PGA resulted in changes in the major peaks of XRD, indicating reactions and structural alterations in the cellulose crystallinity. At lower concentrations (0.5%), an increase in high-level peaks was observed, while at higher concentrations (1.0% and 1.5%), structural changes in crystallinity led to increased stability, consistent with findings by Yu et al. (2024) suggesting that γ-PGA significantly influences the crystallization of BC, especially non-covalent γ-PGA, impacting crystallization properties more significantly than covalent γ-PGA.

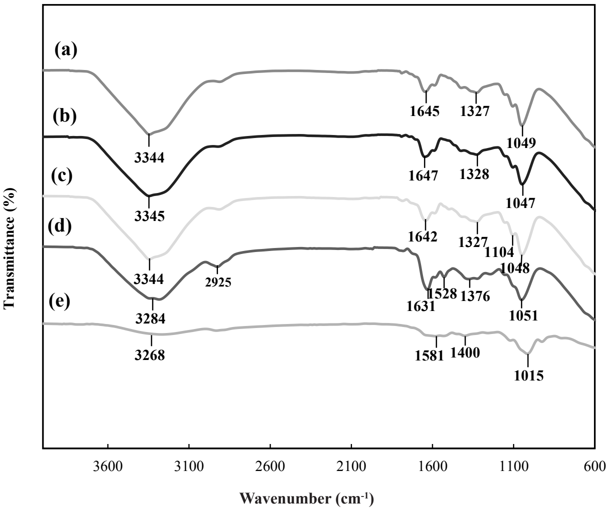

Fig. 1 Fourier Transform Infrared (FTIR) spectroscopy of BC (a), BC/0.5% γ-PGA (b), BC/1.0% γ-PGA (c), BC/1.5% γ-PGA (d) and γ-PGA (e)

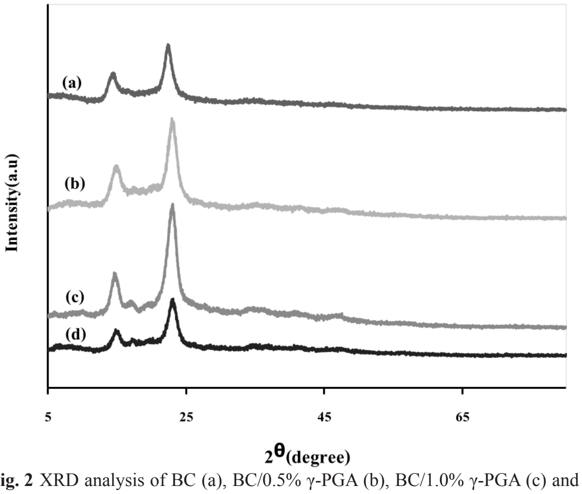

Fig. 2 XRD analysis of BC (a), BC/0.5% γ-PGA (b), BC/1.0% γ-PGA (c) and BC/1.5% γ-PGA (d)

2.3 Analysis of ultrastructure by SEM

The microstructure of BC and BC/γ-PGA was elucidated by SEM, as shown in Fig. 3. The nano-network containing various spaces among the cellulose microfibrils can be clearly seen in the SEM image of unmodified BC (Fig. 3(a)), which is similar to the structure of BC produced by different microorganisms and substrates (Lahiri et al., 2021). In contrast, γ-PGA exhibited a sheet like structure (Fig. 3(b)). Compared with unmodified BC, thicker microfibrils of cellulose could be observed in the nano network of BC/0.5% γ-PGA was observed as shown in Fig. 3(b). Interestingly, increase of γ-PGA to 1.0% and 1.5% clearly exhibited combination of γ-PGA with BC directly and therefore thicker microfibrils and sheet of BC/γ-PGA were observed as shown in Fig. 3(d) and (e), respectively. Besides the thicker microfibrils, the pores of BC/γ-PGA were also bigger. For this *in situ* biochemical BC modification, it is possible that γ-PGA was bonded to the BC microfibril surface and then affected the gathering of microfibrils during cultivation, and the thicker microfibrils and bigger space among the microfibrils were caused by the disordered arrangement of microfibrils (Huang et al., 2010). Different from this study, it was also reported that the higher diameter of the microfibrils might decrease the spaces among the microfibrils during the *in situ* biochemical BC modification also with XG addition (Gao et al., 2020), suggesting that different microorganisms and fermentation media might affect the pattern of BC production and alter the structure of BC even with the same additives for *in situ* biochemical modification (Wang et al., 2021). Overall, the SEM images clearly confirmed the hypothesis that γ-PGA was combined with BC during the *in situ* biochemical modification.

2.4 Thermal analysis

The TGA and DTG curves of BC and BC/1.5% γ-PGA are shown in Fig. 4. Thermal analysis result of BC displayed a slight weight loss at about 119°C, because of water evaporation. The obviously weight loss between 219°C and 333°C was caused by the thermal decomposition and dehydration of cellulose and the decomposition of glucose (Yuan et al., 2016). Whereas BC/1.5% γ-PGA composite showed a slightly higher initial degradation temperature than BC. The weight loss of BC/1.5% γ-PGA were at 226 and 336°C. The melting point (T_m) and decomposition temperature (T_d) of γ-PGA were reported at 219°C and 223°C, respectively (Hoa et al., 2006). However, thermal stability of BC can be influenced by its structure, crystallinity and purity. Dou et al. (2021) reported that post modification of γ-PGA/BC composite hydrogel exhibited higher initial degradation temperature than γ-PGA with addition of BC.

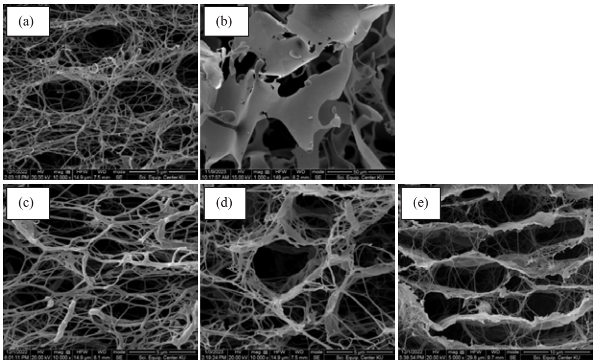

Fig. 3 Microstructure analysis by SEM of BC (a), γ-PGA (b), BC/0.5% γ-PGA (c), BC/1.0% γ-PGA (d) and BC/1.5% γ-PGA (e)

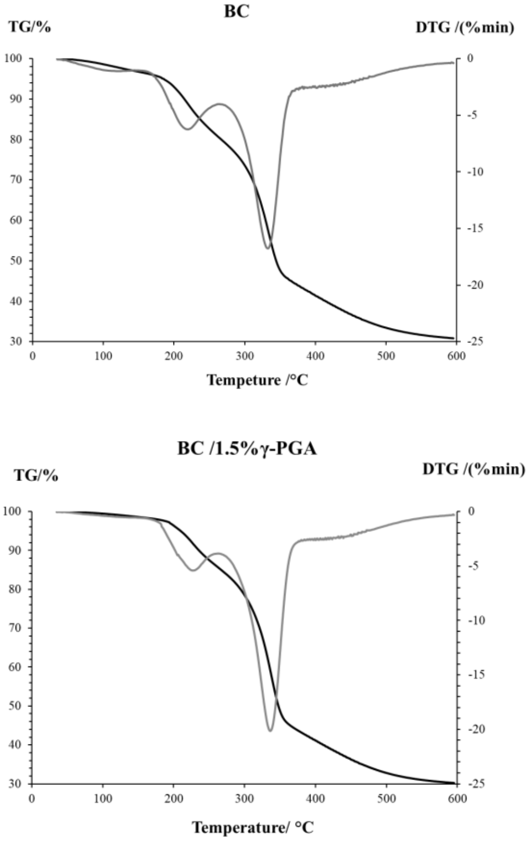

Fig. 4 Thermal properties of BC and BC/1.5% γ-PGA. T_d (decomposition temperature) was measured with thermal gravimetric analysis (TGA)

Conclusion

In summary, *in situ* modification of BC with γ-PGA can produce BC/γ-PGA bio-composite. Incorporating 1.0% γ-PGA optimally increased the water holding capacity of BC/γ-PGA by 11.49% in comparison to the control. Whereas incorporation of 1.5% γ-PGA significantly increased compression force of BC/γ-PGA by 451.65% and decreased water absorption rate by 30.21% in comparison to the control. γ-PGA significantly influences the crystallization of BC and slightly increased thermal stability of BC. The synergistic potential of combining BC and γ-PGA for medical applications such as drug delivery needle and wound healing material. Although γ-PGA provides additional functionalities, such as controlled release and targeted delivery, further studies on drug control release of BC/γ-PGA are required.

References

Abdelnaby, T., Li, Z., Cao, W., & Xue, C. (2023). The effect of gamma-poly glutamic acid as a cryoprotectant on crayfish physicochemical and texture properties during frozen storage. *LWT, 184,* 114905.

Aditya, T., Allain, J.P., Jaramillo, C., & Restrepo, A.M. (2022). Surface modification of bacterial cellulose for biomedical applications. *International Journal of Molecular Sciences, 23,* 610.

Argunşah, G., Gül, A., & Hameş-Tuna, E.E. (2019). Evaluation of a novel antibacterial wound dressing based on bacterial cellulose containing γ-PGA/chitosan complex. In Yazıcı Ş. & K. Köseoğlu (Eds.), *HEZARFEN International Congress of Science, Mathematics & Engineering* (pp.114-125). Izmir, Turkey: Asos Yayinlari.

Cazón, P., & Vázquez, M. (2021). Improving bacterial cellulose films by ex-situ and in-situ modifications: A review. *Food Hydrocolloids, 113,* 106514.

Chawla, P.R., Bajaj, I.B., Survase, S.A., & Singhal, R.S. (2009). Microbial cellulose: Fermentative production and applications. *Food Technology and Biotechnology, 47* (2): 107–124.

Dang, X., Li, N., Yu, Z., Ji, X., Yang, M., & Wang, X. (2024). Advances in the preparation and application of cellulose-based antimicrobial materials: A review. *Carbohydrate Polymers, 342,* 122385.

Dou, C., Li, Z., Gong, J., Li, Q., Qiao, C., & Zhang, J. (2021). Bio-based poly (γ-glutamic acid) hydrogels reinforced with bacterial cellulose nanofibers exhibiting superior mechanical properties and cytocompatibility. *International Journal of Biological Macromolecules, 170,* 354–365.

Gao, H.-L., Zhao, R., Cui, C., Zhu, Y.-B., Chen, S.-M., Pan, Z., ... Yu, S.H. (2020). Bioinspired hierarchical helical nanocomposite macrofibers based on bacterial cellulose nanofibers. *National Science Review, 7,* 73–83.

Gu, J., & Catchmark, J.M. (2012). Impact of hemicelluloses and pectin on sphere-like bacterial cellulose assembly. *Carbohydrate Polymers, 88,* 547–557.

Heinonen, E., Henriksson, G., Lindstrom, M. E., Vilaplana, F., & Wohlert, J. (2022). Xylan adsorption on cellulose: Preferred alignment and local surface immobilizing effect. *Carbohydrate Polymers, 285,* 119221.

Hoa, G.-H., Hoc, T.-I., Hsiehb, K.-H., Sud, Y.-C., Line, P.-Y., Yang, J., ... & Yang, S.C. (2006). γ -Polyglutamic acid produced by *Bacillus subtilis* (natto): Structural characteristics, chemical properties and biological functionalities. *Journal of the Chinese Chemical Society, 53,* 1363-1384

Hu, Z., Liu, D., Wang, M., Yu, C., Han, Z., Xu, M., ... Nie, G. (2023). β-Alanine enhancing the crosslink of chitosan/poly-(γ-glutamic acid) hydrogel for a potential alkaline-adapted wound dressing. *International Journal of Biological Macromolecules, 231,* 123157.

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. *Bioresource Technology, 101*(15), 6084-6091.

Lahiri, D., Nag, M., Dutta, B., Dey, A., Sarkar, T., Pati, S., … Ray, R.R. (2021). Bacterial cellulose: Production, characterization, and application as antimicrobial agent. *International Journal of Molecular Sciences, 22,*12984.

Lee, S.E., & Park, Y.S. (2017). The role of bacterial cellulose in artificial blood vessels. *Molecular Cell Toxicology, 13*(3), 257-261.

Liu, Q., & Zhu, S. (2023). Fractionation of depectinated sugar beet pulp into cellulose, hemicellulose, and lignin with NaOH/urea/H_2O and ionic liquid. *International Journal of Biological Macromolecules, 242*(2), 124706.

Mai, T., Wang, P.- L., & Ma, M.-G. (2024). Promising cellulose-based aerogel composites: Preparation methods and advanced applications. *Composites Part B: Engineering, 281,* 111559.

Ngearnpat, N., Chunhachart, O., Kotabin, N., & Issakul, K. (2023). Comparative assessment of gamma-polyglutamic acid and *Bacillus subtilis* cells as biostimulants to improve rice growth and soil quality. *Journal of Ecological Engineering. 24*(12), 46-59.

Park, J.K., Jung, J.Y., & Khan, T. (2009). Bacterial cellulose. In G.O. Phillips & P.A. Williams (Eds.), *Handbook of hydrocolloids* (pp. 724-739). UK: Woodhead Publishing.

Phruksaphithak, N., Kaewnun, C., & O-Thong, S. (2019). Bacterial cellulose production and applications. *Science, Engineering & Health Studies, 13*(1), 1-7.

Ranjha, M.M.A.N., Shafique, B., Aadil, R.M., Manzoor, M.F., & Cheng, J.-H. (2023). Modification in cellulose films through ascent cold plasma treatment and polymerization for food products packaging. *Trends in Food Science & Technology, 134,* 162–176.

Sadeghi, S., Nourmohammadi, J., Ghaee A., & Soleimani, N. (2020). Carboxymethyl cellulose-human hair keratin hydrogel with controlled clindamycin release as antibacterial wound dressing. *International Journal of Biological Macromolecules, 147,* 1239-1247.

Santoso, S.P., Lin, S.-P., Wang, T.-Y., Ting, Y., Hsieh, C.-W., Yu, R.-C., ... Cheng, K. -C. (2021). Atmospheric cold plasma-assisted pineapple peel waste hydrolysate detoxification for the production of bacterial cellulose. *International Journal of Biological Macromolecules, 175,* 526-534.

Seddiqi, H., Oliaei, E., Honarkar, H., Jin, J., Geonzon, L.C., Bacabac, R.G., & Klein-Nulend, J. (2021). Cellulose and its derivatives: Towards biomedical applications. *Cellulose, 28,*1893–1931.

Shi, Z., Zhang, Y., Phillips, G.O., & Yang, G. (2014). Utilization of bacterial cellulose in food. *Food Hydrocolloids, 35,* 539–545.

Shih, I.L., & Van, Y.T. (2001). The production of poly-(γ- glutamic acid) from microorganisms and its various applications. *Bioresource Technology, 79,* 207-225.

Stumpf, T.R., Yang, X., Zhang, J., & Cao, X. (2018). In situ and ex situ modifications of bacterial cellulose for applications in tissue engineering. *Materials Science and Engineering: C-Material Biological Application, 82,* 372-383.

Su, J., Raghuwanshi, V.S., Raverty, W., Garvey, C.J., Holden, P.J., Gillon, M., … Garnier, G. (2016). Smooth deuterated cellulose films for the visualisation of adsorbed bio-macromolecules. *Scientific Reports, 6,* 36119.

Sun, B., Zhang, L., Wei, F., AL-Ammari, A., Xu, X., Li, W., … Sun, D. (2020). In situ structural modification of bacterial cellulose by sodium fluoride. *Carbohydrate Polymers, 231,* 115765.

Souza, E.F., Furtado, M.R., Carvalho, C.W.P., Freitas-Silva, O., & Gottschalk, L.M.F. (2020). Production and characterization of *Gluconacetobacter xylinus* bacterial cellulose using cashew apple juice and soybean molasses. *International Journal of Biological Macromolecules, 146,* 285–289.

Tunsound, V., Krasian, T., Daranarong, D., Punyodom, W., Jantanasakulwong, K., Ross, S., … Worajittiphon, P. (2023). Enhanced mechanical properties and biocompatibility of bacterial cellulose composite films with inclusion of 2D MoS2 and helical carbon nanotubes for use as antimicrobial drug carriers. *International Journal of Biological Macromolecules, 253,* 126712.

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. *Carbohydrate Polymers, 88*(2), 596-603.

Wang, K., Shen, J., Ma, Z., Zhang, Y., Xu, N., & Pang, S. (2021). Preparation and properties of poly (ethyleneglycol-co-cyclohexane-1,4-dimethanol terephthalate)/polyglycolic acid (PETG/PGA) blends. *Polymers, 13*(3), 452.

Yu, C., Han, Z., Sun, H., Tong, J., Hu, Z., Wang, Y., … Nie, G. (2024). Balancing mechanical property and swelling behavior of bacterial cellulose film by in-situ adding chitosan oligosaccharide and covalent crosslinking with γ-PGA. *International Journal of Biological Macromolecules, 267,* 131280.

Yuan, N., Xu, L., Zhang, L., Ye, H., Zhan, J., Lui, Z., & Rong, J. (2016). Superior hybrid hydrogels of polyacrylamide enhanced by bacterial cellulose nanofiber clusters. *Materials Science and Engineering: C-Material Biological Application, 67,* 221-230.