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Research Article

Eco-friendly Bacterial Cellulose/Castor Oil Hydrogels: Physicochemical Behavior and Biocompatibility

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ABSTRACT

In the present work, novel thermoresponsive hydrogels were developed from renewable resources, and the influence of bacterial cellulose molar ratio on their chemical structure, thermal properties, swelling behavior, morphology, and biocompatibility was systematically investigated. The hydrogels were fabricated using castor oil, 4,4'-diphenylmethane diisocyanate, bacterial cellulose, N-isopropylacrylamide, and N,N'-Methylenebisacrylamide. Structural and physicochemical characterizations were performed by Fourier-transform infrared spectroscopy, scanning electron microscopy, differential scanning calorimetry, and thermogravimetric analysis. The highest equilibrated swelling degree was achieved as 592.6% at the maximum bacterial cellulose content. SEM images revealed that the formation of spongy architecture is caused by the increase in the bacterial cellulose content. In vitro biocompatibility studies revealed that the hydrogel with the highest bacterial cellulose content exhibited the greatest cytocompatibility, with an IC50 value of 11.16 mg/ml. Overall, the findings demonstrate the successful fabrication of a novel bio-based thermoresponsive hydrogel through an eco-friendly approach, highlighting its potential for diverse biomedical applications.

1. Introduction

Hydrogels are crosslinked networks of hydrophilic polymers capable of absorbing large amounts of water. Owing to their structural and functional resemblance to living tissues, they are widely applied in the medical field. A particular class of these materials, known as stimuli-responsive hydrogels, can undergo changes in response to environmental triggers such as temperature, enzymatic activity, electrical signals, pH, light, or ionic strength [1]. These hydrogels exhibit swelling or shrinking behavior in response to external stimuli. Among them, thermoresponsive hydrogels, which respond to temperature changes, are particularly preferred

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for the design of controlled drug release systems (CDRS) [1, 2]. It is crucial to have enhanced CDRS to maximize the efficacy of drug molecules. CDRS may be engineered as self-activated hydrogels by diffusion, decomposition, dissolution, and other methods under the right conditions [3, 4].

The changes of thermoresponsive hydrogels in response to temperature, combined with their crosslinked hydrophilic networks, make them highly valuable for controlled drug release systems and tissue engineering applications. Thermoresponsive hydrogels are generally classified into two types: those that undergo changes in aqueous environments as the temperature increases, known as lower critical solution temperature (LCST) hydrogels, and those that respond to decreasing temperature, referred to as upper critical solution temperature (UCST) hydrogels [5].

Hydrogels made of N-isopropyl acrylamide (NIPAM) exhibit LCST-type behavior, in which they are solvated and swollen below LCST and become insoluble and shrink above LCST. Since the LCST of NIPAM-based hydrogels is close to physiological temperature, they are considered excellent candidates for CDRS applications. Controlling solubility and LCST enables accurate control over drug release behavior. Hydrogel systems can be tailored by adjusting the types and ratios of monomers and crosslinkers. Incorporating hydrophobic monomers lowers the LCST, whereas hydrophilic monomers raise it [6, 7].

Various studies regarding thermoresponsive hydrogels have been reported in the literature. For instance, Cao and coworkers investigated mixed thermoreversible gels [8]. They designed a thermosensitive sol-gel system based on poly(N-isopropyl acrylamide) (PNIPAM) for the controlled and targeted delivery of a short antibacterial peptide. Another study includes PNIPAM-based polymeric nanocomposites [9]. Fundueanu et al. [10] synthesized poly(N-isopropyl acrylamide-co-N-vinylpyrrolidone) as a thermosensitive material and assessed how the comonomer molar ratio influenced the LCST. Park et al. [11] designed poly(NIPAM-co-butyl acrylate) hydrogels using NIPAM and butyl acrylate as comonomers, with N,N'-methylenebisacrylamide as the crosslinker. They demonstrated that the crosslinked poly(NIPAM-co-butyl acrylate) hydrogels have a high potential for application as smart materials. Another study involving controlled release profiles of the hydrogels was conducted by Onaciu et al. [12]. They examined the 3D polymeric network structures of hydrogels, focusing on their compatibility with living tissue and ability to degrade properties, along with their controlled release profiles. Studies have also explored the use of N-vinyl caprolactam in the synthesis of thermoresponsive hydrogels [13, 14]. In these studies, NIPAM was utilized to impart thermoresponsive properties to the hydrogels. NIPAM is widely recognized as one of the most used monomers for producing thermoresponsive hydrogels. Accordingly, NIPAM-based hydrogels swell below their LCST and shrink above it.

Nowadays, scientific efforts are increasingly directed toward developing materials from renewable resources due to their biodegradability, biocompatibility, cost effectiveness, and environmental sustainability [15]. Castor oil (CO) is a kind of vegetable oil and one of the most widely sourced plant-based raw materials. Because of their low toxicity, functional versatility, affordability, and broad availability, plant oil-based compounds are often employed in polymerization reactions instead of petroleum-derived compounds [16]. In addition to plant-based raw materials, functional polymers can also be produced from microorganism-derived sources. Bacterial cellulose (BC) is one of the microbial raw materials. BC exhibits a fibrous network architecture composed of highly organized three-dimensional nanofibers.

This characteristic of cellulose gives the material a porous morphology and significant surface area per unit total space [17]. Other notable characteristics of cellulose include its high purity, extensive polymerization and crystallinity, as well as remarkable biocompatibility [18]. In addition to its flexibility, BC's elasticity, biodegradability, high water absorption, and potential for chemical

modification make it an excellent material for medical research [19-21].

The literature includes several examples of BC and CO-based polymers designed for biomedical applications. Among these works, Alosmanov et al. [22] effectively manufactured grafted PNIPAM brushes from wet BC cellulose sheets. PNIPAM structures included wet BC affected the swelling characters and drying features of PNIPAM brushes. Wang and coworkers [23] designed a novel material for biological applications, including artificial muscles, and investigated how the molar ratio between MDI and the glucose units in BC affects the material's structure. Arikibe et al. [24] explored how pH affects drug release in BC/chitosan-based hydrogels behaviors and swelling profiles.

Similarly, swelling properties were analyzed on scaffolds included BC based hydrogels filled with calcium phosphate by Basu et al. [25]. Moreover, an investigation into the combination of CO and citric acid was reported by Parada Hernandez et al. [26]. Arévalo-Alquichire et al. [27] also designed a polyurethane-based material from CO for biomedical applications. In our previous study, we synthesized thermo-responsive hydrogels from BC and CO and investigated the effect of CO on the hydrogels' physicochemical properties [28]. The hydrogel derived from CO demonstrated improved sensitivity to temperature fluctuations in contrast to the hydrogel without CO. Building on this background, the present study aims to develop thermoresponsive hydrogels from sustainable sources and to examine how changes in the molar ratio of bacterial cellulose (BC) influence their chemical composition, thermal properties, swelling behavior, morphology, and biocompatibility.

2. General Methods

2.1. Materials and microorganisms

N-Isopropylacrylamide (NIPAM), dimethoxy-2-phenyl acetophenone (DMPA), N,N'-Methylenebisacrylamide (MBAM), and quercetin were sourced from Sigma Aldrich. Acetone and triethylamine were obtained from Merck. 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazoline bromide (MTT) and Dulbecco's Modified Eagle Medium (DMEM) were obtained from Sigma Aldrich. 4,4'-diphenylmethane diisocyanate (MDI) was provided by Kimpur Kimteks Polyurethane Corporation. L-glutamine (Gibco) was obtained from Thermo Fisher Scientific, and Castor oil (CO) was obtained from Zag Kimya. All solvents were supplied from Merck. Fetal bovine serum (FBS) was supplied from Lonza. Pure water was obtained by Direct Q-3UV device. UV absorbance readings were acquired using a Photolab 6600 UV-VIS spectrophotometer. UVGL-58 230V 50Hz lamp was used for light-induced polymerization reactions that were realized at 365 nm. 10000 MWt dialysis membranes were used for release experiments. The MMM Vacucell vacuum oven was used to dry the hydrogels. *Komagataeibacter hansenii* DSMZ 5602 was obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ).

2.2. Synthesis of BC from *Komagataeibacter hansenii*

Hutchens et al. [29] and Uzyol et al. [30] detailed the production process of BC. *Komagataeibacter hansenii* and yeast extract-peptone-mannitol medium (YPM) (5 g/L of yeast extract, 3 g/L of peptone, and 25 g/L of mannitol) were used to synthesize BC. BC production occurred at 28°C and pH 6.8. YPM medium served as the preculture medium for all fermentations. After sterilization, a single colony of *Komagataeibacter hansenii* cells was isolated on a petri dish. Subsequently, it was placed to a test tube containing 5 ml of pre-culture medium for further processing.

The incubation occurred at 28°C for two days without agitation. Subsequently, 50 ml of sterile medium was introduced with 10% (v/v) in a 500 ml flask. After 14 days incubation time, the BC was treated with 0.1 N NaOH for 20 min at 80°C to remove bacterial cells and residual medium. Finally, it was extensively washed multiple times with distilled water and stored at 4°C for subsequent procedures.

2.3. Synthesis of Poly(CO-BC-NIPAM) thermoresponsive hydrogels

Two sets of hydrogels were prepared with varying BC molar ratios. Wang et al. [23] described the procedure that was used to create hydrogels from polyurethane. Details of the synthesis method are given in our previous study where the effect of CO on the physical, thermal and morphological characterization of hydrogels was investigated [28]. Furthermore, in our previous study the MDI:BC:CO ratio was kept constant at 1:1:1 and two different hydrogels with and without CO were successfully synthesized. Given the observed positive effects of CO on the hydrogel, it was considered appropriate for all hydrogels in this study to include CO. The present study aimed to explore how variations in the BC ratio influence the thermal, physical, and morphological properties of the hydrogels. The molar ratio of MDI, the glucose units of BC, and CO were set as 1:0.5:1 (poly(CO-BC)-I), and 1:2:1 (poly(CO-BC)-II) (Table 1).

Table 1. Molar equivalency of reactants

Hydrogels	CO[a]	BC[a]	MDI[b]
poly(CO-BC-NIPAM)-I	1	0.5	1
poly(CO-BC-NIPAM)-II	1	2	1

[a)]Molar ratio of glucose units; [b)]molar ratio of MDI.

First, BC was cut up into the smallest possible fragments. Next, BC fragments were mixed with CO and MDI. TEA and dehydrated acetone were introduced into the mixtures. All the mixtures were kept at 40°C for two days. Then, poly(CO-BC) hydrogels were washed three times with acetone. Next, acrylamide-based monomers were incorporated into hydrogels in the second phase of polymerization to provide them thermoresponsive properties. For this purpose, NIPAM (50.0 and 200.0 mg) and MBAM (0.5 and 2.0 mg) were dissolved in distilled water for poly(CO-BC-NIPAM)-I, poly(CO-BC-NIPAM)-II, respectively.

Poly(CO-BC) hydrogels were transferred into acrylamide solutions (NIPAM and MBAM), and the mixtures were kept at room temperature (RT, 25˚C) for 12h. Afterward, radical polymerization was initiated by adding DMPA (1.0% mol of NIPAM). 1h of UV irradiation (365 nm) was applied for photopolymerization. Then, distilled water was used for washing the polymers three times, followed by the drying process under reduced pressure at 40°C for two days. Finally, poly (CO-BC-NIPAM) hydrogels were obtained as opaque crystal structures.

2.4. Characterization of hydrogels

Fourier-transform infrared spectroscopy (FTIR) spectroscopy was used to investigate the functional groups of hydrogels. FTIR analyses were realized by a Tetra JASCO 6600 spectrophotometer. Thermal analysis was performed by Tetra DSC7000X Differential Scanning Calorimeter (DSC) and HITACHI STA7200 Simultaneous Thermogravimetric Analyzer (TGA). FEI QUANTA 450 FEG ESEM Scanning Electron Microscope (SEM) was used for morphological analyses of hydrogels.

2.5. Swelling measurements

Hydrogels were transferred into distilled water at RT to determine the degree of swelling (%). The gravimetric method was conducted. The hydrogels were occasionally taken out of the water, and their weights were reported. Degree of swelling (%) of hydrogels (Equation 1) was calculated as below:

$$\textbf{Degree of swelling (\%)} = ((W_w - W_d) / W_d) \times 100 \quad (1)$$

where Ww is the weight of the hydrogel after swelling at RT, while Wd represents the weight of the hydrogel after drying at RT. The deswelling behavior of the hydrogels was studied at 40°C, which exceeds the LCST. Hydrogels were weighed after being removed from water periodically. Water retention (%) (WR(%)) (Equation 2) is calculated as described below:

$$\textbf{WR (\%)} = [(W_r - W_d)/(W_s - W_d)] \times 100 \quad (2)$$

where Wr denotes the weight of the hydrogel at a specific time at 40°C and Ws represents the weight of the hydrogel after equilibrating its swelling at RT.

2.6. In vitro cytotoxicity assays

In vitro cytotoxicity of poly(CO-BC-NIPAM) hydrogels were analyzed by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay in COS-7 cells (African green monkey kidney fibroblast-like cell line) [31]. COS-7 cells (14×10^4 cells/well) were seeded into 24-well cell culture plates with complete medium consisting of DMEM high glucose with 10% L-glutamine (Gibco), supplemented with 10% FBS, and then cultivated at 37°C in a 5% CO_2 atmosphere.

After the 24-hour growth phase, cells were treated with hydrogels at concentrations ranging from 1 to 20 mg/mL. All experiments were conducted in duplicate. The control group comprised cells that were not treated with hydrogels. After another 24h of incubation, cytotoxicity was determined. 100 µL of sterilized MTT reagent (5 mg/ml) was added to each well, followed by a 4h incubation period. After incubation, the medium was aspirated, and 500 µL of DMSO was added to dissolve the formazan crystals. Following a 15-min incubation in the dark using an orbital shaker, 100 µL of the dissolved formazan solution was transferred to a 96-well plate for absorbance measurement at 570 nm using an AMR-100 Eliza Plate Reader.

The amount of formazan produced is directly proportional to the number of viable cells, and cell viability (%) (CV (%)) was calculated using the following formula:

CV (%) = (Absorbance of the sample/Absorbance of the control group) x 100 (3)

2.7. Statistical analysis

For assessing cell viability, each experiment was independently conducted twice in triplicate. The results are reported as mean ± standard deviation (SD). Statistical evaluation was performed using one-way analysis of variance (ANOVA) with Bonferroni correction for post hoc comparisons to assess comparative statistical analysis between groups, with $p < 0.05$ considered statistically significant.

3. Results and Discussion

3.1. FTIR analyses of hydrogels

FTIR spectra were examined to analyze the functional groups present in the hydrogels. FTIR spectra of poly(CO-BC-NIPAM)-I, and poly(CO-BC-NIPAM)-II were compared with FTIR spectra of starting materials (CO, BC, MDI, and NIPAM). We analyzed FTIR spectra of the BC, MDI, CO, and NIPAM in our previous study [28]. FTIR spectra of poly(CO-BC-NIPAM)-I, and poly(CO-BC-NIPAM)-II are shown in Figure 1. The bands caused by NIPAM are seen in all hydrogels. Amide-II and amide-I groups are observed at 1535 cm^{-1} and 1629 cm^{-1}. The peaks revealed at around 3075 cm^{-1} and 3282 cm-1 indicate NH stretching of NIPAM. There is also a broad peak around 3300 cm^{-1}, likely due to the OH stretching of free hydroxyl groups in BC. The band at 1739 cm^{-1} demonstrates the existence of ester carbonyl groups in CO. [28]. We believe that the absence of the CO band in poly(CO-BC-NIPAM)-I shows that this polymer contains less amount of CO than expected. In our previous study, it was demonstrated that amide II and amide I groups are seen in the 1535 cm-1 and 1629 cm-1 bands [28]. In addition, the bands resulting from the OH stretching of the free hydroxyl groups in BC are also seen in the FTIR analysis of the hydrogels synthesized in our previous study.

Consistent with our findings, Alosmanov et al. [22] noted that the peaks at approximately 3340 cm^{-1} correlated with the existence of amide groups in PNIPAM chains. It was also noted that these bands overlap with those produced by OH stretching vibrations. Moreover, the broad band could have formed as a result involving hydrogen bonds forming among the PNIPAM polymer chains and the BC surface.

3.2. Morphology of hydrogels

Figure 2 illustrates SEM images of poly(CO-BC-NIPAM)-I, and poly(CO-BC-NIPAM)-II. Poly(CO-BC-NIPAM)-I has a layered structure with discrete irregular particles on it, whereas poly(CO-BC-NIPAM)-II has sponge-like structures with spherical particles.

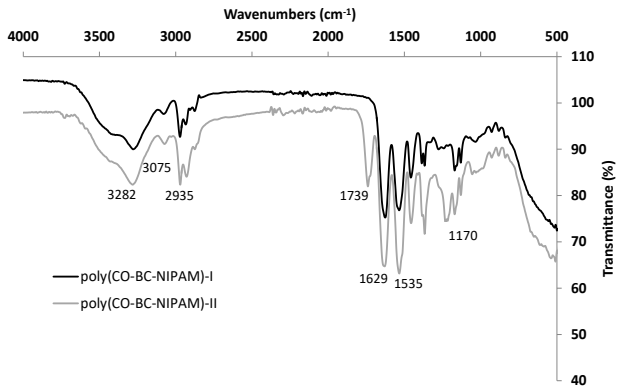

Figure 1. FTIR spectra of the hydrogels

Wang et al. [23] demonstrated that pure BC has a perfect vertical layer structure, whereas BC/PNIPAM composites show porous morphology. In the present study, the sponge-like morphology was observed in the hydrogels with a higher molar ratio of BC. Similarly, Luo et al. [32] showed that there are undulations on the surface of cellulose-based composites and the structure has many folds, resulting in more surface area, and capillary action.

The results from Moura Neto et al. [33] and Alaa et al. [34] are consistent with the hydrogel images obtained in the current study. Moura Neto et al. [33] also reported that CO provided a layered structure in BC/NIPAM composite, which is consistent with our results. As the molar ratio of CO/BC increased, a more layered structure was examined.

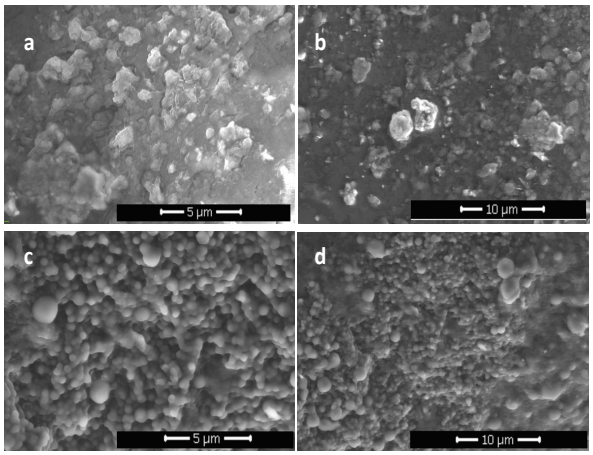

Figure 2. SEM images of produced hydrogels. (a, b) poly(CO-BC-NIPAM)-I, (c, d) poly(CO-BC-NIPAM)-II

Moreover, the quantity of pores in the structures rose as the BC ratio increased because of the porous structure of BC. Layered structures are added to these hydrogels by the addition of CO. As expected from the results of the study, the porosity increases as the BC ratio increases, confirming the results of our previous study [28]. It was also observed that porous and spongy structures were formed with the addition of BC in the hydrogels synthesized in our previous study where the BC ratio was lower compared to poly(CO-BC-NIPAM)-II. The data from this study demonstrates that porosity increases with increasing BC ratio.

3.3. Thermal characterizations

DSC technique gives valuable information about the thermal transition in the polymers such as glass transition and melting. DSC thermograms of the polymers synthesized are shown in Figure 3. Poly(CO-BC-NIPAM)-I showed a glass transition at -45°C. Other polymers do not show any Tg. All polymers showed melting behaviors between certain temperature ranges. Melting temperature of the samples are obtained at 107.2°C, and 114.7°C for poly(CO-BC-NIPAM)-I, poly(CO-BC-NIPAM)-II respectively. The heat of fusion of poly(CO-BC-NIPAM)-I, and poly(CO-BC-NIPAM)-II was determined as 43.1 mJ/mg, and 70.4 mJ/mg, respectively. These melting behaviors might be the results of the degenerative chain transfer reactions of the allylic positions of CO which can cause premature polymerization. Another explanation of these melting peaks would be an action of the long carbon chain domains of fatty acid moieties of CO. These findings are congruent with the literature [28].

TGA (DTA) thermograms give valuable information about the thermal decomposition of the materials. TGA thermograms are illustrated in Figure 4. All samples were heated to 600°C with a 10°C/min heating rate under 200 ml/min nitrogen atmosphere. Thermal stabilities of the materials synthesized can be expressed by 15% and 50% weight loss temperatures. The 15% weight loss temperatures were measured as 272, and 277°C for poly (CO-BC-NIPAM)-I, poly (CO-BC-NIPAM)-II, respectively. The 50% weight loss temperatures of the samples were assessed as 403, and 401°C for poly (CO-BC-NIPAM)-I, poly (CO-BC-NIPAM)-II, respectively.

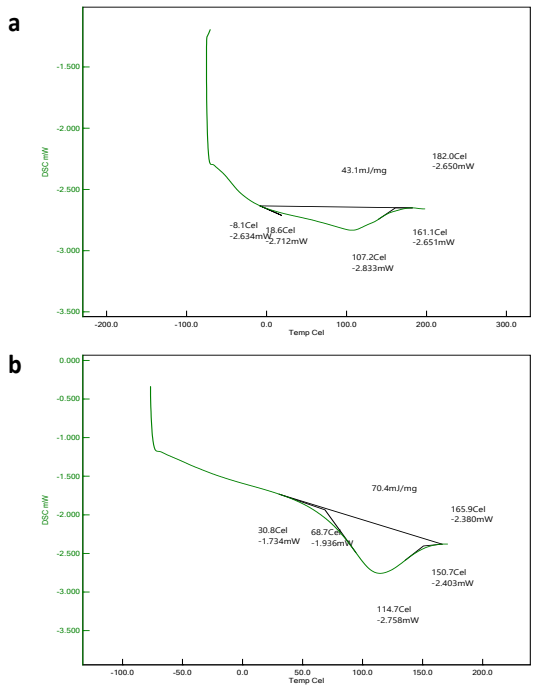

Figure 3. DSC thermograms. **(a)** poly(CO-BC-NIPAM)-I, **(b)** poly(CO-BC-NIPAM)-II

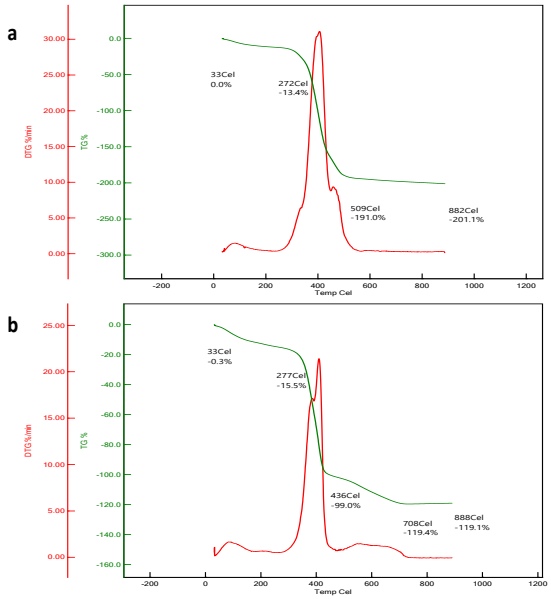

Figure 4. TGA curves. **(a)** poly(CO-BC-NIPAM)-I, **(b)** poly(CO-BC-NIPAM)-II

By using derivative TGA, one can evaluate the thermal degradation rate. In this sense, when the thermal degradation rate increases the intensity of the DTGA increases as well. The lowest one belonged to poly(CO-BC-NIPAM)-II as 22.5 DTG%/min at 401°C.

3.4. Swelling and shrinking dynamics of hydrogels

The hydrogels' swelling properties were investigated. Hydrogels can be visually distinguished from one another when they are dry or wet. Swelling and water retention profiles of the hydrogels over time are demonstrated in Figure 5a and 5b. Equilibrated swelling degree (ESD) of the hydrogels was accomplished in 120 min at room temperature (Figure 5a). ESD of poly(CO-BC-NIPAM)-I, and poly(CO-BC-NIPAM)-II, was calculated as 228.6%, and 592.6%, respectively. Due to BC's greater potential for retaining water than CO, ESD of the hydrogels is dramatically rising as BC's molar ratio is increasing.

A similar correlation between the BC molar ratio and swelling capacity was reported by Lin et al. [35] The exceptional water retention capability of BC can be attributed to robust molecular interactions between the water molecules and OH groups of BC.

In a separate investigation, poly(glycidyl methacrylate)/BC nanocomposites were synthesized through in-situ polymerization of glycidyl methacrylate via free radicals within the BC network. They reported that the increasing poly(glycidyl methacrylate) matrix in composites resulted in a decrease in water uptake ratios. At RT, water uptake capabilities were assessed during a 48h period. For the poly(glycidyl methacrylate)/BC composites, the performances were measured at 32.8%, whereas for BC's hydrophilic nature, they were 138% [36]. In contrast to Faria et al. [36], we found a higher swelling ratio than 138% in the current study. This could be because NIPAM's presence may make the hydrogel network more hydrophilic.

Since the CO ratio was kept constant in the hydrogels of this study, any impact on their swelling and shrinking behavior will be attributed to variations in the BC ratio. The effect of CO on the swelling and shrinking behavior of hydrogels was investigated by our group in our previous study [28]. It was reported that CO has a negative effect on swelling behavior due to its hydrophobic properties. In addition, when the results of this study are examined in the literature, it is shown that increasing the BC ratio has a positive effect on the swelling behavior of hydrogels.

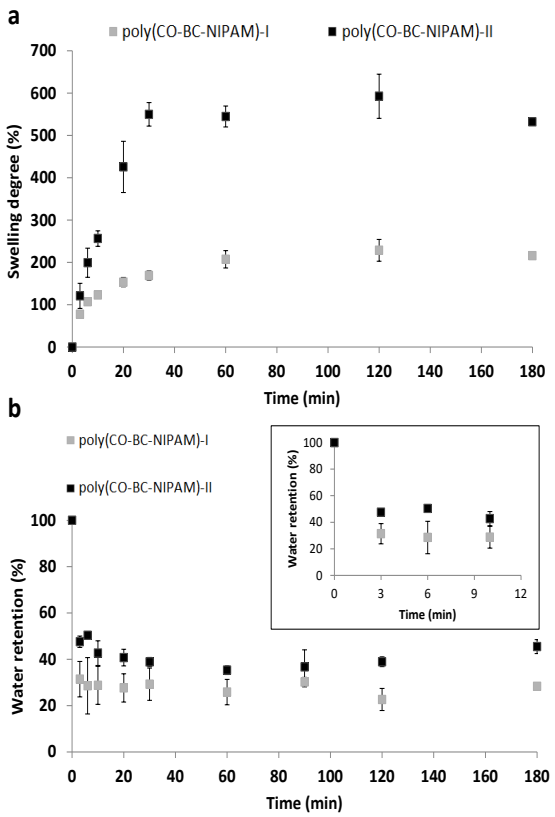

Figure 5. Swelling and water retention behaviors of hydrogels. **(a)** swelling behaviors at RT of the hydrogels; **(b)** water retention profiles of all hydrogels at 40°C for 180 min and 10 min

As the temperature increased above the LCST in this study, the hydrogels' capacity to absorb water rapidly decreased. Figure 5b shows deswelling profiles of the hydrogels. All hydrogels immediately shrink and release water when heated. In comparison to poly(CO-BC-NIPAM)-I, which lost 68.6% of its water in 3 min, and poly(CO-BC-NIPAM)-II suffered losses of 52.4%, respectively. After 180 min, the total water loss for poly(CO-BC-NIPAM)-I, poly(CO-BC-NIPAM)-II, was obtained to be 71.7%, and 54.5%, respectively.

3.5. Cytotoxicity of hydrogels

In the present study, cytotoxicity was evaluated on COS-7 cells for 1 mg/ml, 2 mg/ml, 5 mg/ml, 10 mg/ml, and 20 mg/ml hydrogels which were prepared with different cellulose ratios poly(CO-BC-NIPAM)-I, and poly(CO-BC-NIPAM)-II, as shown in Figure 6. The results revealed that cells exhibited at least 69% viability when exposed to 1 mg/ml of hydrogels and exposing the cells to higher concentrations of hydrogels or cellulose did not change the cell viability significantly indicating that the cytotoxic effect of polymers was independent of the BC concentrations.

IC_{50} values, representing the concentration that inhibits 50% of cell growth, were determined to assess polymer cytotoxicity by plotting cell survival (%) against hydrogel concentration (mg/mL). The IC_{50} concentrations for poly(CO-BC-NIPAM)-I, and poly(CO-BC-NIPAM)-II are 5.33 mg/ml, and 11.16 mg/ml, respectively. These values also support the biocompatibility of the hydrogels as lower IC_{50} values mean more toxicity to the cells.

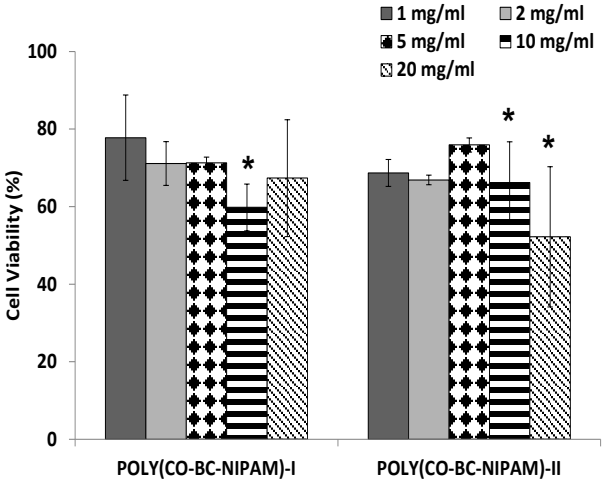

Figure 6. Cell viability values of the hydrogels

Recently, renewable biomaterial-based BC hydrogels are gaining significant attention due to their abundant natural presence and applications in wound care, tissue repair, and pharmaceutical applications. Therefore, researchers emphasize biocompatibility of these novel hydrogels by assessing the cytotoxicity in numerous in vitro and in vivo studies [37-39]. Evaluations regarding the cytotoxicity of composites based on BC in different cellular models and animal studies revealed that these hydrogels are biocompatible, however, the combination of BC with other ingredients such as polymers and natural materials may improve not only the structure and mechanical properties but also their biocompatibility, cell binding ability, and low antigenicity.

For instance, a BC/collagen hydrogel was reported to show a higher adhesive property and was used as wound dressing material in vivo studies [40]. Han et al. [41] on the other hand prepared a BC/polyvinyl alcohol hydrogel

scaffold for corneal reconstruction and the hydrogel showed higher biocompatible property supported by low cytotoxic effect in cell lines and almost no immune response in rabbits. In this study, we evaluated the BC hydrogels prepared with a plant-based castor oil monomer and observed high biocompatibility.

In the following studies, cell binding ability should be evaluated to determine its suitability as a scaffold for wound dressing and tissue engineering applications.

4. Conclusion

In the present study, CO and BC based thermoresponsive hydrogels poly(CO-BC-NIPAM)-I, and poly(CO-BC-NIPAM)-II were successfully synthesized. The influence of the BC ratio on the physical and structural properties of the BC/PNIPAM hydrogels was investigated. FTIR analysis was performed to evaluate structural differences between poly(CO-BC-NIPAM)-I and poly(CO-BC-NIPAM)-II, while SEM studies were employed to examine their morphological variations. The thermal properties of the hydrogels were characterized using TGA and DSC. Additionally, their swelling and deswelling behaviors were examined, and biocompatibility was assessed via the MTT assay.

The BC ratio in the hydrogels influences their swelling and deswelling profiles. The increased BC content of poly(CO-BC-NIPAM)-II resulted in the highest swelling degree. Poly(CO-BC-NIPAM)-II reached the highest equilibrium swelling degree of 592.6% within 120 min at room temperature. These findings may be attributed to the strong affinity of cellulose for water. Furthermore, the swelling and deswelling profiles demonstrated that all the hydrogels exhibited thermosensitive characteristics. SEM images revealed that the formation of spongy structure in hydrogels is caused by the increase in BC ratio and the presence of CO.

When the results of the thermal analysis were examined, the highest 15% weight lost temperature for poly(CO-BC-NIPAM)-II was found to be 299°C and the maximum absorbed heat was determined as 183 mJ/mg. IC50 values are 5.33 mg/ml, and 11.16 mg/ml for poly(CO-BC-NIPAM)-I, and poly(CO-BC-NIPAM)-II, respectively. The cell viability increased with the molar ratio of BC.

Overall, the findings highlight the potential of renewable resources in hydrogel development. This study introduces a hydrogel derived from renewable raw materials using an eco-friendly process. The resulting thermoresponsive hydrogels exhibit high swelling capacity and biocompatibility, with those containing bacterial cellulose showing enhanced properties. These novel hydrogels hold promise for a wide range of biomedical applications.

Article Information Form

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Authors' Contribution
Conceptualization, E.I.K., Ö.Y.Ç., D.K. and P.Ç.H..; methodology, E.I.K., Ö.Y.Ç., G.Ç., D.K. and P.Ç.H.; validation, E.I.K., O.P., Ö.Y.Ç., G.Ç., D.K. and P.Ç.H.; formal analysis, E.I.K., O.P., Ö.Y.Ç., G.Ç., D.K. and P.Ç.H.; resources, E.I.K., O.P., G.Ç., D.K. and P.Ç.H.; data curation, E.I.K., and P.Ç.H.; writing—original draft preparation, E.I.K., O.P., G.Ç., D.K. and P.Ç.H.; writing—review and editing, E.I.K., O.P., Ö.Y.Ç., D.K. and P.Ç.H. All authors have read and agreed to the published version of the manuscript.

The Declaration of Conflict of Interest/ Common Interest
No conflict of interest or common interest has been declared by the authors.

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During the preparation of this work, the authors used ChatGPT for grammar checking and editing. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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