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Production of Bacterial Cellulose Based On Bio Nonwoven / Nonwoven Composites for Medical Textile Applications

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ABSTRACT

Bacterial cellulose (BC) is a popular biomaterial which is used in innovative research in many fields thanks to its unique properties. In this study, BC as bio nonwoven structures are produced in Kombucha culture using '*Acetobacter xylinum*' bacteria in a static culture setting. Bio nonwoven surfaces are produced with the sandwich composite model. They use 15-25-60% cotton/ viscose/ polypropylene nonwoven surface fabric and 80-20% polypropylene /viscose nonwoven surface fabric while creating bio nonwoven surfaces. Water retention, porosity, dust retention, FTIR (Fourier Transform Infrared Spectrophotometer), SEM (Scanning Electron Microscope), and TGA (Thermogravimetric Analysis) analysis of the obtained BC structures are investigated. As a result of the analysis, it is determined that the BC and BC composite structures, which have undergone hydrogen peroxide and sodium hydroxide applications, have properties that can be used for medical purposes.

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KEYWORDS

Bacterial Cellulose, Static culture, Medical textiles, Biomaterials

1. INTRODUCTION

Biomaterials have benefited human civilization for thousands of years. The design of biomaterials in various fields is extremely important for its sustainability. With the advances in materials science, it has become easier to manufacture, process, and functionalize biomaterials because of their' structural authenticity [1]. One of the raw materials used as a biomaterial is cellulose. Cellulose is the most plentiful, natural polymer in nature derived from renewable resources [2, 3]. Cellulose, which forms a dense part of the chemical structure of vegetable fibres, is a polysaccharide with the general formula $(C_6H_{10}O_5)n$ [4, 5]. Cellulose consumption is increasing day by day directly proportional to the increase in the world's population. Researchers have sought alternative cellulose sources to meet this consumption by taking advantage of the rapid development of biotechnology [6, 7]. One of these alternative sources is the production of cellulose from bacteria. The cellulose synthesized in this way is called bacterial cellulose (BC) or microbial cellulose [9, 10]. The BC layer is also defined as the BC nonwoven surface or bio nonwoven surface [11, 12]. In studies done to date, it has been proved that bacteria such as *A. xylinum, Agrobacterium, Pseudomonas, Rhizobium, Salmonella, Sarcina,* and *Zoogloea* can synthesize high purity cellulose [8, 20].

BC is a natural polymer variety which is produced by a class of acetic acid bacteria. Both systems use different sugar sources and produce β 1-4 glucan chains. They exit the cell through the pores in the cell membrane and form highly ordered and porous bio nonwoven structures [13]. Temperature, nutrient sources (carbon, nitrogen source, and microelements), pH, production methods (static culture, shaken culture, bioreactor), oxygen yield, and bacterial variety are the main parameters affecting BC production [14, 15, 16, 17, 39].

The most preferred culture media in BC production are HS (Hestrin - Schramm), Yamanaka, Zhou, CSL (corn steep liquor)-fructose media [8, 18]. In addition to these culture media, a symbiotic combination of bacteria and yeasts known as kombucha (SCOBY) is also used to produce BC [19].

Kombucha solution consists of water, sugar, tea, and kombucha culture (tea mushroom) [18]. BC layer is

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obtained by incubating the relevant solution at room temperature, preferably in a dark environment for about 1-3 weeks [21, 22]. It is predicted that BC will continue to be used increasingly in the following years due to its biocompatible, flexible, high purity, high Young's modulus, high water retention capacity, high crystallinity, and environmental friendliness [23, 24, 25, 26, 27].

BC to be used as a raw material in different application areas needs to undergo some preliminary processes such as cleaning, bleaching, and drying [31]. The great structural, physicochemical, mechanical, thermal, and biological properties of BC have made it sufficient in biomedical [32], environment [33], energy [34], electronics [35], and many other fields [13]. BC shows hydrogel properties in aqueous media. Between the wet and dry states of BC; there are differences in mechanical properties, dimensional stability, thickness, and tissue structure [28]. The nanofibers in BC have a large surface area per unit and a diameter of 20 to 100 nm. When this property is combined with the hydrophilic nature of BC, high water absorption capacity results in better adhesion, and increased moisture content, and thanks to these properties, it can be used as a biomedical material for wound dressing [29, 30, 44].

Solway et al. (2011) showed in their study that the application of BC to diabetic ulcers increases the rate of wound healing [55]. Heleni et al. (2006) evaluated the *in vivo* biocompatibility of BC and implanted it in mice for 1, 4, and 12 weeks. It was stated that the conditions such as chronic tissue damage and inflammation in implanted BC were not observed. As a result of this study, it was accepted that BC is biocompatible *in vivo* applications [56].

Bacterial cellulose is used in many medical fields such as temporary artificial skins, treatment of burns and ulcers, artificial implants, and artificial vein production. In these areas, BC is used in its raw form, its modified form (*in situ, ex situ*), and different forms by regenerated [29, 54].

Our study focuses on BC, a highly versatile natural biomaterial. In this study, BC is produced in a static culture medium by using *A. xylinum* bacteria. To control the restrictive properties of BC, the production of composite structures was obtained by combining them with non-woven surfaces suitable for medical textile applications, which were not used together in composite production before, and their usability in the field of medical textiles was investigated. Based on the idea that the pure cellulose content, porous structure, and high water holding capacity of BC will provide high performance in wound dressing applications.

2. MATERIAL AND METHOD

2.1 Material

Kombucha SCOBY is provided by a company in Turkey to produce BC. Glucose (carbon source), black tea (nitrogen source), distilled water, and kombucha starter are used in the preparation of kombucha culture. Acetic acid is used to provide the necessary acidic environment for BC production. For the cleaning process of the BC, sodium hydroxide (NaOH) and ethanol (C_2H_5OH) are used [38].

Hydrogen peroxide (H_2O_2) is used for bleaching BC. To create a composite structure with BC, 52 g/m^2 15% cotton (CO)/25% viscose (VI)/60 polypropylene (PP) nonwoven fabric and 37 g/m^2 80% PP/20 VI nonwoven fabric were used. The codes, properties, and processes of the samples used in the study are shown in Table 1.

2.2 Method

2.2.1 BC produced in static culture

There are various methods for the production of BC. In this study, the static culture method is used due to the low technological cost and high production capacity.

In the first stage of BC culture production, 8.2 grams of black tea is added to 1 liter of boiled distilled water and infused for 15 minutes. 100 grams of glucose is added to the brewed tea and mixed until it dissolves. When the temperature of the mixture drops below 30 ºC, 10% acetic acid and 1 BC culture (4x4 cm^2 layer) are added to the obtained tea. The mixture is covered in such a way that it can absorb oxygen, and it is kept in an environment without the sun for 10 days. The implementation was carried out at 25.8 °C and 34% humidity [40]. A cleaning process was required to purify the obtained

Table 1. The code of samples to use in analyses

Code	Sample Properties	Process
BCW	Raw BC wet	Not processed
BCD	Raw BC dry	Drying in sunlight
BCN	After treatment with NaOH	Treated with NaOH after washing in distilled water
BCNP	After treatment with H_20_2	Treated with H_2O_2 after treatment with NaOH
52NF	52 g/m^2 PP/VI/CO nonwoven fabric	Not processed
37NF	37 g/m^2 PP/VI nonwoven fabric	Not processed
BC52NP	52 g/m^2 PP/VI/CO nonwoven BC composite	BC composite treated with H_2O_2 after treatment with NaOH
BC37NP	37 g/m^2 PP/VI nonwoven BC composite	BC composite treated with H_2O_2 after treatment with NaOH
BCCD	BC sprinkled with coal dust	Coal dust was added to the surface of the BC and vacuumed.

BC layers from bacteria and other foreign substances. For the cleaning process, primarily, the BC layers were kept in C_2H_5OH for 10 minutes. As a second process, it was treated with distilled water at boiling temperature for 40 minutes. As the last step, it was kept in 0.1 M NaOH at 90 °C for 20 minutes 4 times (Figure 1). The cleaned BC layers must be dried. To carry out the drying process, the material is kept on a wooden surface that does not absorb water, in an environment that receives sunlight, until it dries. The reason for drying in the daylight is to ensure that the dehydration takes place slowly and to preserve the properties of the structure [41].

When this product is intended to be used as a wound dressing, it is thought that it may cause the liquids (inflammation, blood, etc.) formed on the wound to be overlooked, especially due to its colour. In addition, the colour of the BC structure, which becomes white after the bleaching process, stands out visually. To bleach the obtained BC layers after the cleaning process, it was processed at 40 ml/l H_2O_2 (35%) pH 7 for 1 hour at boiling temperature [42]. At the end of the cleaning and bleaching process, the BC layer is ready for the preparation of the composite structure.

Figure 1. BC layer produced in static culture medium

2.2.2 Preparation of BC composite structures

A sandwich composite structure was created to design the wound dressing. Figure 2 shows the schematic representation of the composite sandwich structure. BC structures are used in the outer layer. In the inner layer, biocompatible 52 g/m^2 15% CO/25% VI/60 PP and 37 g/m^2 80% PP/20 VI nonwoven surfaces are used. It is thought that the content of nonwoven surfaces used in composite structures will contribute positively to the properties of the produced samples.

In composite structures which are used for the reason of low cost, easy workability, low density, high strength, and excellent chemical resistance, nonwoven surfaces with high polypropylene (PP) ratio are used. In addition to that, the main reason for the presence of cotton and viscose in the contents of the used nonwovens is the high water absorption ability, which is one of the necessary features in the design of the wound dressing.

Figure 2. Schematic representation of the composite sandwich model

2.2.3 Porosity analysis of BC and BC composite structures

Especially in interbody applications, porosity is one of the main components. The use of structures with high porosity increases the speed of healing in the long term. BC, its composite samples, whose dry weight was taken, were then kept in 10 ml of distilled water for 2 hours. After this process, it was vacuumed in a vacuum desiccator for 1 hour to fill all the pores with water. Secondly, the wet weight and wet volume of the samples were measured to calculate their size [43]. The specific gravity of pure water (ρwater) was accepted as 1 g/cm³ and porosity results were obtained using the equation (1, 2, 3) below.

First Step: (1)

The weight of the water filling the pores= Sample wet weight – Sample dry weight

ρwater=Weight/Volume

1 g/cm³ = weight of water filling pores /volume of water filling pores

Second Step: (2)

Weight of water filling pores = volume of water filling pores

If it is thought to be;

Third Step: (3)

It can be expressed as

% Porosity=[(Sample wet weight – Sample dry weight)/ (Wet sample volume)] x 100

2.2.4 Water retention capacity analysis

Today, BC structures can be used as wound dressings. The water retention capacity of composite BC which are obtained by combining with nonwoven surfaces consisting of different contents is investigated. And it is thought that it will benefit the development of composite products with different contents.

For the analysis of the water retention capacity of the samples, the wet weights of the samples were taken after they were kept in water for 1 hour. Then, it was then left to dry at room temperature for 24 hours and their dry weights were taken [45, 46]. The water retention capacity analysis was carried out with equation (4) given below.

$$\text{Water retention ratio}(\%) = [(W_{wet} - W_{dry})/W_{dry}] \times 100 \quad (4)$$

2.2.5 Dust permeability analysis

Coal dusts which are smaller than 100 microns (μ), 100-200 μ, and larger than 200 μ were sieved to simulate the foreign objects that may cause infection or delay healing. The usability of the BC structures as wound dressings was investigated. The sieved charcoal powders were transferred to the BC structure to prevent dust and microorganism transmission to the wound surface. Then vacuuming was applied for 1 hour. To analyze whether the coal dust has passed into the sample, images were taken under the microscope with a camera at 60x, 120x, and 200x magnification.

2.2.6 SEM analysis

SEM analysis was performed with a Fei Quanta 250 Feg machine. For the morphology analysis of BC samples, images were taken by applying an adjustment scheme between 15-20 kV in a scanning electron microscope (SEM) in a low vacuum environment.

2.2.7 FTIR analysis

FT-IR analysis was performed by using the Perkin Elmer Spectrum BX instrument. Analyzes were performed at room temperature, using the KBr pellet technique with a resolution of 4 cm^{-1}, in the mid-infrared region of 4000-400 cm^{-1} with 2 cm^{-1} intervals and 16 scans.

2.2.8 Thermal analysis

For the thermal analysis, Perkin Elmer Instruments, diamond TG/DTA/DSC was used. The thermal properties of the raw BC which were dried at room temperature and H_2O_2 treated BC; It was determined by making it in a nitrogen environment between 10 °C/min and 25 – 650 °C.

3. RESULTS AND DISCUSSION

As a result of the bleaching process after the cleaning step, the BC structure has become white as in the cotton fibre. While the colour of the raw BC layer has been brown, the colour has changed to white after bleaching. After the cleaning process (NaOH), the BC structures have been off-white. Figure 3 shows the BC structures after raw (BCW) and NaOH treatment (BCN) and after H_2O_2 treatment (BCNP). The colour change of the obtained BC structures obtained after cleaning and bleaching can be seen clearly from the images.

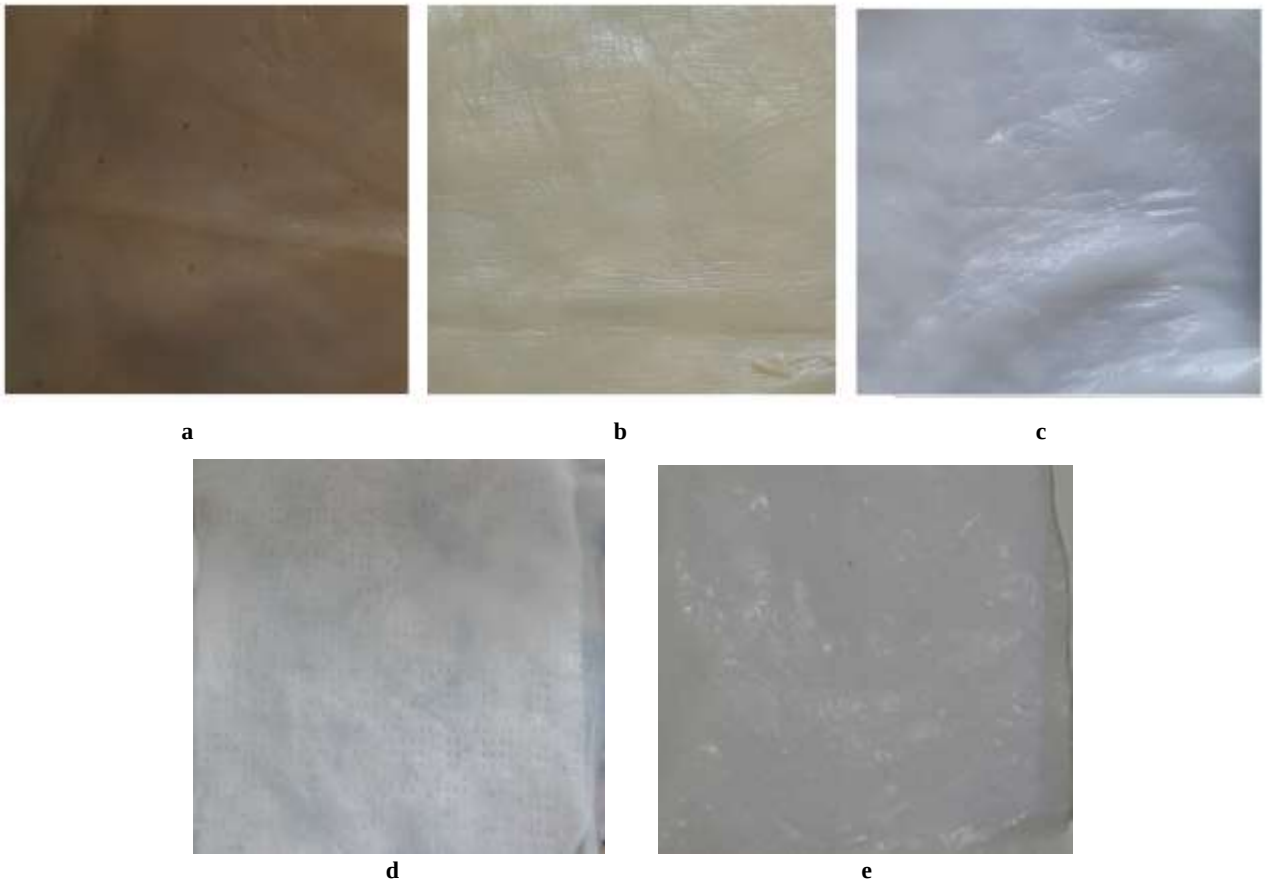

Figure 3. (a) BCW, (b) BCN, (c) BCNP, (d) BC37NP, (e) BC52NP

3.1 Porosity analysis

It is seen that BCW structures have less porosity than BCD structures due to the presence of water in them. Obtained BC structures after the NaOH treatment, have higher porosity than obtained BC structures obtained after H_2O_2 treatment. The reason for this is that the amount of the used NaOH is less than H_2O_2, which causes less damage to the chemical structure of BC. The % porosity graph of BC layers and BC composite structures is given in Figure 4. The reason why the porosity results of nonwoven surfaces are higher than the porosity results of BC structures is that the nonwoven surface area in the existing unit area is larger and has more voluminous structures compared to the BC structures. In addition, it has some damage to the structure as seen in the porosity results although it is known that H_2O_2 causes less damage to the structure than other bleaching chemicals [47]. The fact that the porosity of 37 g/m^2 80/20 PP/VI nonwoven-raw BC composite structure is higher than the raw BC structure is derived from the effect of increasing the porosity of the composite structure of the nonwoven surface.

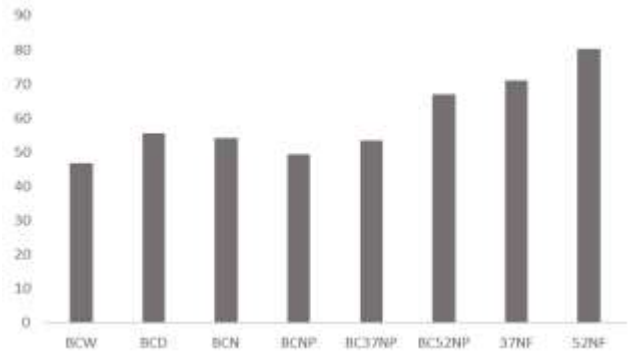

Figure 4. % porosity plot of BC and BC composite structures

3.2 Water retention capacity analysis

It is observed that the BCN structure has the highest water retention capacity in BCD, BCN, and BCNP samples. NaOH and H_2O_2 treatment decrease the water retention capacity of the structure. It is thought that the reason for the high water retention capacity of the composite structures with nonwoven surfaces is because the contacting area of the nonwoven surface is larger than the BC structures. It is observed that the water holding capacities of BC52NP and BC37NP are higher than BCNP and BCW due to the viscose and cotton fibers they contain. The graph of the water retention values of the BC layer and the BC composite structures is given in Figure 5. One of the features that should be in wound dressings as medical textile material is the ability to hold liquid. It is expected to act as a barrier in the retention of fluids such as inflammatory fluid and blood caused by an infection in the wound. In addition, it is necessary to keep the damaged tissue moist.

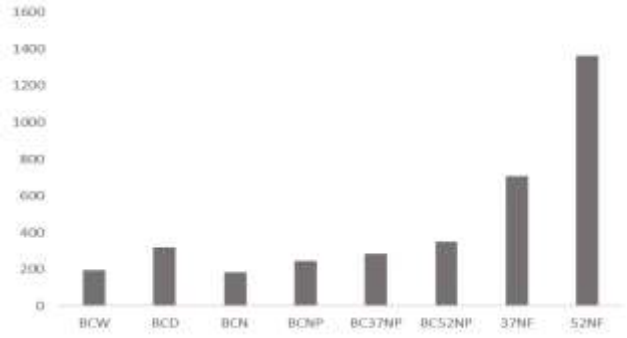

Figure 5. % water retention graph of samples

3.3 Dust permeability analysis

In the dust permeability analysis, images of coal dust are smaller than 100 microns, between 100-200 microns, and larger than 200 microns, taken under the microscope at 60x, 120x, and 200x magnifications, which are given in Figure 6.

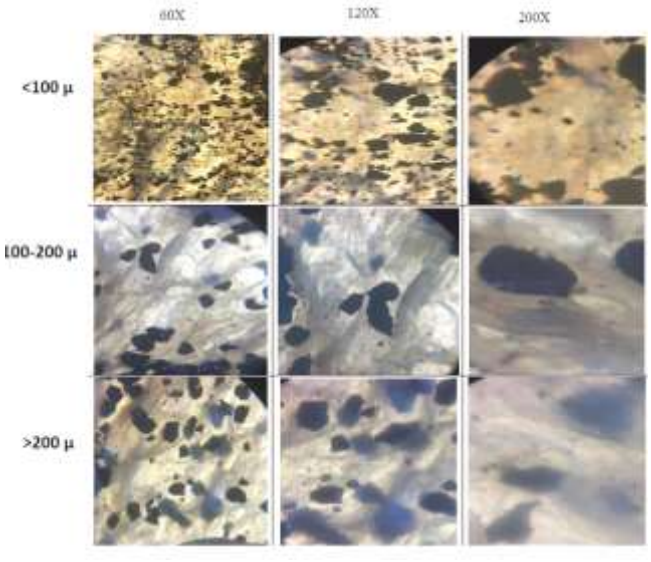

Figure 6. BC sprinkled with various sized curry powders

When the images given above are examined, it is seen that the BC structure to be used in the dressing design does not contain the coal dust after the vacuuming process. This finding is important in terms of protecting the wound from external factors and acting as a barrier during wound healing.

3.4 SEM analysis

It is seen that BCNP and BCN structures have nano-sized cellulose fibres as stated in the literature. Average fibre diameter was measured as ~65 nanometers from SEM images. It is seen that coal dust remains on the surface after vacuuming BCCD samples. This finding is important in terms of protecting the wound from external factors and acting as a barrier during wound healing. SEM images are shown in Figure 7.

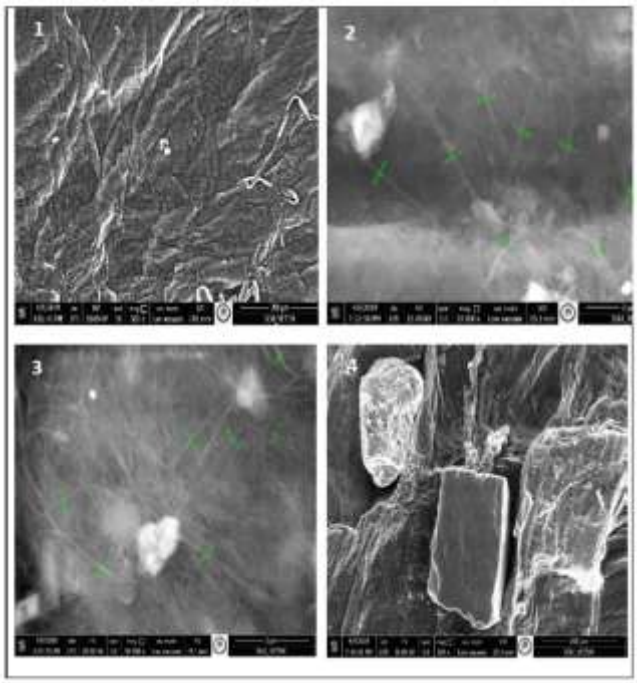

Figure 7. SEM images of samples 1. BCD (500x) 2. BCN (50000x) 3. BCNP (50000x) 4. BCCD (500x)

3.5 FTIR Analysis

The FTIR analysis was made to understand the differences in the chemical groups among the various produced BC structures and the results were given. The results of the analysis of the cotton (CO), BCD, BCN, and BCNP coded samples obtained as a result of the studies were given.

While analyzing the structures, the bond and wavenumber data given in Table 2 are used. When these four structures are examined, it is seen that they are almost the same characteristics. However, it is observed that, unlike the others, the BSD structure, which means the raw BS structure, gives stretched O-H cellulose I peak at 3389 and 3337 cm^{-1} wavenumbers. In the other three structures, since they undergo the application of sodium hydroxide (which enables cellulose I to transform into cellulose II) versus cellulose I peak, these peaks appear as a single peak at 3400 cm^{-1} and these peaks are known as stretched O-H cellulose II [36, 37].

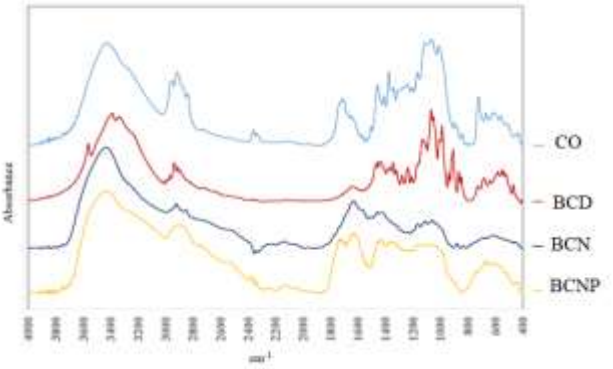

Figure 8. FT-IR analysis graph of CO, BCD, BCN, BCNP coded samples

There is an interaction between H_2O_2 and cellulosic structure in CO and BCNP structures. Therefore, it has been observed that stretched C=O bonds, that is, carboxylic acid groups, appear at wavenumbers of 1700-1730 cm^{-1}.

C(O-H) ie O-H groups belonging to the absorbed water are seen at wavenumbers of 1635-1645 cm^{-1} in BCD, BCN, and BCNP structures. So, from these peaks, it is understood that the BC structures given in the FTIR analysis are not completely dry. Characteristic lignin peaks are observed in P structures around 1510 cm^{-1} and 1470-1430 wavenumbers [48, 49]. This result proves that the BC structures do not contain lignin. O-H bonds observed in cellulose I structures at a wavenumber of 1455 cm^{-1} are observed in all structures. This result shows that even when NaOH is applied to the BC layers, not all cellulose 1 is converted to cellulose II. Additively, absorbed water peaks are observed in all BC structures. This supports that BC has high water and thus liquid absorbency.

3.6 Thermal Analysis

Thermal analysis has been investigated to determine the maximum temperature to be applied in the following thermal processes such as subsequent sterilization and drying, depending on the structure of the new obtained products. The graph of TGA analysis of raw BC structure and H_2O_2-treated BC structure after NaOH treatment is shown in Figure 9.

Table 2. FT-IR analysis bond, wavenumber matching

Bond	Number of Waves cm^{-1}	References
υ(O-H) Cellulose II	3495	[50]
υ(O-H) Cellulose II	3443	[50]
υ(O-H) Cellulose I	3345	[50]
υ(O-H) Cellulose I	3278	[50]
υ(C=O) Carboxylic acid from H_2O_2	1700-1730	[51]
υ(C=O)	1655-1685	[52]
C(O-H) of water absorbed	1635-1645	[52]
Characteristic Lignin Point	1510	[48]

The Tg temperature value is a polymer structure-specific value and as it is known, it expresses the glass transition temperature of the polymer structures. The polymer structure exposed to the temperature above this value melts. For this reason, this study needs to determine the Tg value of the biopolymer, which will be exposed to heat treatments such as sterilization and drying in the future.

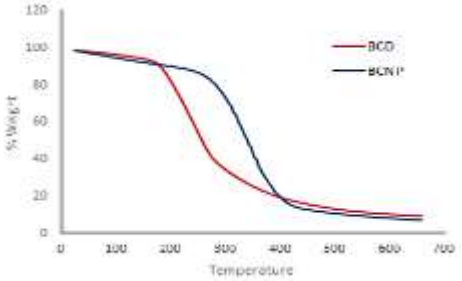

Figure 9. BCD and BCNP TGA graph

DSC analysis graph of raw BC structure and H_2O_2-treated BC structure after NaOH treatment is shown in Figure 10. When Figure 10 is examined, an endothermic peak is formed in the samples due to the water loss at around 130 °C. A wide exothermic peak is observed in both samples around 350°C. The reason for this is partial pyrolysis, which is due to the breakdown of carbon or monoxide carbon-yielding units from anhydrous glucose into carbonyl and carboxylic bonds. Tg values are approximately 220°C when they are viewed from the graph [53, 54].

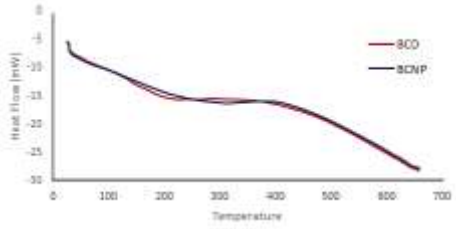

Figure 10. BCD and BCNP DSC graph

4. CONCLUSION

BC is used as a wound dressing in different applications. However, in this study, unlike previous studies in the literature, a new surface is produced in combination with nonwoven surfaces. This situation reveals the original part of the study. As a result of the studies, the fact that the BC structure is self-fibrous shows that it is extremely suitable for tissue requirements in interbody applications. The fact that there is no need to produce extra nanofibers is one of the factors that reduce the cost. In addition, since no organic pollutants are used, there will be no chemicals in the structure, so the more ecological product has been obtained. As a result of the data obtained, it was observed that the BC structures, whose properties were investigated in the study, were porous, nano-fibrous, had high water retention capacity, and provided a barrier against solid particles. These properties are known to be sufficient for wound dressing applications [57, 58]. It has been observed that BC composite structures are particularly enriched with BC and non-woven surfaces, which have undergone cleaning and bleaching, in particular, are suitable for the usage as a medical textile product and increase the absorbency of the structure. In addition, it is predicted that nonwoven surfaces affect the properties of composite structures positively and can be used especially to protect wounds.

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