ONION WASTE VALORIZATION FOR BACTERIAL CELLULOSE-BASED VEGAN LEATHER PRODUCTION VIA SUSTAINABLE CULTIVATION

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This study examined the possibility of utilizing onion waste extract as a substitute for the conventional culture medium for bacterial cellulose (BC) production by *Gluconacetobacter hansenii*. The nutrient components present in the onion waste extract were identified as fermentable sugars and other low-molecular-weight nutrients essential for BC biosynthesis. Essential bioactive compounds in onion waste were effectively recovered using hot water extraction, which was further confirmed using ^{1}H NMR spectroscopy with distinct peaks at 3.2-4.2 ppm for carbohydrates, 0.8-2.5 ppm for aliphatic amino acids, and minor peaks at 6.0-8.0 ppm for aromatic compounds. BC production by *G. hansenii* was successfully achieved when the waste onion extracts were used as the culture medium. The Fourier transform infrared and X-ray diffraction analyses verified the retention of the cellulose I crystal structure for BC produced from the waste onion extracts, as evidenced by the presence of similar characteristic peaks. The mechanical properties of BC and OBC also showed some differences, as the OBC material had higher stiffness and tensile strength, while the material produced from the waste onion extracts had lower elongation and toughness. The thermal stability of the BC and OBC materials also showed a decrease for the BC produced from the waste onion extracts. *In vitro* cytocompatibility tests with NIH 3T3 cells indicated the cell viability on BC and OBC surfaces. The results demonstrate the potential of onion extract as a nutrient substitute for BC production, while impacting the BC property–structure relationship. The paper presents experimental results for the valorization of agricultural waste materials for the production of microbial cellulose biosynthesis.

Keywords: sustainable materials, bacterial cellulose, agricultural waste, leather substrate

INTRODUCTION

The global sustainability drive has led to the development of sustainable and cruelty-free alternatives to resource-intensive animal leather materials.[1] The conventional process of leather production poses serious environmental and ethical concerns, including greenhouse gas emissions and the production of harmful wastewater during the leather tanning process, and cruelty to animals.[2-3] In this context, the past decade has seen the rapid development of sustainable and cruelty-free vegan leather materials made from biogenic and plant-based materials, with emphasis on the upcycling of agricultural and organic waste materials.[4-7] Bacterial cellulose (BC) has also emerged as an alternative leather material with superior purity, biodegradability, and structural properties. BC is biosynthesized by *Gluconacetobacter hansenii* and other microorganisms in an extracellular matrix through the polymerization of glucose units to β-1,4 glucans, which are arranged in a 3D network of nanofibrils. BC is highly suitable for leather applications due to its excellent mechanical properties, elasticity, and water retention. Significantly, BC production is highly dependent on the composition of the culture medium used for its production, which is composed of carbon and nitrogen sources. Therefore, it is vital to utilize low-cost substrates for its production. In addition, structure–property correlations of BC are of significant importance in defining its functional properties.[8-11]

Among emerging bio-based materials with potential for alternative leather, fungal mycelium and plant fiber-based materials have shown promise due to their biodegradable and sustainable properties.[7,12,13] However, limitations in their mechanical properties and structural uniformity are major drawbacks. On the other hand, BC possesses several unique properties, such as chemical purity, defined nanoscale fibrillar network, and excellent mechanical properties. BC is biosynthesized in an

interconnected cellulose I structure with inherent integrity. Unlike traditional leather, mycelium-based leathers eliminate toxic tanning chemicals, reducing environmental and health risks. For instance, Amobonye *et al.* highlighted the carbon-neutral cultivation of mycelium and its potential in sustainable fashion.[14] Wijayarathna *et al.* further demonstrated the feasibility of producing multilayered vegan leathers by growing fungal biomass on food waste and integrating waste valorization with advanced materials engineering.[15] Plant-based leathers made from agro-waste, such as pineapple leaves, apple peels, and coconut husks, offer biodegradable and aesthetically appealing alternatives.[16] Reinforced with natural binders or biopolymers, these fibrous materials show promise in replicating the mechanical and tactile properties of animal leather. Akhter *et al*. reported improved performance in jute–mycelium composites crosslinked with polyhydroxyalkanoate biopolymers, highlighting the potential of plant–fungus hybrids in fashion applications.[17] However, challenges persist in achieving durability, flexibility, and structural integrity of genuine leather, and usable feedstocks remain limited. Expanding this scope to include onion waste, a nutrient-rich, widely available by-product of food processing, presents a promising yet underexplored opportunity.

Onion waste is rich in essential nutrients for microbial growth and biosynthesis of cellulose, including reducing sugars, such as glucose, fructose, and sucrose, amino acids, vitamins, and minerals.[18,19] These reducing sugars are the main source of carbon for the biosynthesis of cellulose, which is metabolized to activate glucose, leading to the production of UDP-glucose, which is then used to produce the polysaccharide chains of cellulose. Onion waste also contains organosulfur compounds, such as quercetin, which may be beneficial due to their antioxidant activity. The use of onion waste as a substrate for BC biosynthesis can be advantageous in two ways: biosynthesis of BC at lower production costs, and functional improvements in the final product.

Research on the use of onion waste in BC-based vegan leather is still in the early stages. In order to replicate the structural complexity of animal leather, various approaches have been taken to develop the multi-layered structure of the product. The structure of the ECM is such that it provides mechanical strength and elasticity. In the context of designing the structure of vegan leather, the optimization of the scaffold and the functionalization of the surface have been addressed. Recent studies indicate that mammalian fibroblasts can be cultured on BC membranes to develop tissue-like structures. Despite the existing BC–mammalian cell integration, the simultaneous employment of onion waste valorization, BC biosynthesis, and mammalian cell culture in the context of vegan leather fabrication is limited. Onion waste is extremely common in the world, and vegan leather is becoming increasingly popular. Therefore, it is of great importance to explore this simultaneous employment of these techniques. In this research, we propose a green and sustainable methodology for developing multilayered vegan leather through: (1) valorization of onion waste for efficient BC biosynthesis using *G. hansenii*, (2) optimization of scaffold properties through ECM-mimetic surfactant treatment and multilayered fibroblast cell culture, and (3) physicochemical, mechanical, and biological assessment of the resulting biocomposite.

This study aims to bridge the gap between microbial biotechnology, agricultural waste recycling, and tissue engineering to advance sustainable materials science. Transforming low-value by-products into functional biomaterials through eco-friendly methods helps reduce environmental impact, decrease reliance on synthetic polymers, and support the development of high-performance, animal-free leather alternatives. The proposed platform is beneficial in the context of sustainability and circular bioeconomy because it helps in the reuse of agro-industrial waste and the reduction of the use of harmful chemicals. In addition, the proposed platform has the potential for scaling up and can be used in the fields of fashion, packaging, and biomedical materials.

EXPERIMENTAL

Materials

Discarded onions used for preparing the microbial growth medium were purchased from a traditional market in Yeongcheon, Gyeongbuk, South Korea. Glucose ($C_6H_{12}O_6$) was purchased from Samchun Chemical Co. Ltd., South Korea. Yeast extract, peptone, and acetic acid (CH_3COOH, 99.7% purity) were purchased from Daejung Chemicals, South Korea. Mouse fibroblast cells (NIH3T3) were obtained from the Korean Cell Line Bank. All chemicals were used as received, and all experiments were conducted using double distilled (DI) water.

Production of BC sheet

BC sheets were produced by culturing *Gluconacetobacter hansenii* PJK using standard procedures.[20,21] The MAE basal medium, which contained 0.2 g/L succinate, 1.5 mL/L acetic acid, 7 g/L peptone, 10 g/L glucose, and 10 g/L yeast extract, was prepared in deionized water, and the pH was adjusted to 5.0 using 1 M NaOH. Bacterial colonies were inoculated in the MAE medium, which was then incubated for 24 h at 30 °C with shaking. After that, 5% (v/v) bacterial suspension was inoculated in fresh MAE medium, which was then statically cultured at 30 °C, pH 5, for 14 days to form BC sheets. The resulting sheets were purified using 0.1 M NaOH solution in an autoclave system (121 °C, 15 min), keeping the BC:NaoH solution volume ratio constant at 1:20 (w/v), with sufficient volume to totally immerse the sheets. In the case of the medium containing onion extract, it was sterilized by autoclaving at 121 °C for 15 min before inoculation. The purified BC sheets were rinsed thoroughly with deionized water until the pH became neutral and kept refrigerated at 4 °C before use.

Production of BC sheet using onion extract medium

Onion-derived BC was prepared by growing biomass on the onion extract as the growth medium. The growth medium was prepared according to the process described in Figure 1. In this process, the waste materials such as the outer layers of the onion were collected and washed with deionized water. The washed materials were then chopped into small pieces and subjected to hot water extraction at 90 °C for 1 h at a ratio of 1:10. The process was carried out under continuous stirring. The extracted materials were then cooled and subjected to filtration with muslin cloth and Whatman filter paper three times. The materials were then sterilized at 121 °C for 15 minutes and subjected to autoclaving. The sterilized materials were then subjected to BC production by mixing the materials with MAE growth medium at specific volume ratios such as 25%, 50%, 75%, and 100% v/v. In this process, the MAE growth medium was replaced with the onion extract without the addition of any nutrients. The process was also carried out with the standard MAE growth medium, without the addition of onion extract. To ensure consistency and prevent variability in BC yield, a standard inoculum size of 5% (v/v) *Gluconacetobacter hansenii* was applied for all conditions. Static cultures were carried out at 30 °C for 14 days to obtain OBC sheets. After the static culture, BC pellicles were purified using a solution of 0.1 M NaOH in a laboratory autoclave at 121 °C for 15 minutes, ensuring a BC-to-NaOH solution ratio of 1:20 (w/v), along with sufficient volume to completely immerse the BC. The purified OBC sheets were then rinsed completely with deionized water until a neutral pH was achieved, and then stored at 4 °C.

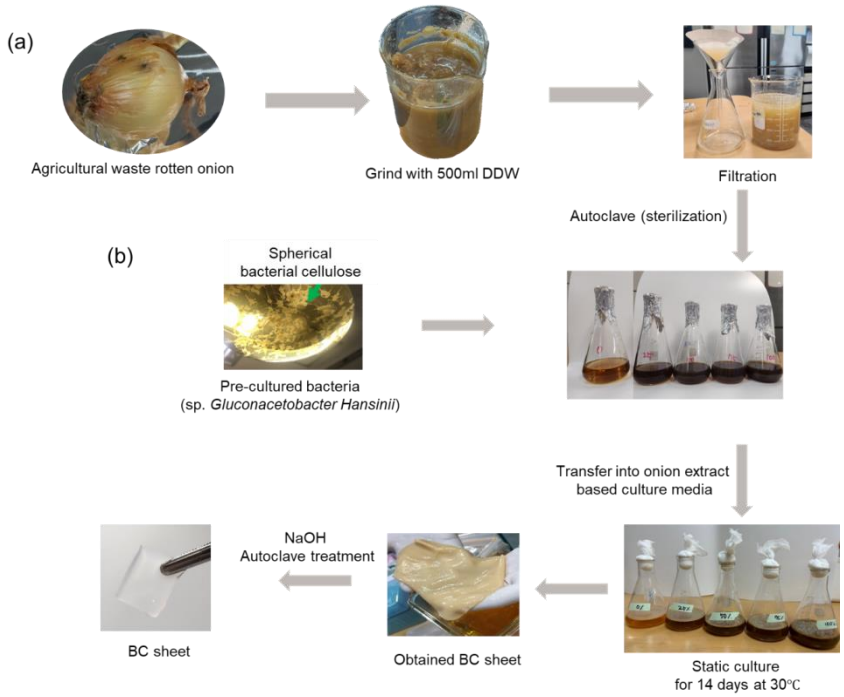

Figure 1: Schematic representation of (a) agricultural waste onion extract, (b) production process of BC using onion extract as medium

Characterization

BC and OBC were chemically characterized by Fourier transform infrared spectroscopy (FTIR, Perkin Elmer) in the ATR mode in the wavenumber range of 4000-450 cm^{-1}. For XRD analysis of BC and OBC, X-ray diffraction data were recorded using a Bruker AXS D8 X-Ray Diffractometer with Cu-K α radiation of 0.154 nm

wavelength. The scan range of 2θ was set between 10° and 60° with a scan speed of 5°/min. The dried films were placed on a flat sample holder and scanned in ambient atmosphere.

To determine the degree of crystallinity of the samples, the crystallinity index (CrI) was calculated using the Segal method, which is based on the crystal index of the (200) peak appearing at 2 θ = 22.4° and the amorphous portion appearing at 18°. The crystallite size of the samples was determined using the Scherrer equation and the full width at half maximum (FWHM) of the (200) peak.

Differential scanning calorimetry (DSC, TA Q200) analysis of BC and OBC was carried out in the range of 10 °C to 300 °C, with a scan speed of 10 °C/min under a nitrogen environment (50 mL/min).

The yield of BC was calculated by measuring the dry weight of purified and freeze-dried BC per unit volume of culture medium.

Mechanical properties were tested using a universal testing machine (MCT 2150, A&D Co., Japan). To determine tensile properties, rectangular strip samples with dimensions of length ~30 mm, width ~5 mm, and thickness ~0.5–1 mm were used. The samples were tested at a gauge length of 20 mm with a crosshead speed of 10 mm/min. The strain was also calculated using the formula for strain. At least five independent samples were tested for reproducibility.

Field emission scanning electron microscopy (FE-SEM, Hitachi S-4800) was used to analyze the microstructure of the samples. The samples were subjected to freeze-drying and air-drying, and then, were sputter coated with platinum (90 seconds) to improve the conductivity of the samples.

Cell culture

The biocompatibility of BC and OBC composites was evaluated *in vitro* using the MTT assay. NIH3T3 fibroblast cells were cultured in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum and maintained at 37 ^{o}C in a humidified 5% CO_2 atmosphere. Before seeding, the BC and OBC sheets were sterilized by immersion in 70% ethanol, rinsed with phosphate-buffered saline (PBS), and exposed to ultraviolet light for 30 min. After sterilization, 1×10^3 NIH3T3 cells were seeded onto each hydrogel disc and placed in 24-well plates. The cells were incubated for 1, 3, and 5 days, followed by MTT treatment and incubation for 2 h to allow for formazan crystal formation. Subsequently, formazan crystals were dissolved by adding 100 μL of dimethyl sulfoxide to each well, and the resulting solution was transferred to 96-well plates for absorbance measurement at 570 nm using a microplate reader. Cell viability and morphology were assessed using a Live/Dead assay. The cells were stained with calcein-AM solution (live cells) and ethidium homodimer (dead cells), incubated at room temperature for 30 min, and imaged using a Nikon Eclipse Ti fluorescence microscope. For SEM imaging, 5-day cultured samples were fixed with 2.5% formaldehyde in PBS at 4 °C for 10 min, dehydrated through graded ethanol, subjected to critical point drying, gold-coated, and imaged using FE-SEM to observe cell attachment and morphology on both BC and OBC sheets.

RESULTS AND DISCUSSION

Preparation of BC sheets

To further assess the suitability of onion waste extract as a nutrient source for BC production, freeze-dried extracts were characterized by ^{1}H NMR spectroscopy (Fig. 2), and compared with reference spectra of MAE medium and individual components of the medium, *i.e.*, glucose, yeast extract, and peptone. The ^{1}H NMR spectrum of the onion waste extract showed characteristic peaks at δ 3.2-4.2 ppm for soluble sugars. The presence of amino acid and small peptides, *i.e.*, compounds showing peaks in the δ 0.8-2.5 ppm region, was also confirmed, as these are required for microbial growth. In addition, small peaks in the δ 6.0-8.0 ppm region of the spectrum may correspond to phenolic compounds, *e.g.*, quercetin, which is involved in antioxidant activity.

To confirm this, BC was synthesized from different concentrations of onion extract ranging from 25 to 100% v/v (Fig. 3). In this study, the 75% onion extract solution resulted in the highest BC yield of ~4.8 g/L, similar to ~5.2 g/L for the control MAE medium. The BC sheets produced from this solution had an average thickness of ~0.45 mm and a weight per unit area of ~56 g/m². Although there are differences in the features of the spectra, this study confirms that the onion extract solution contains a balanced amount of both carbon and nitrogen sources required for BC biosynthesis. This study, therefore, confirms that the onion waste solution can be used as a potential alternative solution for BC biosynthesis. However, further studies are necessary to assess the yield of BC, as well as the cost and mechanical properties of BC, for vegan leather production. The OBC with 100% onion extract was used for comparison of physicochemical and biological performance with BC produced from MAE medium alone.

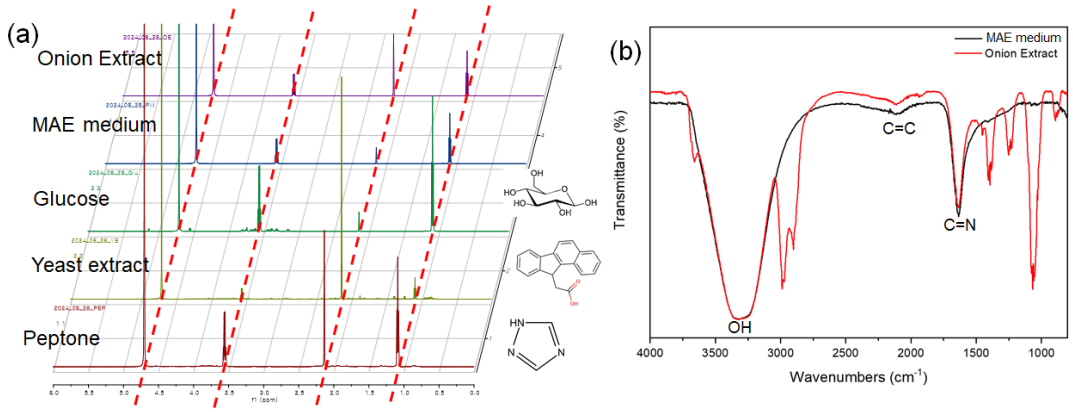

Figure 2: (a) 1H NMR spectra of various components, (b) FTIR spectra of MAE medium and onion extract

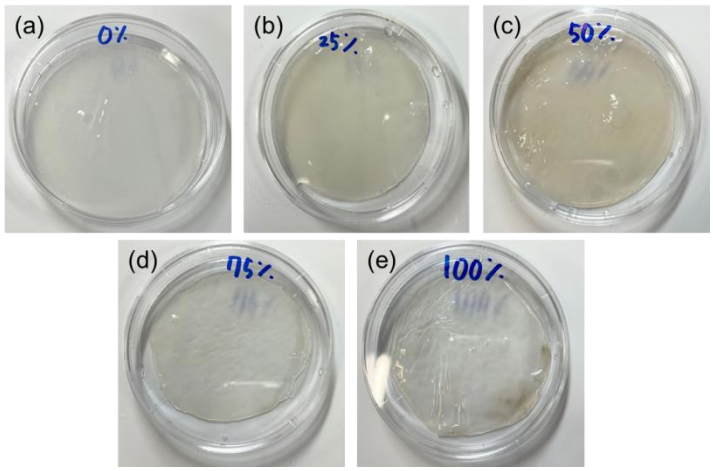

Figure 3: Digital photograph images of produced BC using onion extract mixed with MAE medium in varying ratios (a) 100% MAE medium, (b) 25% onion extract, (c) 50% onion extract, (d) 75% onion extract, and (d) 100% onion extract with respect to MAE (v/v)

FTIR spectra

FTIR spectra of BC and OBC are shown in Figure 4. Both BC and OBC have characteristic bands for cellulose, *i.e.*, around 3335 cm^{-1} for O–H stretching, 2892 cm^{-1} for C–H stretching, and 1645 cm^{-1} for O–H bending of adsorbed water. In addition, specific bands are observed around 1432 cm^{-1} for CH_2 bending, 1371 cm^{-1} for C–H bending, and 1317 cm^{-1} for CH_2 wagging, all of which are characteristic of a Cellulose I structure.[20,21] Additionally, the bands around 1160 cm^{-1}, 1105 cm^{-1}, and 1055 cm^{-1} are characteristic of C–O–C asymmetric stretching and C–O stretching of the glucopyranose ring. The band observed around 897 cm^{-1} corresponds to β-1,4-glycosidic linkages, proving the presence of a polysaccharide structure of cellulose.

The highly similar peak positions of BC and OBC spectra are obvious, whereas small differences in peak intensity are observed, especially for BC, around 3335 cm^{-1} and in the range of 1000-1200 cm^{-1}. The overall spectral features of BC and OBC are highly similar. This implies that the fundamental chemical structure of cellulose is maintained when the onion extract is used as the growth medium. However, it was observed that there were small variations in the relative intensities of the peaks in the O-H stretching and the fingerprint region. The variations might be due to the hydrogen bonding interactions and the presence of moisture in the samples.

It is important to note that the FTIR analysis indicates the presence of cellulose functional groups, but does not indicate the source of the cellulose. Therefore, the source of the cellulose is not confirmed. The overall results obtained in the FTIR analysis indicate that the functional groups present in the cellulose produced by the onion extract are similar to the functional groups of cellulose I. This implies that the structure of the cellulose produced by the onion extract is similar to the structure of the original cellulose.

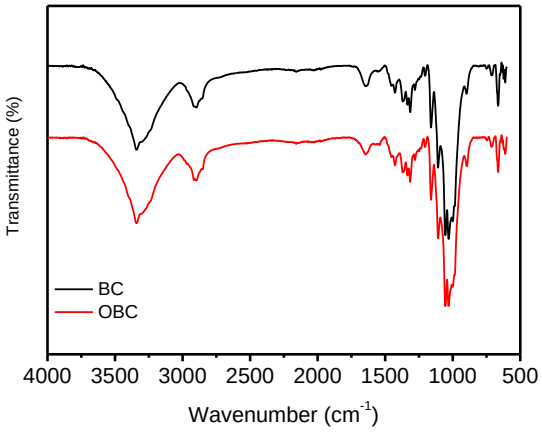

Figure 4: FTIR spectra of BC and OBC

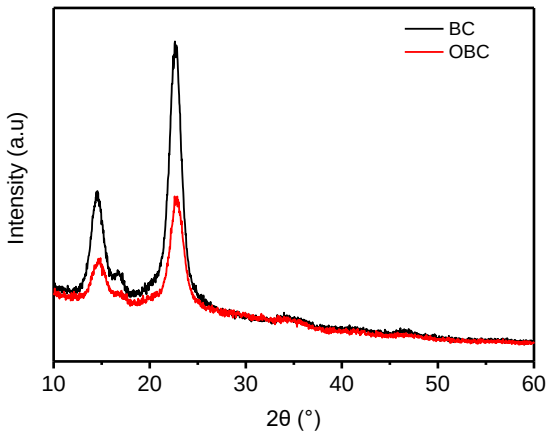

Figure 5: XRD patterns of BC and OBC

X-ray diffraction (XRD) patterns

X-ray diffraction (XRD) analysis was carried out in order to assess the crystalline arrangement of BC (Fig. 5). Both BC and onion-based BC (OBC) have distinct peaks at 2θ values of around 14.5°, 16.6°, and 22.4°, which correspond to (100), (010), and (200) planes of cellulose I, respectively. The peaks have been linked with cellulose Iα and Iβ allomorphs, which are characteristic of bacterial cellulose. This indicates that it is semi-crystalline in nature.[20,21] Though both samples have peaks at similar positions, indicating that both have maintained their crystalline form of cellulose I, differences in peak intensities were noticed. The OBC sample indicated that the (200) peak is broader with lower intensity than that of BC. This indicates that there is a decrease in crystallinity and crystallite sizes. This is in agreement with the crystallinity index (63.3% for OBC *vs.* 68.0% for BC) and crystallite sizes (3.55 nm for OBC *vs.* 3.92 nm for BC). From these findings, it is concluded that though onion extract as a growth medium does not affect the basic crystalline structure of BC, it affects the crystallinity and structural ordering. In conclusion, it is found that OBC possesses the major structural characteristics of cellulose I and that onion extract is an effective growth medium with minimal effects on crystallinity.

Mechanical properties

The mechanical properties of native BC and OBC have been provided in Figure 6. The mechanical properties of native BC were found to be 0.55 ± 0.03 mm/mm for elongation at break, 0.34 ± 0.02 MPa for tensile strength, 0.52 ± 0.05 MPa for Young's modulus, and 0.117 ± 0.01 MJ/m³ for toughness. For OBC, the tensile strength was higher at 0.524 ± 0.03 MPa, whereas Young's modulus was also higher at 3.63 ± 0.20 MPa. However, the elongation at break was lower at 0.164 ± 0.01 mm/mm, whereas the toughness was also lower at 0.05 ± 0.004 MJ/m³. The mechanical properties were calculated from the stress-strain diagram. The toughness value is defined by the area under the stress-strain diagram.

The results have provided valuable insights that OBC is more mechanically strong and stiffer, whereas native BC is more flexible with more energy absorption. However, it is also evident that both materials have lower mechanical properties than that of natural leather. When compared with the literature, it is evident that the tensile strength and modulus of BC lie in the range of typical reported values for bacterial cellulose. The differences in the properties of BC and OBC could be attributed to differences in fibril orientation, which is evident from the XRD results. However, it is not clear whether there is any difference in density or chemical incorporation. For cyclic mechanical testing, 10 loading-unloading cycles were applied. From the results, it is evident that both materials have structural integrity without any signs of failure. However, in leather products, there is a need for more cycle numbers than what was tested in this study.

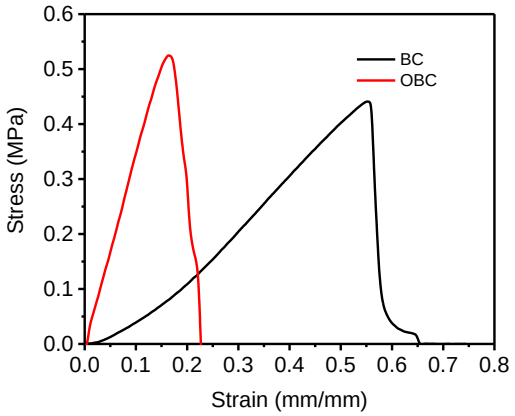

Figure 6: Tensile stress-strain curves of BC and OBC

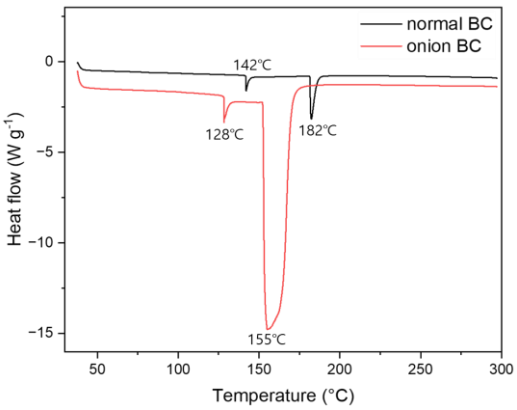

Figure 7: DSC thermograms of BC and OBC

Differential scanning calorimetry

The thermograms obtained from the DSC analysis of BC and OBC are illustrated in Figure 7. Both materials exhibit characteristic thermal transitions associated with bacterial cellulose materials. An intense broad endothermic transition is visible in both thermograms at temperatures below ~120 °C, which is associated with the removal of physically absorbed and bound water in both materials. This is characteristic of hydrophilic polysaccharides like bacterial cellulose, which have high moisture retention capacity. An intense endothermic peak is visible in both thermograms in the range of ~200–260 °C, which is associated with thermal transitions in relation to relaxation of the cellulose chains. The onset of thermal degradative processes is also visible in both materials. The BC sample shows this transition at a slightly higher temperature with greater intensity than that of OBC, indicating stronger intermolecular interactions. On the other hand, the OBC thermogram shows a shift to a lower temperature with reduced peak height, indicating relatively weaker intermolecular interactions and reduced thermal resistance.[22] This may be explained by the effects of the medium, the onion extract, which could affect the microstructural organization of the material. It is worth noting that DSC is more related to the thermal properties of the material and the enthalpic changes, but the determination of the temperature of decomposition and the degree of crystallinity is not possible using DSC. As seen above, the thermograms of BC and OBC show similar trends, which are characteristic of bacterial cellulose. Although the thermogram of OBC shows reduced thermal stability, the above findings show that the onion extract has no significant effect on the thermal properties of bacterial cellulose.

Morphology

The morphology of BC sheets derived from conventional medium (BC) and onion extract (OBC) was studied by SEM (Fig. 8). Both samples revealed a three-dimensional network comprising interwoven nanofibers, which is characteristic of bacterial cellulose. Although the fibrous structures appear similar, it is important to note that this is qualitative evidence and does not confirm structural integrity. Furthermore, the effect of the drying method on the final morphology was also assessed. The freeze-dried samples revealed a more open and porous network, which is likely due to ice crystal formation and subsequent sublimation, causing pores in the cellulose network. On the other hand, heat-dried samples (50 °C for >24 h) revealed a denser and more compact network, likely due to slow evaporation of water, causing cellulose fibers to pack tightly together. It is essential to note that although differences in porosity and network structures are apparent, these are qualitative results and not quantitative evidence. Thus, it is observed that both BC and OBC exhibit the typical nanofibrous structure of bacterial cellulose, and porosity is mainly affected by the drying method rather than the medium used in the culture. It is also concluded that the presence of the onion extract does not affect the general morphology of bacterial cellulose.

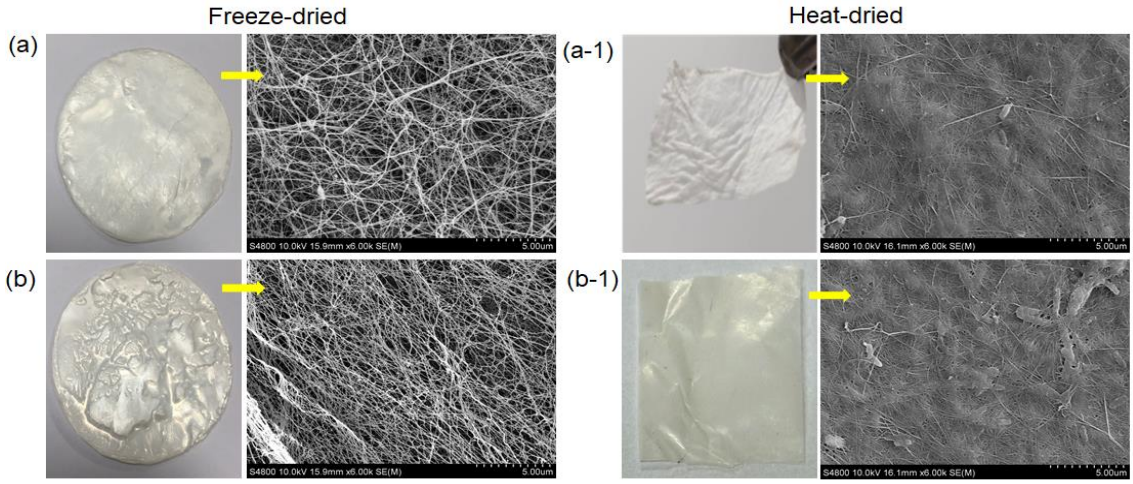

Figure 8: SEM images of (a) freeze-dried and (a-1) heat-dried BC, and of (b) freeze-dried and (b-1) heat-dried OBC

Cell culture

Cell culture studies performed using NIH3T3 fibroblast cells showed similar cytocompatibility results for both BC and OBC after 1, 3, and 5 days of culture, as determined using the MTT assay (Fig. 9a). All cell culture groups showed increased cell viability over time, which is a normal phenomenon for cell growth. No significant differences were found in cell viability for BC and OBC, indicating that the onion extract does not negatively influence cell metabolism.

The SEM images of NIH3T3 cells cultured on BC and OBC surfaces for 5 days are presented in Figure 9 (b,c), showing cell attachment and growth on both cellulose fibrous surfaces, with a normal cell shape for a fibroblast cell line. The images indicate that both BC and OBC provide a cell culture surface for cell adhesion, as evidenced by the presence of attached and elongated cells on both materials. The presence of cell aggregates on OBC surfaces, as seen in the images, cannot be validated quantitatively. Fluorescence imaging is another tool used to demonstrate the distribution of the cells and their interaction with the materials (Fig. 10). As shown in Figure 10a, DAPI-stained images of the control surfaces reveal a uniform distribution of nuclei, while in OBC surfaces (Fig. 10b), there are areas of closely packed nuclei, possibly due to layered distributions of the cells. These images are qualitative in nature and do not reveal any increase in proliferation rate, as observed in the MTT assay results. Confocal images of live and dead cells grown on OBC surfaces (Fig. 10c) reveal mostly live cells as shown by the presence of green fluorescence. This confirms that the material supports the survival of the cells during extended periods of culture. From these 3D images, it appears that the cells interact with the surface as well as the structure of the material, although the extent of infiltration cannot be quantitatively determined from these images alone.

BC and OBC support cell adhesion and viability, showing no significant differences in metabolic activity. The results suggest that onion-derived media can be used for BC production, without affecting cytocompatibility. However, SEM and fluorescence analyses are qualitative in nature, and further quantitative studies need to be carried out to confirm differences in cell organization and behavior. Future work should aim at developing leather-like sheets through the controlled formation of multilayer cell sheets on OBC by optimizing cell density, culture time, and multilayer cell sheet formation. Moreover, mechanical conditioning, crosslinking, and surface treatments can also be employed for enhanced interlayer adhesion of cell sheets. Quantitative evaluation of the thickness, uniformity, mechanical contribution, and durability of cell sheets is also required to assess the possibility of multilayer cell sheets for leather-like material development.[23]

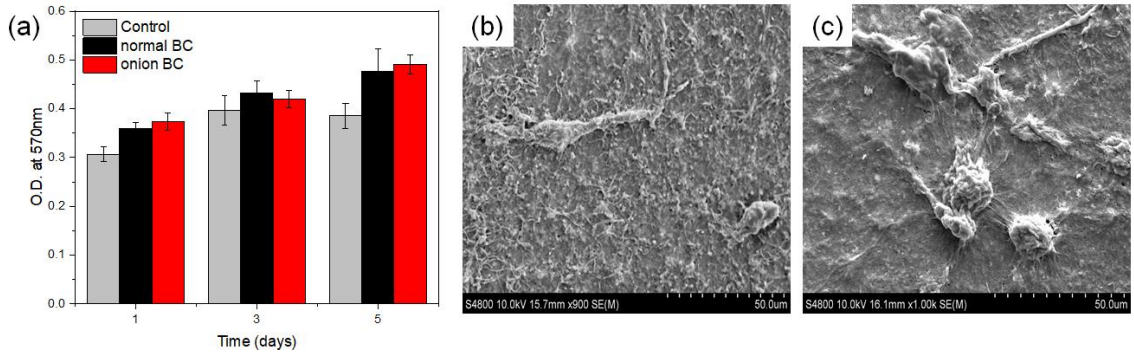

Figure 9: (a) Cell viability of BC and OBC treated with NIH3T3 cells using MTT assay after 1, 3 and 5 days of incubation, and SEM images of NIH3T3 cells adhered on (b) BC and (c) OBC after 5 days of incubation

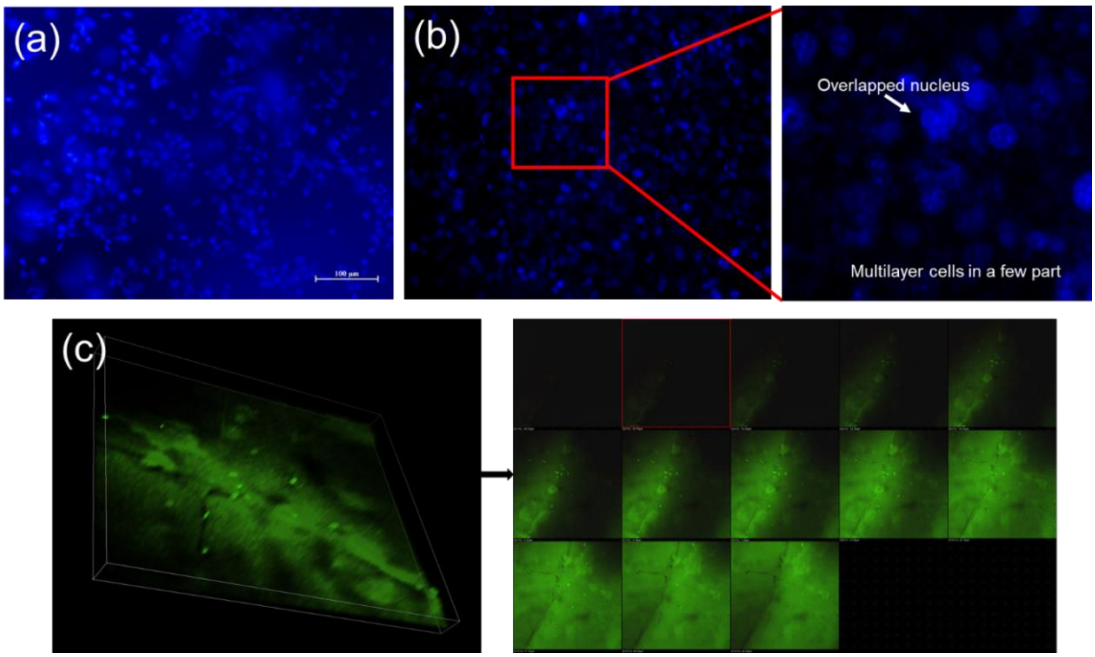

Figure 10: Florescence images of DAPI stained NIH3T3 cells illustrating cell adhesion on (a) control and (b) OBC, and CLSM live/dead cell image of NIH 3T3 cells cultured on OBC for 30 days of incubation (c)

CONCLUSION

This investigation has proved that onion extract, which is a by-product of the food industry, has potential as a viable alternative medium in BC synthesis. The OBC sheets were found to have similar physical, chemical, and morphological properties to those produced using conventional MAE medium, as confirmed through spectroscopy, crystallinity and microscopic studies. The mechanical properties of OBC were found to show higher stiffness and tensile strength, along with lower ductility and toughness, than those of native BC, which indicates that using onion extract affects its properties rather than preserving them. Cyclic mechanical testing revealed that both materials retained their integrity for a limited number of cycles. However, durability needs further research. Cell culture studies for NIH3T3 fibroblasts showed that BC and OBC have comparable cell viability. No significant differences were seen in the study. Qualitative imaging revealed that cells adhered to BC and OBC surfaces. Layered cell formation on OBC needs further research for quantitative validation. The use of onion waste for BC synthesis is a novel method for valorizing waste materials. However, no quantitative analysis of sustainability is conducted in this research. Therefore, only the sustainability of the use of waste materials is demonstrated, but not guaranteed by quantitative analyses, such as life cycle analysis and cost analysis. In conclusion, this research is a proof of concept for demonstrating the use of onion waste and onion-derived medium for BC production. The BC and OBC have tunable properties and are potentially useful for fields such as bio-based materials and engineered leather.

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