MANUFACTURING OF SCOBY NANOCELLULOSE FOR BIOMEDICAL APPLICATION

Anna Baran[1]*, Katarzyna Reczyńska-Kolman[1], Elżbieta Pamuła[1]

[1] Department of Biomaterials and Composites, Faculty of Materials Science and Ceramics, AGH University of Krakow, Al. Mickiewicza 30, 30-059 Kraków, Poland

* Corresponding author: annabaran@agh.edu.pl

Abstract

This study aimed to produce, purify, and characterize SCOBY nanocellulose for biomedical use. The material was obtained through a biotechnological fermentation process of tea broth using a symbiotic culture of bacteria and yeast (SCOBY). The tea broth consisted of black tea extract supplemented with sucrose. The SCOBY pellicle formed at the air-liquid interface after 10 days of fermentation was collected. Pellicle purification involved washing with Milli-Q water, sonication combined with ultraviolet-C (UV-C) treatment, followed by washing with hot 1.5 M NaOH and 1.5% H_2O_2 solutions. Fourier transform infrared (FTIR) spectroscopy confirmed the effectiveness of the purification process, leading to the acquisition of a transparent nanocellulose hydrogel. Scanning electron microscopy (SEM) revealed the characteristic nanofibrous morphology of SCOBY nanocellulose, with nanofibers exhibiting a mean diameter of 88 ± 19 nm. The nanofibers formed a highly porous internal structure, whereas the outer layer consisted predominantly of densely packed nanofibers, effectively protecting the culture from the external environment. The pores within the structure exhibited diameters ranging from 4 to 6 µm. The mean density of the structure was 0.024 ± 0.006 g/cm^3. In vitro studies using L929 murine fibroblasts demonstrated that SCOBY nanocellulose was not cytotoxic, as confirmed by the Alamar Blue assay and live-dead fluorescence staining.

Keywords: *symbiotic culture of bacteria and yeast (SCOBY), SCOBY nanocellulose, biomaterial, L929 cells*

Introduction

A symbiotic culture of bacteria and yeast (SCOBY) is used to produce kombucha, a beverage obtained through the fermentation of sweetened tea broth. During fermentation, an exopolysaccharide film forms at the air-liquid interface, referred to as the SCOBY pellicle. This unique structure is made of nanocellulose and provides a microporous environment for microorganism immobilization while simultaneously allowing gas exchange and limiting water evaporation and external contamination [1, 2]. In the SCOBY consortium, the dominant bacterial strains include *Acetobacter xylinum* and *Gluconobacter hansenii*, while yeasts belong to a group of *Zygosaccharomyces*, *Saccharomyces*, and *Schizosaccharomyces* [1]. The formation of nanocellulose is the result of microbial metabolism. Yeasts hydrolyse sucrose into glucose and fructose, which are later converted to ethanol and organic acids. Acetic acid bacteria oxidize these intermediates and utilize glucose as a precursor for cellulose biosynthesis via uridine diphosphate glucose (UDP-glucose), leading to the formation of β-(1→4) linked glucan chains organized in a nanofibrillar network [1]. The composition of kombucha and SCOBY pellicle is strongly influenced by fermentation parameters such as substrate composition, temperature, and fermentation duration [2, 3]. Kombucha contains a wide range of compounds, including organic acids, enzymes, vitamins, and polyphenols [2]. Owing to the presence of enzymes, fermentation improves the bioavailability of polyphenols in kombucha, while microbial cells and their metabolites contribute to its probiotic and postbiotic nature [3, 4].

In recent years, SCOBY-derived nanocellulose has attracted increasing attention as a sustainable and cost-effective biomaterial. Its production is based on simple and widely available substrates, and the pellicle itself is often considered a byproduct of fermentation, which makes the process environmentally and economically advantageous. From a materials science perspective, SCOBY nanocellulose exhibits a highly porous structure, a high-water absorption capacity, and the ability to form flexible but mechanically stable films [5]. These properties make it particularly promising for biomedical applications, especially as wound dressing materials, where its structure promotes moisture retention and facilitates the incorporation of bioactive compounds [6]. Furthermore, wound dressings can be enriched with gold [7], silver nanoparticles [8], or chitosan [6], thereby providing antibacterial properties. In addition to biomedical use, microbial cellulose is evaluated for use in food packaging and, more interestingly, in the fashion industry as faux leather material due to its excellent pigment absorption capacity [1–3, 9, 10].

Despite growing interest, SCOBY-derived nanocellulose remains poorly characterized compared to conventionally produced bacterial cellulose synthesized exclusively by bacterial strains. Variations in microbial composition, fermentation conditions, and purification protocols can significantly affect its structure and properties [1–3]. Therefore, further investigation is required to better understand these relationships and evaluate the potential of SCOBY nanocellulose as a reproducible and functional biomaterial.

The aim of this study was to develop a reproducible method for the production of SCOBY nanocellulose for biomedical applications, with particular emphasis on optimizing processing conditions and pellicle purification. To this end, FTIR spectroscopy was employed to monitor the purification process and confirm the purity of SCOBY nanocellulose, whereas SEM was used to characterize its microstructure. Furthermore, in vitro studies using L929 murine fibroblasts were conducted to evaluate the cytocompatibility of SCOBY nanocellulose.

Materials and methods

Materials

The primary kombucha starting culture was purchased from delikatna.bio, Poland. Black tea was from Remsey, Jeronimo Martins, Poland. Sucrose, AlamarBlue reagent, sodium hydroxide (NaOH), calcein AM, and propidium iodide (PI) were purchased from Sigma-Aldrich. L929 fibroblast cell line was from the European Collection of Cell Cultures (Salisbury, UK). Dulbecco's modified Eagle medium (DMEM), foetal bovine serum (FBS), penicillin/streptomycin antibiotic, and

[Engineering of Biomaterials 174 (2026) 10]

doi:10.34821/eng.biomat.174.2026.10

Submitted: 2026-05-02, Accepted: 2026-06-05, Published: 2026-06-11

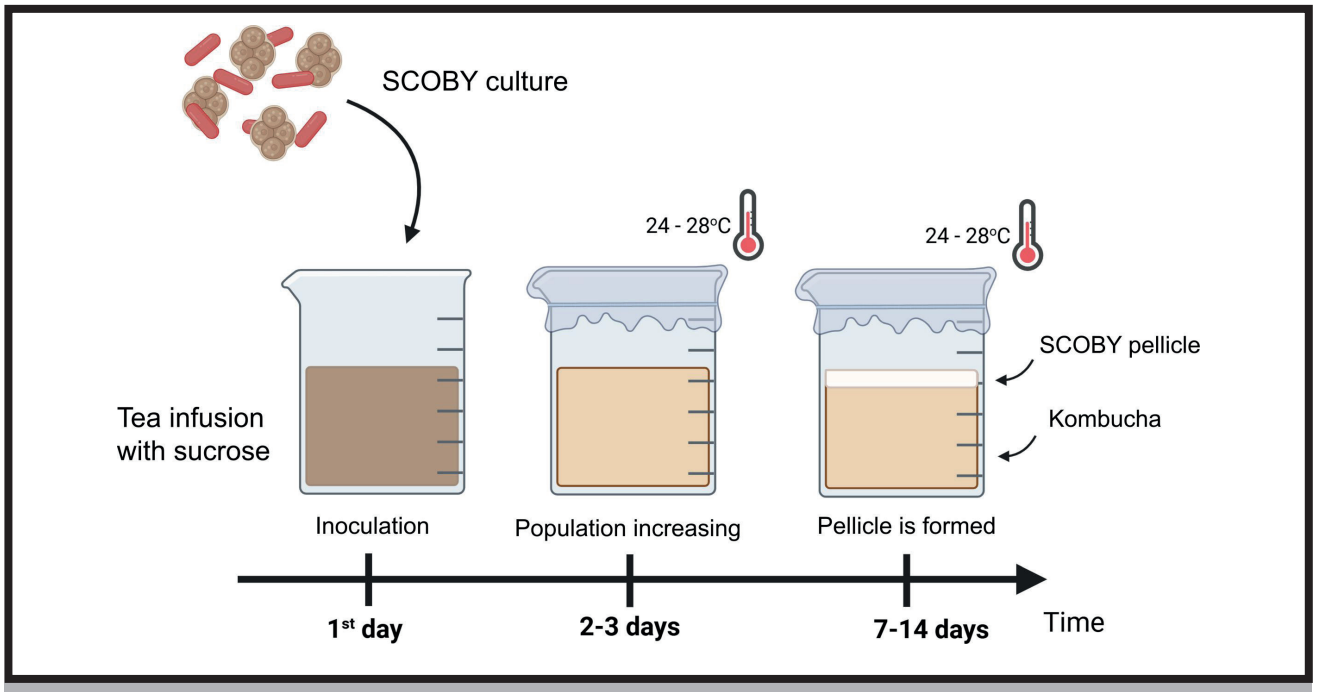

FIG. 1. Tea broth fermentation and SCOBY pellicle formation

Dulbecco's phosphate-buffered saline without calcium or magnesium (DPBS) were purchased from PAN BIOTECH, Aidenbach, Germany.

SCOBY nanocellulose production

SCOBY nanocellulose was obtained from the purified pellicle formed during kombucha production. For material production, 8 g of black tea was steeped in 1 000 ml of sterilized Milli-Q water, followed by the addition of 95 g of sucrose. Fermentation was carried out at 27°C in previously autoclaved beakers containing the tea broth and SCOBY inoculum, which were covered with single-use facial tissue. The production process is shown in FIG 1. After 10 days of fermentation, the SCOBY pellicle formed at the air-liquid interface was collected.

Purification of the SCOBY pellicle involved washing to remove the remaining tea broth, followed by sonication (42 kHz, Emag Emmi-06 UVC, Conrad, Poland) in a water bath combined with UV-C treatment, followed by double washing with 1.5 M sodium hydroxide heated to 90°C, bleaching with 1.5% hydrogen peroxide, and thorough rinsing with Milli-Q water until neutral pH was achieved after 24 h. Washing, bleaching, and rinsing were performed on a digital rocker (IKA 3D Rocker Digital); constant movement during the purification process ensured efficient penetration of the solutions into the samples.

In this study, three types of samples were analysed. The first was the SCOBY pellicle collected directly from the fermentation container and rinsed with Milli-Q water for 24 h until a neutral pH was achieved. The second sample consisted of a SCOBY pellicle subjected to two NaOH washing steps and rinsed with Milli-Q water over 24 h until a neutral pH was reached. The third sample was the fully purified material, hereafter referred to as "SCOBY nanocellulose". This material was subjected to multistage rinsing with water and treatment with both NaOH and H_2O_2.

Fourier transform infrared spectroscopy

Fourier transform infrared (FTIR) spectroscopy in attenuated total reflection (ATR) mode was performed using a Tensor 27 FTIR spectrophotometer (Bruker, Germany) equipped with a diamond crystal. Three types of samples were analysed: untreated SCOBY pellicle, NaOH-treated pellicle, and fully purified SCOBY nanocellulose. Before FTIR analysis, the samples were freeze-dried (Alpha 1–2, Martin Christ, Osterode am Harz, Germany).

Morphology and fibre size

Scanning electron microscopy (SEM) was employed to examine the microstructure of SCOBY nanocellulose. Before imaging, samples were freeze-dried, fixed on metal holders with carbon tape, and sputter-coated with a carbon layer (MED 010 carbon coater, Blazers Union Limited, Liechtenstein) to ensure electrical conductivity. To examine cross-sections of chosen samples, they were first immersed in liquid nitrogen and subsequently fractured to ensure brittle fracture and minimize alterations to the microstructure. SEM images were acquired using ThermoFisher Scientific Apreo 2, Scios 2 microscope (Waltham, MA, USA). The high voltage was set to 5.00 kV, Everhart-Thornley Detector was used. The dwell time was 3 µs, and images were acquired at magnifications of 5 000 ×, 20 000 ×, and 50 000 ×. Fibre diameter was analysed using ImageJ software (1.54d, Wayne Rasband and contributors, National Institutes of Health, USA) based on measurements of 200 fibres per sample.

Porosity

The porosity of SCOBY nanocellulose was determined by comparing the apparent and true densities of cellulose. Cylindrical samples (n = 8) were obtained from the purified SCOBY nanocellulose using a biopsy punch, followed by freeze-drying. The diameter and height of each sample were measured. Sample volume was calculated using the cylinder volume equation (1):

$$V_{cylinder} = \pi \cdot r^2 \cdot h \quad (1)$$

where: $V_{cylinder}$ – volume of cylinder, r – radius, h – height.

The apparent density was calculated as the ratio of sample mass to its volume. The assumed true density of pure cellulose was 1.6 g/cm^3 [11]. Porosity was calculated according to equation (2).

$$\phi\ (\%) = 1 - \frac{\rho_{app}}{\rho_t} \times 100 \quad (2)$$

where: ϕ – porosity, ρ_{app} – apparent density, ρ_t – true density.

In vitro cytotoxicity

In vitro cytotoxicity tests included the AlamarBlue assay (resazurin-based) and live/dead staining (calcein AM and propidium iodide). Material extracts were evaluated in contact with L929 murine fibroblasts. Cells were cultured in DMEM supplemented with 10% FBS and 1% penicillin/streptomycin. Culture was maintained at 37°C, in a humidified atmosphere containing 5% CO_2. To prepare extracts, samples were freeze-dried, treated with UV light from both sides for 20 min, and immersed in culture medium for 24 h. Two extract concentrations were prepared: 10 mg/mL and 5 mg/mL. Extracts were sterilized by filtration using syringe filters (pore size 0.22 µm, PES, GoogLab Scientific, Poland). Cells were seeded in a 96-well plate (n = 6) at a density of 3 000 cells per well in 200 µl. After 24 h, the culture medium was replaced with the prepared extracts, and cells were further cultured for 1, 3, and 7 days.

For the AlamarBlue assay, the cell culture medium was aspirated at each time point and 200 µl of 5% AlamarBlue reagent was added to each well, incubated for 3 h and transferred to a 96-well black plate. Fully reduced AlamarBlue, used for the calculations, was obtained by autoclaving 5% reagent for 15 min at 121°C. Fluorescence intensity was measured at excitation/emission wavelength of λex = 530 nm and λem = 590 nm using the FluoroSTAR Omega reader (BMG Labtech, Ortenberg, Germany). Cell viability was evaluated from the AlamarBlue assay using equation (3):

$$AlamarBlue\ reduction = \frac{F_{sample} - F_{blank}}{F_{100\%\ reduction}} \qquad (3)$$

where: F_{sample} – fluorescence measured for a sample, F_{blank} – fluorescence measured for a mixture of media and AlamarBlue mixture incubated in empty wells along with samples, and $F_{100\%\ reduction}$ – fluorescence measured for a fully reduced sample via autoclave (pure resazurin).

The results were shown as mean ± standard deviation (SD). Statistical analysis was performed using OriginLab 2025 software and a one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Differences were considered statistically significant for $p \leq 0.05$.

The live/dead assay consisted of staining cells with a DPBS solution containing 0.1% calcein AM and 0.1% propidium iodide for 20 min at 37°C. Cells were observed by fluorescence microscopy (ZEISS Axiovert 40 CFL, Carl Zeiss, Oberkochen, Germany).

Results and discussion

SCOBY nanocellulose production and purification

The SCOBY pellicle is formed during kombucha fermentation at the air-liquid interface of the culture medium and can be purified to obtain transparent SCOBY nanocellulose layers. The purification process used in this study consisted of sonication combined with UV-C irradiation, followed by washing with water, NaOH, and H_2O_2. As shown in FIG. 2, differences in colour and transparency are clearly visible depending on the stage of purification. The SCOBY pellicle is yellow-brown and exhibits the lowest transparency. The pellicle treated with NaOH shows increased transparency but remains yellow-brown, while the pellicle treated with hydrogen peroxide and multiple washing steps becomes white and transparent. Transparency is an important property of materials intended for biomedical applications, particularly for wound dressings. Transparent dressings enable continuous monitoring of the wound bed without the need to remove or lift the dressing. SCOBY nanocellulose is commonly reported in the literature as exhibiting limited transparency [6, 12].

Fourier transform infrared spectroscopy analysis

FIG. 3 shows the FTIR spectra of the SCOBY pellicle, the sample treated with NaOH, and the fully purified SCOBY nanocellulose. The main FTIR bands in the SCOBY pellicle are at 3 350, 2 900, 1 720, 1 585, 1 430, 1 120, 1 050 and 550 cm^{-1}. After NaOH treatment, the intensity of these bands was reduced and the band at 1 720 cm^{-1} disappeared. The FTIR spectrum of SCOBY nanocellulose does not exhibit a band at 1 585 cm^{-1}.

Within the 1 500 – 450 cm^{-1} fingerprint region, the absorbance spectrum of SCOBY nanocellulose is similar to that of pure cellulose [13]. This region contains the band at 1 160 cm^{-1} originating from C-O-C antisymmetric bridge stretching vibrations associated with the presence of β-(1→4)-glucosidic linkage in microbial cellulose [14]. The bands at 1 120 and 1 050 cm^{-1} are associated with the presence of C-N groups of proteins and nucleic acids; their

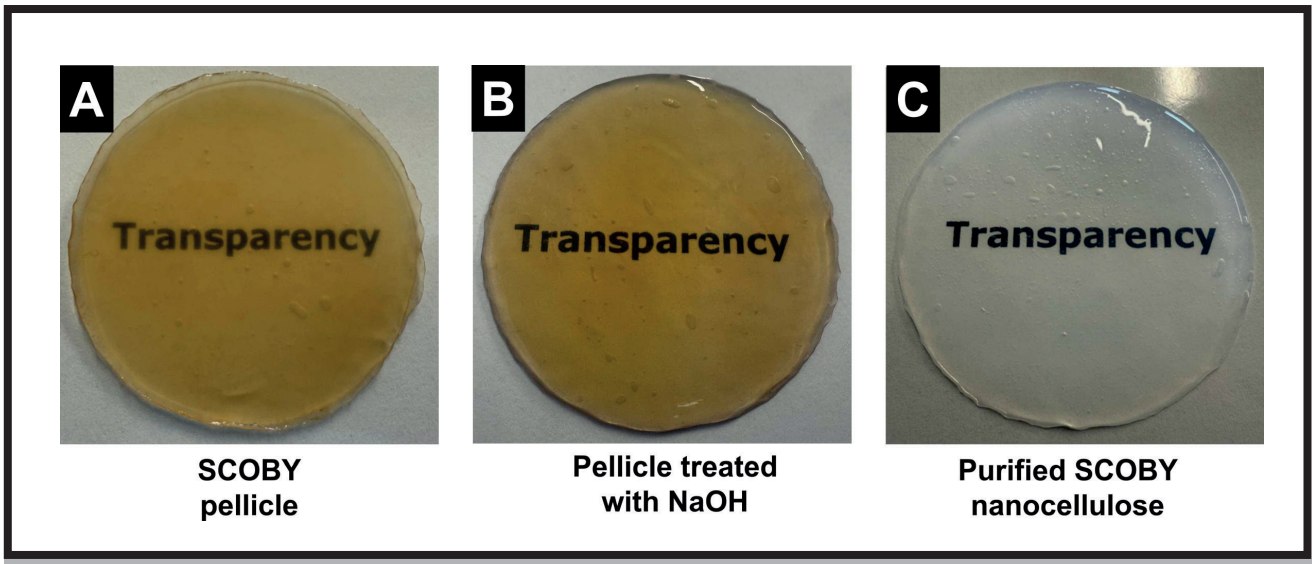

FIG. 2. Exopolysaccharide film in different stages of the purification process: SCOBY pellicle after washing with Milli-Q water (A); SCOBY pellicle after washing with water and NaOH (B); SCOBY nanocellulose: pellicle after washing with water, treatment with NaOH, and bleaching with H_2O_2 (C).

intensity decreases significantly after purification [14–16]. The band at 1 430 cm^{-1} originates from CH_2 scissoring or symmetric bending vibrations, as well as O-H in plane bending. The band at 1 050 cm^{-1} is due to C-O stretching vibrations in primary alcohol and C-O-C skeletal vibrations, which explains why the intensity is higher for the untreated SCOBY pellicle. This band is also associated with the presence of C-O-H bonds in carbohydrates and is an expected part of the fingerprint region of pure cellulose [14].

Outside of the fingerprint region, there are high-intensity bands at 3 350, 2 900, 1 720, and 1 585 cm^{-1}. The band at 1 720 cm^{-1} is associated with stretching vibrations of the C=O bonds in amide I, while the band at 1 585 cm^{-1} originates from the amide II bond in proteins; both peaks disappear after purification of the SCOBY pellicle, indicating the removal of compounds other than cellulose [17]. The band at 2 900 cm^{-1} is associated with C-H stretching vibrations; this can come from lipids present in bacterial cells and after purification it disappears [4, 15, 17]. The band at 3 350 cm^{-1} originates from stretching vibrations of O-H groups in cellulose [14], but it can also come from the presence of absorbed water molecules; the intensity of this peak decreases after NaOH treatment [16].

The results show that the non-cellulose impurities present in the SCOBY pellicle are progressively removed during NaOH treatment and further eliminated after H_2O_2 treatment. On the basis of FTIR analysis, it can be concluded that SCOBY nanocellulose has been effectively purified using the presented protocol. Additionally, the proposed purification protocol in this work could be considered relatively harsh compared to commonly reported methods for microbial cellulose. However, this purification is proposed for material that is required to be suitable and safe for biomedical applications. Applied purification also influences the transparency of the material. FTIR analysis and sample transparency indicate the high quality of the obtained material and the effectiveness of the purification protocol.

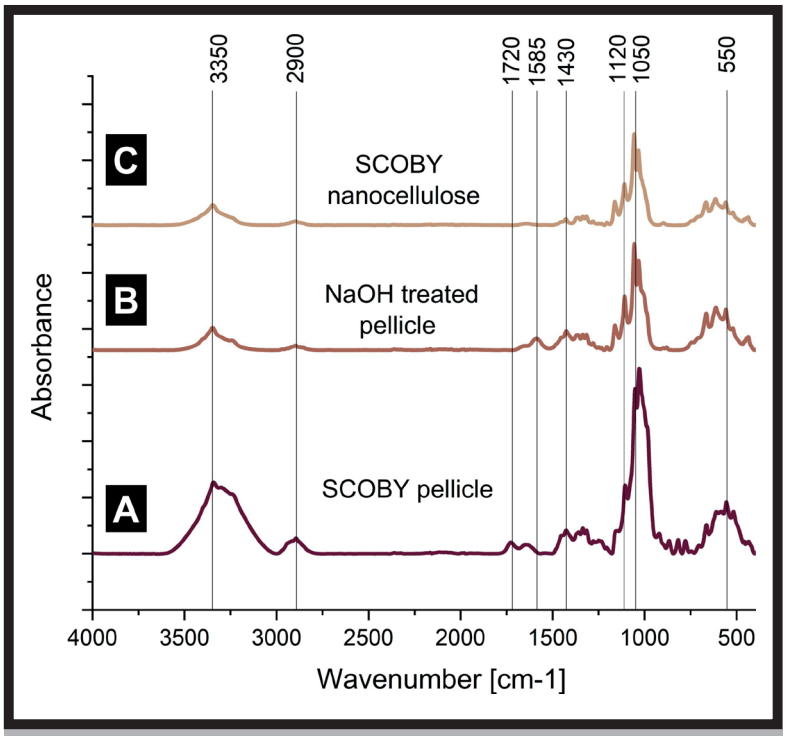

FIG. 3. FTIR spectra of: SCOBY pellicle after washing with Milli-Q water (A); SCOBY pellicle after washing with water and NaOH (B); SCOBY nanocellulose: pellicle after washing with water, treatment with NaOH and bleaching with H_2O_2 (C).

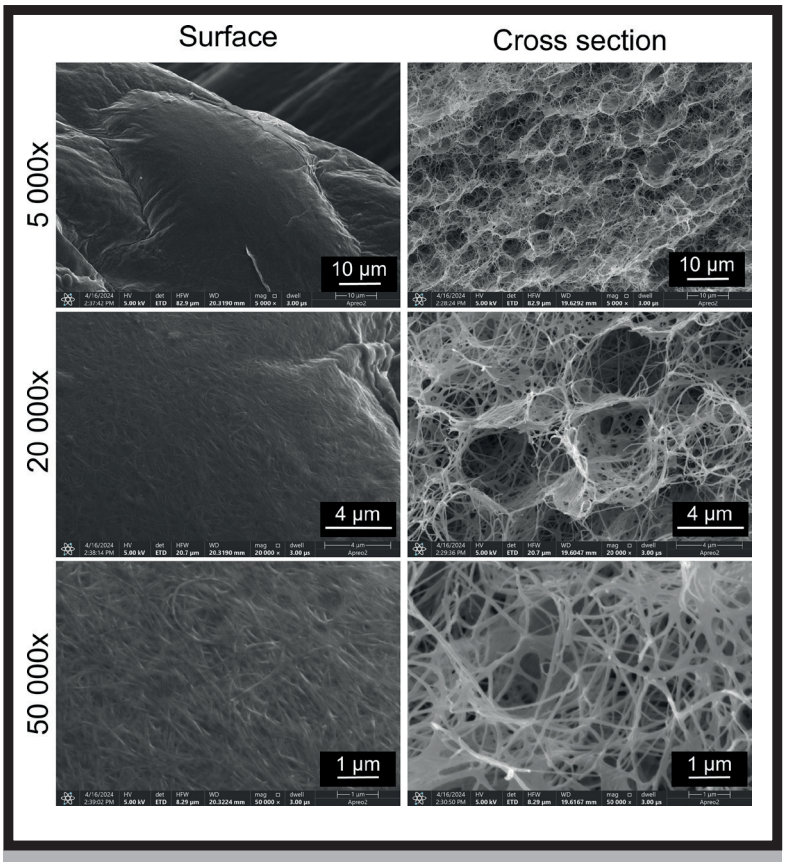

FIG. 4. Scanning electron microscopy (SEM) pictures of the top surface (A, C, E) and cross-section (B, D, F) of SCOBY nanocellulose at magnification of 5 000× (A, B), 20 000× (C, D) and 50 000× (E, F).

Microstructure and porosity

SEM images of freeze-dried SCOBY cellulose (FIG. 4) show that the surface of the material is made up of tightly packed nanofibers, while the cross-section exhibits a regular, highly porous nanofibrous structure, creating micropores with diameters ranging from 4 to 6 µm.

FIG. 5 A, B, C show the microstructure of SCOBY nanocellulose originating from three different batches, respectively. Fibre diameter measurements performed on images from these batches confirm the high reproducibility of the production method (FIG. 5D, E, F, respectively).

The results show that SCOBY nanocellulose fibres have a diameter between 40 and 130 nm, with median values of 85, 91, and 86 nm, respectively. Some differences in diameter distribution can be seen between the samples but were considered negligible. These samples were produced independently over the span

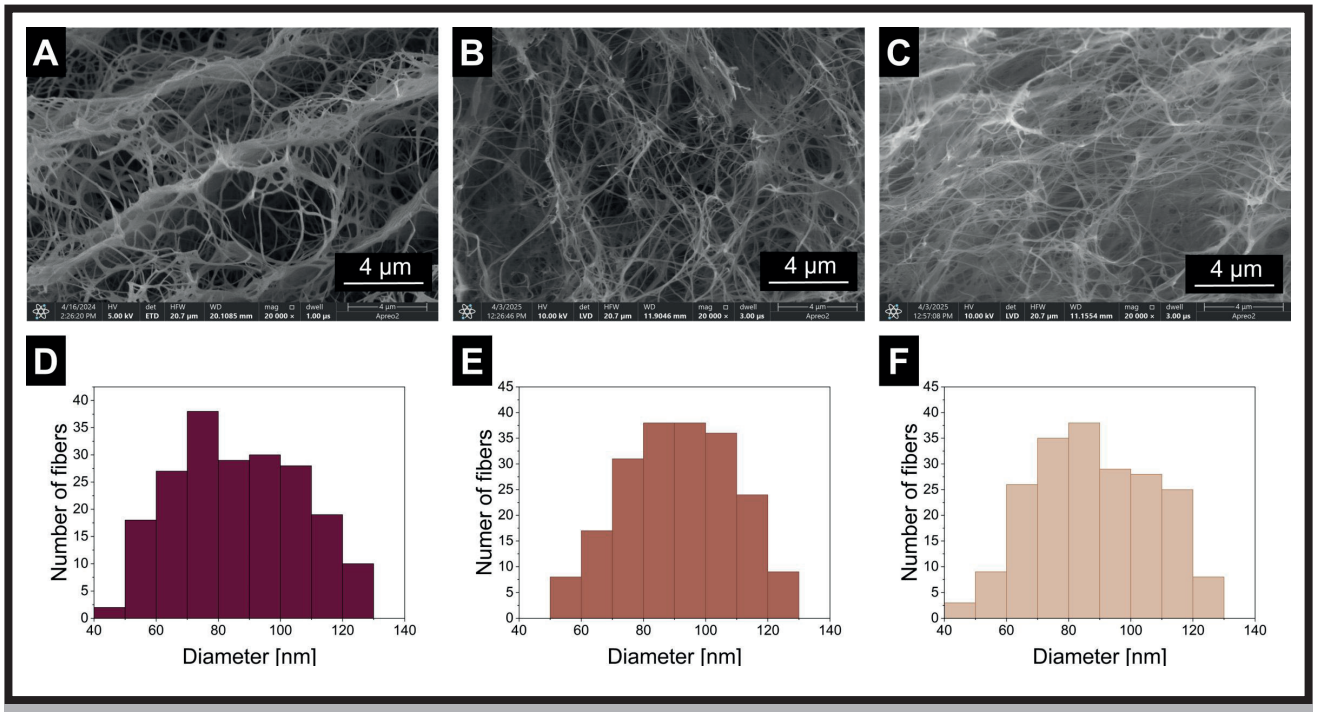

FIG. 5. SEM images (A, B, C) and histograms of the fibre diameter distribution (D, E, F) of SCOBY nanocellulose originating from different batches: batch I (A, D), batch II (B, E), batch III (C, F) at magnification of 20 000 ×. Histograms were performed on the basis of the measurements of 200 individual fibres.

of 13 months; conditions such as temperature and media compositions remained the same, as well as the preparation method for SEM imaging. This finding suggests that the presented method of SCOBY nanocellulose manufacturing yields reproducible results with respect to fibre size, morphology, and microstructural features of the material. Reproducibility is important for scaling up the manufacturing process.

The porosity of SCOBY nanocellulose was measured by comparing the apparent and true density of cellulose. Cylindrical samples (n = 8) were weighed and measured with a calliper (diameter and height). The mean sample height was 2.91 ± 0.87 mm, while the mean diameter was 11.02 ± 0.56 mm. The mean apparent density of freeze-dried SCOBY nanocellulose was 0.024 ± 0.006 g/cm³, while the true density of cellulose is 1.6 g/cm³ as reported in the literature [11]. Based on these data, the porosity of the freeze-dried material was calculated to be 98.5 ± 0.4%. In comparison with other published studies, our material shows much higher porosity compared with previously reported values of 66–87% [18] and 93.6 ± 0.3% [19]. Fibre diameters measured in this work are in good agreement with those reported in the literature [18]. The general morphology is also in agreement with literature reports; SCOBY nanocellulose exhibits tightly packed fibres on the external layers, while the cross-section shows a more layered, porous and fibrillar structure. The material obtained

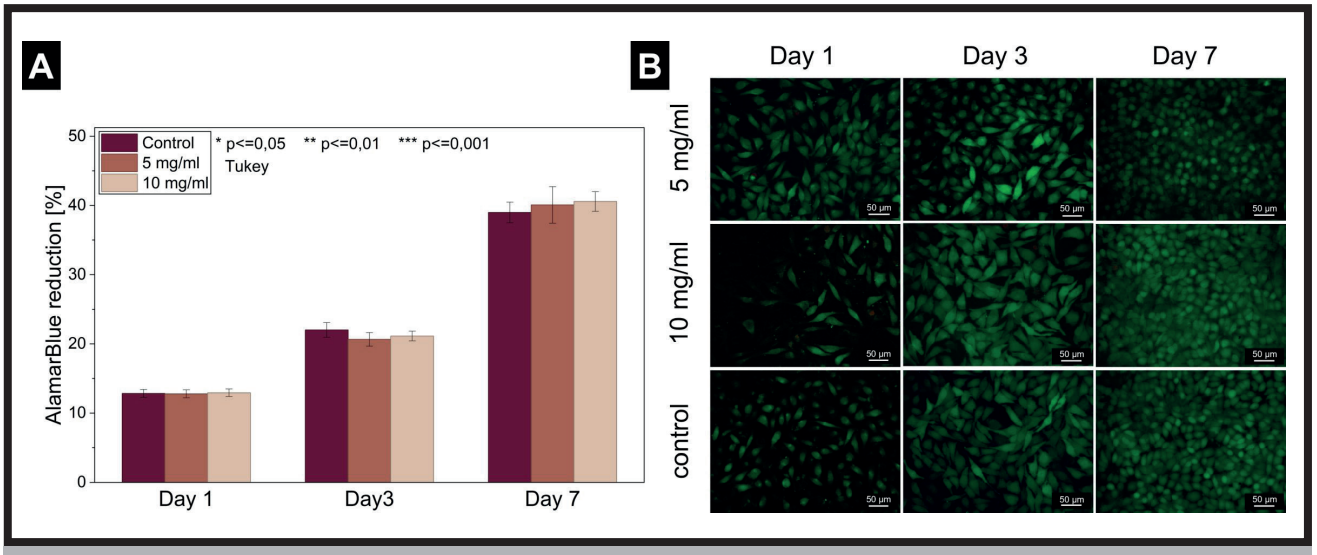

FIG. 6. Average viability of L929 cells cultured in material extract (A) of n=6 samples with SD and statistically significant differences at **p≤0.01. No statistically significant changes were observed between the samples for each time point. Live/dead images (calcein AM and propidium iodide staining) taken by fluorescence microscopy (B).

in this study shows a more uniform microstructure, without separation between layers; however, this microstructure may be related to sample preparation, which can be more or less damaging [18].

In vitro tests

L929 cells were cultured for 24 h in a 96-well plate before exchange of the culture medium for SCOBY nanocellulose extracts. Extracts of 5 and 10 mg/mL were chosen instead of the typical 5 and 10% because of the high liquid absorption capacity of SCOBY nanocellulose samples. Both the AlamarBlue assay and live/dead staining were performed after 24 h, 72 h, and 7 days.

FIG. 6 A shows the reduction of AlamarBlue by cells grown in DMEM (control) and in 5 and 10 mg/mL of material extracts in DMEM. The increase in AlamarBlue reduction, which confirms cell viability and proliferation, is observed as a function of culture time. Cells cultured in extracts exhibited comparable metabolic activity to those cultured under control conditions.

Live/dead staining (FIG. 6 B) shows that most of the cells in all groups are stained green, therefore viable. The images show an increase in the number of cells and a slight change toward a less elongated shape but maintain spindle-shaped morphology. This is normal and expected, as cells cannot occupy as much surface area with increasing population. Live/dead staining shows the absence of cytotoxic effects of material extracts and supports the findings of the AlamarBlue test.

Taking into account the metabolic activity assay, viability staining, and cell morphology observations, it can be concluded that SCOBY nanocellulose does not have a toxic effect on cells and can be further evaluated for biomedical use. These results are in agreement with other reported studies on the cytocompatibility of microbial cellulose [20, 21]. It is important to note that the cytotoxic effect in this study could also arise from inadequate removal of the solutions used for purification (sodium hydroxide and hydrogen peroxide). This appears not to be the case; therefore this observation further supports the conclusion that the pellicle was adequately purified and that potentially harmful chemicals were successfully removed, suggesting effective elimination of cytotoxic residues.

Conclusions

The method of producing SCOBY nanocellulose described in this study emphasises both ecological and economic aspects as well as the successful processing of the material for biomedical use. The production of SCOBY-derived nanocellulose through fermentation of a tea infusion with sucrose is significantly cheaper and more accessible than using a single-strain bacteria approach. Moreover, by optimising the purification process, it is possible to utilise a material that is usually considered a post-fermentation byproduct. The purification process has been shown to be effective when applied to complete samples, that is, when the samples are not divided into smaller fragments before treatment. This is particularly important for the use of the material as a wound dressing, since its dimensions can be tailored during the fermentation process by controlling the size and shape of the container.

Despite the fact that the fermentation environment and the composition of the tea broth remained the same, the materials obtained through biotechnological processes can differ batch by batch. However, this was not observed for SCOBY nanocellulose in this study. The described biotechnological process enables the production of a microporous material composed of cellulose nanofibers with diameters between 40 and 130 nm, with medians for three independent batches being 85, 91, and 86 nm, respectively, based on 200 measurements of individual nanofibers per sample. These results show that this method yields consistent fibre sizes. The material exhibits a unique morphology, as nanofibers form micropores with diameters around 4 to 6 µm. The porosity of this material after freeze-drying is extremely high and accounts for 98.5 ± 0.4%.

FTIR spectroscopy enabled the analysis of functional groups present in the examined material and provided information on its chemical composition. It was used to examine whether non-cellulose impurities are still present within the SCOBY exopolysaccharide matrix during and after the purification process. We found that after the appropriate purification and processing, SCOBY nanocellulose does not demonstrate cytotoxic effects, as confirmed by in vitro tests on L929 murine fibroblasts. The high transparency of the material is particularly favourable for wound dressing applications, as it enables continuous visual monitoring of the wound bed, eliminating the need for dressing removal. Consistent fibre diameters across three independently analysed samples suggest a stable fabrication process, important for large-scale production. The combination of high porosity, nanofibrous microstructure, transparency, reproducibility, and cytocompatibility demonstrates strong potential of the SCOBY nanocellulose produced in this study in biomedical applications, particularly for wound dressings and tissue engineering. Future studies on this material are intended to focus on wound healing, as it is believed that it can be applied as an environmentally friendly and low-cost matrix for active wound dressings.

Acknowledgments

This research was funded by the Ministry of Science and Higher Education, Poland from the Program “Excellence Initiative—Research University” and from the subsidy (no. 16.16.160.557) for the AGH University of Krakow.

References

[1] Laavanya D, Shirkole S, Balasubramanian P. Current challenges, applications and future perspectives of SCOBY cellulose of Kombucha fermentation. *Journal of Cleaner Production* 2021; 295: 126454.

[2] de Melo LM, Soares MG, Bevilaqua GC, et al. Historical overview and current perspectives on kombucha and SCOBY: A literature review and bibliometrics. *Food Bioscience* 2024; 59: 104081.

[3] Priyadharshini T, Nageshwari K, Vimaladhasan S, et al. Machine learning prediction of SCOBY cellulose yield from Kombucha tea fermentation. *Bioresource Technology Reports* 2022; 18: 101027.

[4] Içen H, Corbo MR, Sinigaglia M, et al. Microbiology and antimicrobial effects of kombucha, a short overview. *Food Bioscience* 2023; 56: 103270.

[5] Chawla P, Bajaj I, Survase S, et al. Microbial Cellulose: Fermentative Production and Applications. *Food Technology and Biotechnology* 2009; 47: 107–124.

[6] Yakaew P, Phetchara T, Kampeerapappun P, et al. Chitosan-Coated Bacterial Cellulose (BC)/Hydrolyzed Collagen Films and Their Ascorbic Acid Loading/Releasing Performance: A Utilization of BC Waste from Kombucha Tea Fermentation. *Polymers* 2022; 14: 4544.

[7] Candra A, Ahmed YW, Kitaw SL, et al. A green method for fabrication of a biocompatible gold-decorated-bacterial cellulose nanocomposite in spent coffee grounds kombucha: A sustainable approach for augmented wound healing. *Journal of Drug Delivery Science and Technology* 2024; 94: 105477.

[8] Alkhalifawi I. Silver Nanoparticles Synthesis by Hamza's Khubdat (A.S.) (Kombucha) Tea and used in Burn Wounds Treatment. *Journal of Global Pharma Technology* 2019; 10: 489–500.

[9] Lee S. Suzanne Lee: Grow your own clothes. TED Talk, https://www.ted.com/talks/suzanne_lee_grow_your_own_clothes (accessed 28 June 2024).

[10] State AH-I. This sustainable 'leather' comes from kombucha tea. *Futurity*, https://www.futurity.org/scoby-fabric-kombucha-leather-1149392-2/ (2016, accessed 28 June 2024).

[11] Yano H, Omura H, Honma Y, et al. Designing cellulose nanofiber surface for high density polyethylene reinforcement. *Cellulose* 2018; 25: 3351–3362.

[12] Kołodziejczyk AM, Silarski M, Kaczmarek M, et al. Shielding properties of the kombucha-derived bacterial cellulose. *Cellulose* 2025; 32: 1017–1033.

[13] Goh WN, Rosma A, Kaur B, et al. Microstructure and physical properties of microbial cellulose produced during fermentation of black tea broth (kombucha). II. *International Food Research Journal* 2012; 19: 153–158.

[14] Wang S-S, Han Y-H, Ye Y-X, et al. Physicochemical characterization of high-quality bacterial cellulose produced by Komagataeibacter sp. strain W1 and identification of the associated genes in bacterial cellulose production. *RSC Advances* 2017; 7: 45145–45155.

[15] Fuller ME, Andaya C, McClay K. Evaluation of ATR-FTIR for analysis of bacterial cellulose impurities. *Journal of Microbiological Methods* 2018; 144: 145–151.

[16] Sousa L, Leite P, Vieira A, et al. Effect of water and alkali on purification bacterial cellulose membrane from Kombucha. Research, *Society and Development* 2021; 10: e526101523267.

[17] Orlovska I, Podolich O, Kukharenko O, et al. Bacterial Cellulose Retains Robustness but Its Synthesis Declines After Exposure to a Mars-like Environment Simulated Outside the International Space Station. *Astrobiology* 2021; 21: 706–717.

[18] Daus F, Montroni D, Pesavento L, et al. Influence of the thickness of Symbiotic Culture of Bacteria and Yeast on purification and final properties of bacterial cellulose. *Carbohydrate Polymer Technologies and Applications* 2025; 9: 100645.

[19] Bergottini VM, Bernhardt D. Bacterial cellulose aerogel enriched in nanofibers obtained from Kombucha SCOBY byproduct. *Materials Today Communications* 2023; 35: 105975.

[20] Popa-Tudor I, Tritean N, Dima Ștefan-O, et al. Kombucha Versus Vegetal Cellulose for Affordable Mucoadhesive (nano)Formulations. *Gels* 2025; 11: 37 .

[21] Bağlan İ, Yanbakan E, Tuncel T, et al. 3D printed kombucha biomaterial as a tissue scaffold and L929 cell cytotoxicity assay. *Journal of Cellular and Molecular Medicine* 2024; 28: e18316.