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Synthesis and structure of silver-containing bacterial cellulose composites with chitosan

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Materials and Methods

The following materials were used in this study: chitosan (Chit) from Wirud C100001 (Germany, deacetylation degree >75%), glutamic acid (GA) from Sigma-Aldrich (USA, 98.5%), isopropanol ($CH_3CH(OH)CH_3$, USA, Aldrich, 99.7%), and Ag (99.99%).

Bacterial cellulose

Bacterial cellulose (BC) was obtained by culturing the Novacetimonas hansenii GH-1/2008 producer strain for 7 days at 28 °C under dynamic conditions on a modified Gestrin S. and M. Schramm medium: glucose (Thermo Fisher Scientific, Waltham, MA, USA) 40 g/L, yeast extract (Thermo Fisher Scientific, Waltham, MA, USA) 5 g/L, Na_2HPO_4 (AppliChem GmbH, Darmstadt, Germany) 2.7 g/L, K_2HPO_4 (AppliChem GmbH, Darmstadt, Germany) 2 g/L, $(NH_4)_2SO_4$ (Sigma-Aldrich RTC, Inc., Laramie, Wyoming, USA) 3 g/L, citric acid monohydrate (Thermo Fisher Scientific, Waltham, MA, USA) 1.15 g/L. The nutrient medium was sterilized in an autoclave (All American 50×, Wisconsin Aluminum, Manitowoc, WI, USA) at 120 °C for 30 minutes. At 22 °C 5% of the inoculum and 0.5% of the total nutrient medium volume were added to the flasks with the medium. The addition of the producer and alcohol to the flasks with the media was carried out in a laminar flow hood (NEOTERIK BMB-II-Laminar-S-0.9b, Industrial Group "Laboratory", St. Petersburg, Russia). For dynamic cultivation 250 ml spherical round-bottomed flasks with 125 ml of nutrient medium were used which were placed in a shaker-incubator (KS 4000 control IC, IKA, Staufen, Germany) rotation speed 150 rpm. To purify the polymer from the culture fluid and the producer RIPA buffer (Thermo Fisher Scientific, Waltham, MA, USA) was used. The following composition (g/L) was used: SDS 1 g, sodium chloride 10 g, tris-(oxymethyl)-aminomethane 10 g and deoxycholate 5 g. The RIPA solution was washed with vigorous shaking in a shaker for 48 hours, changing the solution every 24 hours. The polymer was then washed in distilled water with vigorous shaking in a shaker until the washing solution reached a neutral pH

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Preparation of a bacterial cellulose suspension

Bacterial cellulose, grown under dynamic conditions, was placed in a glass container and ground with an immersion blender (Braun MQ 7075 X, Romania) for 15-30 minutes at 13300 rpm. The resulting polymer suspension was centrifuged to remove water (Janetzki T 24, Germany) for 5 minutes at 5000 rpm. The process was repeated three times, after which the sample was placed in isopropanol to yield a gel containing 0.1 g of dry BC substance per 4.7 g of gel mass.

Modification of Chit powder and BC suspension with Ag nanoparticles (Ag NPs) synthesized by the MVS

The MVS method was described in detail in references.[1,2] Isopropanol was dried and distilled over 4 Å zeolites under purified argon. Before synthesis the solvent was degassed using alternating freeze-thaw cycles. Silver was evaporated from a tantalum boat. In a typical experiment 150 ml of organic reagent and 0.2 g of silver were used for synthesis. The co-condensation of metal vapor and the organic ligand was carried out in a vacuum of 10^{-2} Pa for approximately 50 min in a stationary 5 L quartz reactor cooled with liquid nitrogen. After metal evaporation was complete the supply of organic reagent was stopped. The cooling was removed, and the co-condensate matrix melted at room temperature to form an organosol. The organosol was withdrawn from the reactor "*in situ*" via a siphoning system and transferred in equal portions into two evacuated flasks containing Chit powder and a BC suspension. Modification was carried out under an argon atmosphere for 20 min with vigorous stirring. Excess isopropanol was then removed from the flasks. Silver-containing chitosan (Chit@Ag) was dried to constant weight under a vacuum of 10^{-1} Pa and a temperature of 60 °C. Isopropanol was decanted from the flask containing silver-containing bacterial cellulose (BC@Ag) to yield a gel-like mass, which was used for the synthesis of a hybrid composite.

Preparation of the BC-Chit-GA composite

0.1 g of bacterial cellulose was mixed with 0.1 g of chitosan (previously dissolved in 10 mL of a 0.1 M glutamic acid solution) and stirred for 1.5 h using a magnetic stirrer to obtain a gel-like mass, which was then freeze-dried.

Preparation of the GA@Ag conjugate

An organosol containing 0.15 g of Ag NPs in 120 mL of isopropanol obtained immediately "*in situ*" after MVS and was added to a solution of 0.15 g of GA and stirred at room temperature for 0.5 h using a magnetic stirrer. The solvent was evaporated to yield the GA@Ag conjugate, which was then dried to constant mass under a vacuum of 10^{-1} Pa at 60 °C.

Preparation of the Chit@Ag-GA composite

0.1 g of the Chit@Ag composite was dissolved in 10 mL of a 0.1 M GA solution, stirred at room temperature for 0.5 h using a magnetic stirrer, and dried to constant mass under a vacuum of 10^{-1} Pa at 60 °C.

Preparation of the BC-Chit-GA@Ag composite

Chit@Ag (0.1 g) was placed in a flask and dissolved in 10 ml of a 0.1 M GA solution. BC@Ag (4.7 g of gel, equivalent to 0.1 g of dry matter) was then added to the flask and stirred for 1.5 hours on a magnetic stirrer at 1500 rpm.

Freeze-drying of BC-Chit-GA and BC-Chit-GA@Ag composites

BC-Chit-GA and BC-Chit-GA@Ag samples were placed in Petri dishes frozen at −18 °C for 24 h and dried at −50 °C and 0.1 mbar using an Alpha 1-2 LD freeze dryer (Christ, Germany) for 24 h.

Research Methods

The metal concentration in the composite materials was determined by X-ray fluorescence analysis. The analysis was performed using a VRA 30 X-ray fluorescence analyzer (Germany) using the Kα line of the X-ray fluorescence spectrum.

Scanning electron microscopy (SEM) images were obtained in secondary electron mode on a JCM-6000 PLUS benchtop electron microscope (JEOL, Japan) equipped with an energy-dispersive X-ray spectrometer.

X-ray photoelectron spectroscopy (XPS) studies were performed using an Axis Ultra DLD spectrometer (Kratos Ltd., Manchester, UK) with a monochromatic Al Kα X-ray source (1486.6 eV). The spectrometer's energy scale was calibrated according to a standard procedure based on the following binding energies: Au $4f_{7/2}$ 83.96 eV, Cu $2p_{3/2}$ 932.62 eV, Ag $3d_{5/2}$ 368.21 eV. To eliminate the effect of sample charge, the spectra were recorded using a neutralizer. The surface charge for the samples was corrected by referencing the NH_2 group identified in the N 1s (399.6 eV) spectra.[3]

X-ray diffraction analysis was performed on a D8 Advance diffractometer (Bruker AXS, Karlsruhe, Germany). Diffraction profiles were analyzed using TOPAS 5 software (Bruker AXS, Karlsruhe, Germany).

Thermogravimetric analysis was performed using a Shimadzu DTG-60H thermal analyzer (Shimadzu Corporation, Kyoto, Japan) on samples weighing approximately 5 mg at a heating rate of 10 °C/min in an argon atmosphere. The temperature at which a 10% mass loss was observed was taken as the onset temperature of decomposition.

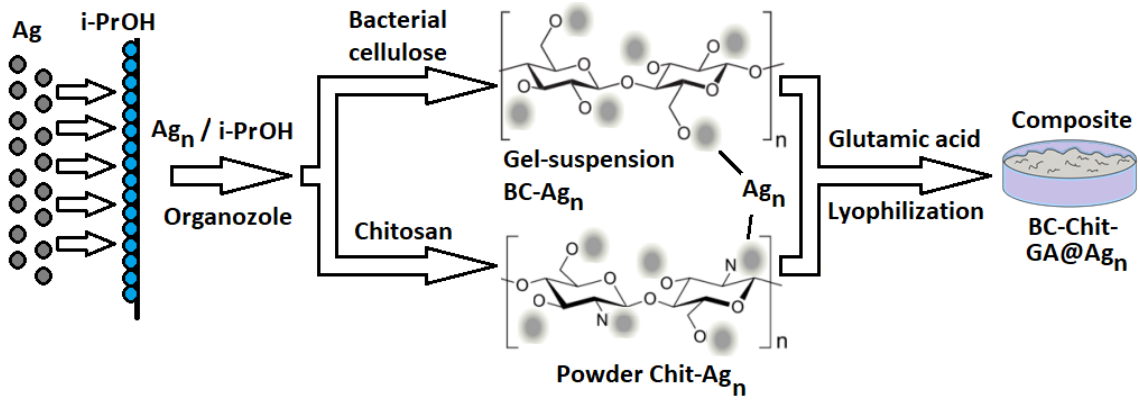


Figure S1. Schematic diagram for the preparation of BC-Chit-Ga@Ag composites.

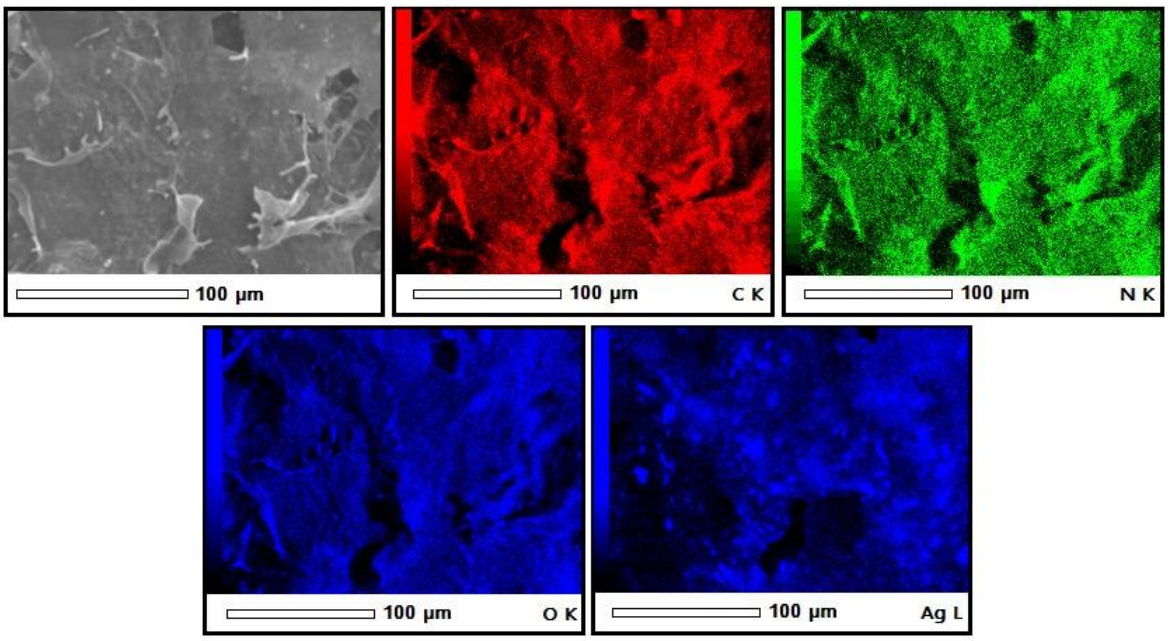


Figure S2. Elemental distribution of C, N, O, and Ag in the BC-Chit-GA@Ag sample.

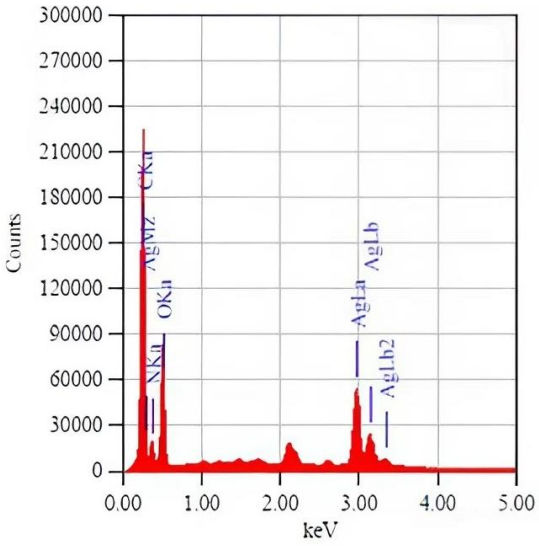


Figure S3. Energy-dispersive X-ray spectrum of BC-Chit-GA@Ag: C—26.1 wt.%; N—16.2 wt.%; O—39.0 wt.% and Ag—18.8 wt.% (b).

Figure S2 shows the element distribution maps of C, O, N, and Ag, and Figure 3S shows the energy-dispersive X-ray spectra of the BC-Chit-GA@Ag nanocomposite. Elemental mapping analysis of the BC-Chit-GA@Ag composite confirmed the presence of Ag in the sample and demonstrated its uniform distribution within the porous material.

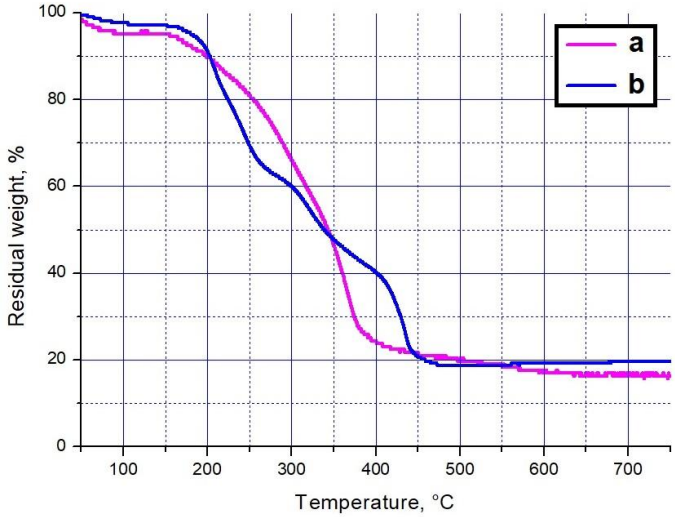


Figure S4. TGA curves of BC-Chit-GA (a) and BC-Chit-GA@Ag (b) samples at a heating rate of 10 °C/min in an argon atmosphere.

The thermal stability of the BC-Chit-GA (a) and BC-Chit-GA@Ag composites was evaluated by TGA in the temperature range of 25–750 °C in an argon atmosphere. Both samples lost 1.5–2.5% of their mass between 0 and 100 °C, a loss attributed to the removal of external and weakly bound water. In the 200–350 °C range, the Ag-containing product lost mass more rapidly than the BC-Chit-GA composite. However, BC-Chit-GA@Ag exhibited a higher residual mass in the 350–

450 °C range. This is likely due to the presence of silver nanoparticles, which enhance the composite's thermal stability in this temperature range, promote the formation of carbonaceous material, and retard the generation of volatile pyrolysis products.

Table S1. Characteristics of the N 1s photoelectron spectra of Chit and BC-Chit-GA@Ag: binding energies (E_b), Gaussian widths (W), and relative intensities (I_{rel}) of photoelectron peaks belonging to different chemical groups.

Sample		NH_2	N(O)C	NH_3^+ (Glut)	NH_3^+ (Chit)
Chit	E_b, eV	399.6	400.7	-	402.1
	W, eV	1.43	1.43	-	1.43
	I_{rel}	0.84	0.12	-	0.04
BC-Chit-GA@Ag	E_b, eV	399.6	400.7	401.5	402.1
	W, eV	1.6	1.6	1.6	1.6
	I_{rel}	0.26	0.04	0.69	0.01

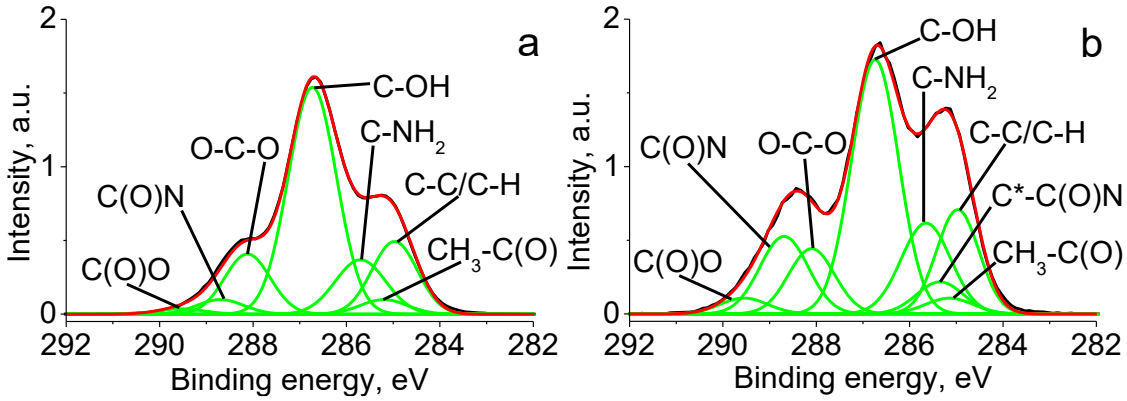


Figure S5. Photoelectron spectra of C 1s samples Chit (a), Chit@Ag-GA (b).

Table S2. Characteristics of C 1s photoelectron spectra for Chit and Chit@Ag-GA: binding energies (E_b), Gaussian widths (W), and relative intensities (I_{rel}) of photoelectron peaks corresponding to various chemical groups

Sample	Parameters	C-C/C-H	CH_3-C(O)	C*-C(O)N	C-NH_2	C-OH	O-C-O	C(O)N	C(O)O
Chit	E_b, eV	285	285.2	-	285.7	286.7	288.1	288.7	289.6
	W, eV	1.15	1.18	-	1.27	1.22	1.15	1.18	1.2
	I_{rel}	0.15	0.03	-	0.13	0.52	0.13	0.03	0.01
Chit@Ag-GA	E_b, eV	285	285.2	285.4	285.7	286.7	288.1	288.7	289.6
	W, eV	1.07	1.2	1.18	1.25	1.19	1.16	1.22	1.2
	I_{rel}	0.14	0.02	0.05	0.15	0.4	0.1	0.12	0.02

Table S3. Characteristics of Ag 3d photoelectron spectra of BC-Chit-GA@Ag: binding energies (E_b) and spin-orbit splitting (ΔE_{Ag}).

Sample	E_b		ΔE_{Ag}	state
	Ag $3d_{5/2}$, eV	Ag $3d_{3/2}$, eV	Ag $3d_{3/2}$ - Ag $3d_{5/2}$, eV	
BC-Chit-GA@Ag	368.0	374.0	6	Ag^+
	368.7	374.7	6	Ag^0

References

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