Electronic Supplementary Material (ESI) for Biomaterials Science.

Supplementary Information

Construction of Cellulose Nanofibers/Quaternized Chitin/Organic Rectorite Composites and Their Application as Wound Dressing

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Experimental

Waste BA was treated with 5 wt% NaOH, 7% (v/v) HCl and 5 wt% NaOH at room tempertaure successively for 12 h, the mixture was washed with deionized water between each pair of steps. Then the pigments of waste BA were eliminated by using 4 % (v/v) hydrogen peroxide for 6 h at 80 °C, followed by washing with distilled water and freeze-drying to obtain cellulose.[1] BA cellulose (1 g) was dispersed in water (100 mL) containing 2,2,6,6-tetramethylpiperidin-1-oxyl (TEMPO) (0.016 g) and sodium bromide (0.1 g). A 5% NaClO solution was adjusted to pH 10 by the addition of 0.1 M HCl. The TEMPO-mediated oxidation was started by adding NaClO (5 mmol) solution, and continued at room temperature under vigorous stirring. The pH was maintained at about 10 by adding 0.5 M NaOH using a pH meter until no NaOH consumption was observed.[2] The TEMPO-mediated oxidized cellulose was dialyzed for 3 d with deionized water to remove salt and alkali. A slurry of oxidized cellulose (0.1%) was sonicated for 10 min by using an ultrasonic processor (JY98-IIID, Ningbo Scientz Biotechnology Co., Ltd, China) at 20 kHz and an output power of 720 W. The suspension was centrifuged to remove course particles. Then the suspension was evaporated to different concentrations of 2 mg/mL, 5 mg/mL and 10 mg/mL, then freeze dried to obtain BANCFs sponges, and the sponges were noted as BACNF2, BACNF5, BACNF10, respectively. Bamboo pulp cellulose nanofibers (BpCNFs), cotton linter pulp cellulose nanofibers (ClpCNFs) and wood pulp cellulose nanofibers (WpCNFs) were prepared by the same methods.

The carboxylate content of the TEMPO-oxidized cellulose was determined using an electric conductivity titration method. Freeze dried cellulose (0.3 g) was added to 0.1mmol/L NaCl (60 mL) solution and the mixture was sufficiently stirred to prepare a well-dispersed slurry. Then, 0.1 M HCl was added to the mixture to set the pH value in the range of 2.5-3.0. A 0.05 M NaOH solution was added at the rate of 0.1 mL/min up to pH 11 by using a pH stat. The carboxylate content of the sample was determined from the conductivity curves.[2]

The morphologies of cellulose nanofibers were observed with a Transmission Electron Microscope (TEM, JEM-2100, HR, JEOL, Japan). A 20 μL cellulose nanofibers suspension (1 mg/mL) was dropped onto a copper grid and one drop of sodium phosphotungstate was added. Excess solution was blotted out with a filter paper and allowed to stand for drying by natural evaporation. Then the samples were observed with TEM.

The squid pen was treated with 5 wt% NaOH for deproteination and 7 % (v/v) HCl for demineralization in turn at room temperature for 12h. The mixture was washed with deionized water after each step until neutral. Then the product was freeze dried to obtain β-chitin. β-chitin (2 g) was dispersed in 2-propanol (100 mL) with stirring for 0.5 h at room temperature, and then NaOH solution (25 g, 40 wt%) was added into the β-chitin suspention with stirring for 2 h. Then Hydroxypropyl trimethylammonium chloride solution (HPTAC) (19.46 g, 60 wt%) was added into the suspension. After stirring at 40 °C for 6 h, the reaction was stopped.[3] The mixture was filtered and the solid was dissovled and dialyzed with deionized water until neutral. Then the dialyzed solution was centrifuged to remove insoluble part followed by freeze dried to get QC. The degree of substitution (DS) of QC was determined by titrating the amount of chloride ions with $AgNO_3$ solution and calculated as follows[4]:

$$DS = V \times c / [Vc + (W - Vc \times 354.5) / 203] \quad (1)$$

where V (mL) is the volume of $AgNO_3$ solution, c (mol/mL) is the concentration of $AgNO_3$ solution, and W (g) is the weight of QC. DS of QC was 0.41.

REC (4 g) was dispersed in water with stirrer for 30 min and then left standing for 24 h. Cetyltrimethyl Ammonium Bromide (CTAB) (2 g) was dissolved in water, and then dropped slowly into the REC suspension at 90 °C under stirring. After stirring for 5 h, the product was washed with deionized water and filtered to ensure the complete removal of bromide ions, which were detected with $AgNO_3$ until no AgBr precipitate was found. The product was freezw dried to obtain organinc rectorite (OREC).[5]

The interlayer distances of REC, OREC, and QCRs were calculated by Bragg equation:

$$d = \lambda / 2\sin\vartheta \quad (2)$$

where d is the interlayer distance of the sample, λ is the wave length of X-ray, ϑ is the diffraction angle of the sample.

LB broth (0.5 g beef extract, 1 g peptone, 0.5 g NaCl, 100 mL water) and LB agar (0.5 g beef extract, 1 g peptone, 1.5 g agar, 0.5 g NaCl, 100 mL water) were autoclaved at 121 °C for 20 min before using. A single bacterial colony was picked and inoculated into 5 mL LB broth, then incubated at 37 °C with shaking at 150 rpm for 10 h. The inoculated bacteria suspension was diluted by sterile distilled water to 10^6 CFU/mL and 100 μL bacteria suspension was added to culture tube containing 10 mL QC or QCRs solution at concentrations ranging from 1 to 100 μg/mL. The mixtures were incubated at 37 °C with shaking at 150 rpm for 10 h and then the 100 μL mixtures were coated on LB agar and incubated at 37 °C for 24 h, and the resulting bacterial colonies were counted. Bacteria suspensions with sterile saline were used as negative control. The minimum inhibitory concentration (MIC) is defined as less than 5 bacterial colonies were visible.

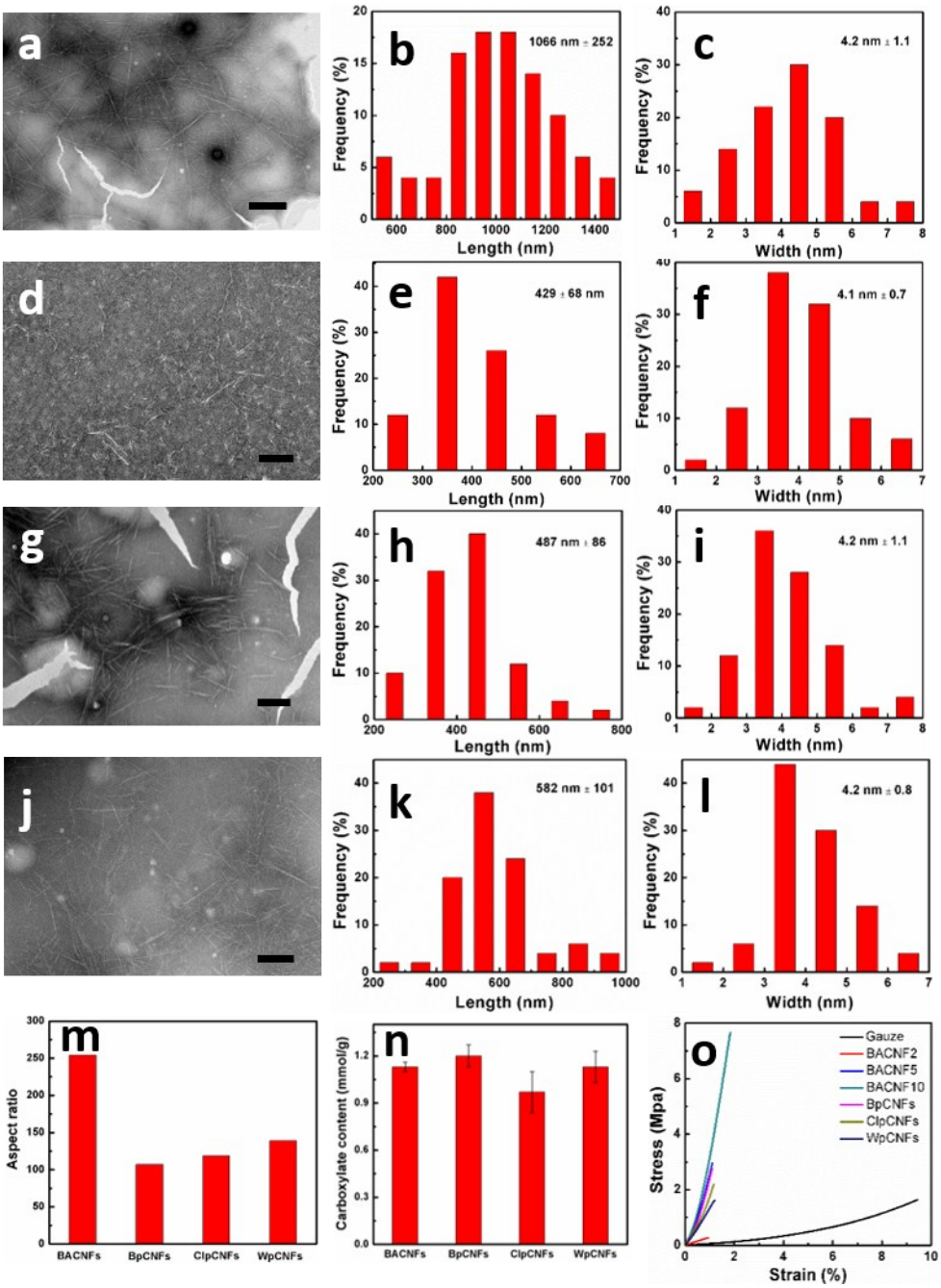

Fig. S1 TEM images, length and width distributions of BACNFs (a-c), BpCNFs (d-f), ClpCNFs (g-i) and WpCNFs (j-l), the scale bars were 500 nm; aspect ratios (m) and carboxylate contents (n) of different CNFs; tensile stress-strain curves of gauze and CNFs sponges (o).

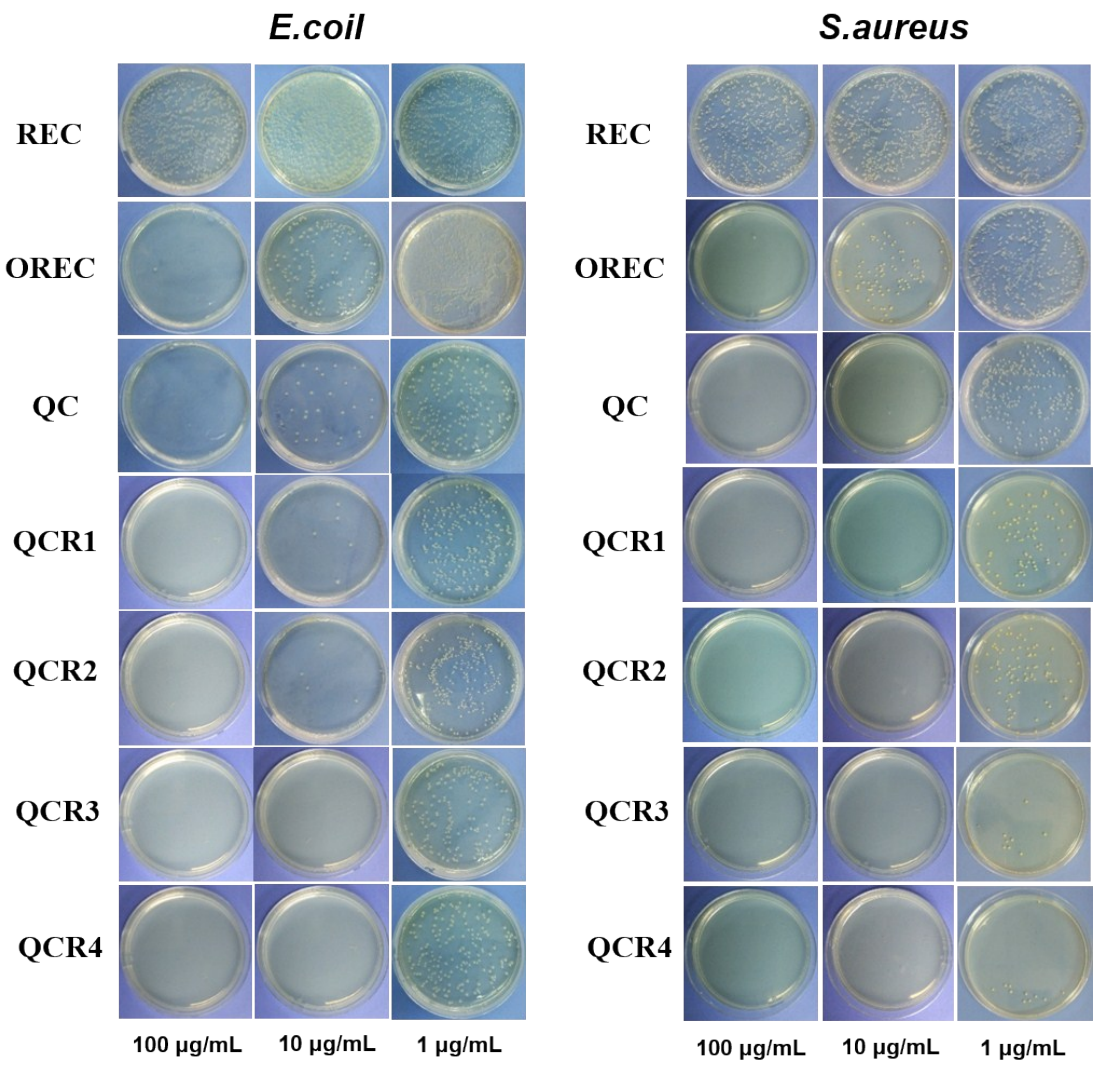

Fig. S2 Antimicrobial activities of different concentrations of REC, OREC, QC and QCRs against *E.coil* and *S. aureus*. The experiments repeated three times.

Table S1 Experimental results of diffraction angles, interlayer distances and the minimum inhibitory concentrations (MICs) of REC, OREC, QC and QCRs.

Sample	Mass ratio of OREC:QC	θ (°)	d (nm)	MIC (μg/mL)	
				E. coli	*S. aureus*
REC	-	1.8	2.45	-	-
OREC	-	1.35	3.27	200	100
QC	-	-	-	20	10
QCR1	1:1	1.05	4.2	10	5
QCR2	1:2	1.06	4.16	10	5
QCR3	1:5	0.9	4.9	5	2
QCR4	1:10	0.94	4.69	5	2

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