Electronic Supplementary Material (ESI) for Materials Horizons
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Supplementary Information

Bioinspired stiff and flexible composites of nanocellulose-reinforced amorphous $CaCO_3$

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This Supplementary Information contains experimental details and 7 figures (Figs. S1 to S7).

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Experimental

Preparation of ACC/PAA Precursor Suspension. A colloidal suspension of the ACC/PAA precursor was prepared according to a method previously reported (Oaki *et al.*, *Adv. Mater.*, 2008, *20*, 1). To a stock solution of 0.1 M PAA ($M_w = 2 \times 10^3$; Aldrich, St Louis, MO, USA), anhydrous $CaCl_2$ (Wako Pure Chemicals, Osaka, Japan) was added to be 0.1 M and completely dissolved. An equal volume of 0.1 M Na_2CO_3 (Kanto Chemical, Tokyo, Japan) was added to the solution of PAA and $CaCl_2$. A sealed bottle of the mixed solution was allowed to stand at 25 °C for 1 h. The resulting precipitate, or ACC/PAA precursor, was separated and rinsed once with distilled water by centrifugation at 16000 *g*. The paste-like ACC precursor, collected by centrifugation, contained 5% water, 93% $CaCO_3$, and 2% PAA. Distilled water was again added to prepare a colloidal suspension of the ACC precursor containing 1.7% w/v $CaCO_3$. The surface-charge content of the colloidal ACC/PAA precursors is unmeasurable because these precursors have no distinct grain boundaries (Oaki et al. *Adv. Mater.*, 2008, *20*, 3633).

Preparation of CACell. The hydrogels of bacterium-produced cellulose, a biosynthetic product of *Acetobacter xylinum* strains, have submicron-scale network structures consisting of cellulose nanofibrils with rectangular cross-sections of 10 nm × 50−100 nm and contain water up to 99% w/v (Iguchi *et al.*, *J. Mater. Sci.*, 2000, *35*, 261). Cellulose samples for the preparation of CACell were produced by *A. xylinum* JCM10150. A gel-like cellulose pellicle was obtained from a culture standing at 28 °C in a mixture of 4% sucrose and 4% corn-steep liquor for 3–5 d. The pellicle was purified with 4% NaOH at room temperature for 1 d and then with 0.3% $NaClO_2$ at 60 °C and pH 5 for 1 h. The purified pellicle was finally rinsed with distilled water, and stored at 4 °C in wet state. The surface oxidation of the cellulose was carried out according to a previously reported method (Isogai *et al.*, *Nanoscale*, 2011, *3*, 71). The purified cellulose pellicle (0.5 g dry weight) was suspended in 0.1 M sodium phosphate buffer (90 mL, pH 6.8) with dissolved TEMPO (0.016 g) and

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$NaClO_2$ (1.13 g, 80%). A 2 M NaClO solution (0.5 mL) was then added in one step, and the reaction flask was immediately stoppered. The flask was heated up to 40 °C and gently shaken. After 6 h, the oxidized cellulose pellicle was removed and rinsed with distilled water. The pellicle was then soaked in 0.01 M HCl (100 mL) at room temperature for 1 h and again rinsed with distilled water. The carboxylate content of the oxidized cellulose or CACell was approximately 0.4 mmol per gram of CACell as determined by electrical conductivity titration method. In order to exchange the proton carboxyl counter-ions for calcium ions (see Supplemental Figure S7), the CACell pellicle was then shaken in 0.1 M $CaCl_2$ (100 mL) for 3 h and rinsed with distilled water. All the chemicals used for the preparation of CACell were of laboratory grade (Wako Pure Chemicals, Osaka, Japan).

Fabrication of CACell/ACC/PAA Composites. The CACell pellicle was cut into pieces of thickness about 3 cm × 3 cm × 0.5 cm and dewatered by compression with filter papers. The mat-like squeezed CACell was soaked in the 2.5% colloidal suspension of ACC/PAA precursors (20 mL) and shaken at 100 rpm with an orbital shaker for 4 h to swell the squeezed mat with the colloidal suspension. The white swollen CACell mat was then briefly dipped in distilled water to rinse off the ACC/PAA precursors deposited on the surfaces of the swollen mat, and dried on a flat acrylonitrile–butadiene–styrene plate for 1 d at 23 °C and 50% relative humidity. The film-like CACell/ACC/PAA composite (thickness: 10–15 μm) was spontaneously formed by drying, and stored under ambient conditions.

Analyses. SEM observations of the film cross-sections were carried out with a Hitachi S-4000 (Hitachi, Tokyo, Japan) field emission microscope at 5 kV. The SEM samples were pre-coated with osmium, using a Meiwafosis Neo Osmium Coater (Meiwafosis Co., Ltd., Tokyo, Japan) at 10 mA for 5 s. The osmium-coated layers are estimated to be about 2.5 nm thick under the set conditions. Tensile tests were performed using a Shimadzu EZ-TEST instrument (Shimadzu, Kyoto, Japan)

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equipped with a 500 N load cell. Rectangular strips of width 3 mm and length 20 mm were cut from the samples and tested at a rate of 1.0 mm/min and a span length of 10 mm. Indentation hardness tests were performed using a Shimadzu DUH-201 in a load-controlled mode. A Berkovich-shaped tip was used to indent the film surface, and the peak load was set to 1.0 mN. The load and displacement data were analysed according to the method of Oliver and Pharr (*J. Mater. Res.*, 1992, *7*, 1564). As a reference, strips cut from carapaces of *M. japonicus* were air-dried and subjected to the mechanical tests. FTIR spectroscopy was performed using a JASCO FT/IR-660 Plus (JASCO, Tokyo, Japan). Wide-angle XRD measurements were made using a Rigaku RINT 2000 (Rigaku, Tokyo, Japan) with monochromator-filtered Cu-Kα radiation (λ = 0.1548 nm) at 40 kV and 40 mA. TG analyses were carried out using a Rigaku Thermoplus TG-8120 with a nitrogen flow of 100 mL/min and a heating rate of 4 °C/min. The sample compositions were estimated from the TG curves. $CaCO_3$ decomposes to CO_2 and CaO at around 600 °C, and the released CO_2 corresponds to the rapid weight loss at around 600 °C on the TG curves; this was used for calculation of the $CaCO_3$ ratios in the composites. The moisture content of the sample was taken as the weight loss at 150 °C. The remainder was assigned to the organic components.

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Supplemental figures

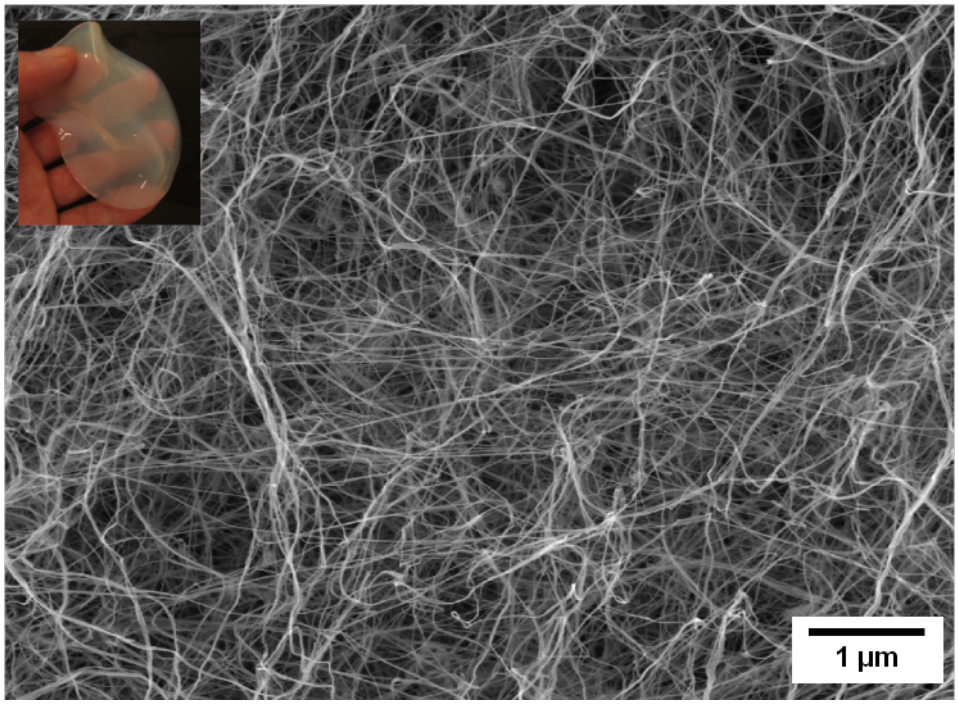

Figure S1. SEM image of the CACell network. The inset shows the CACell hydrogel.

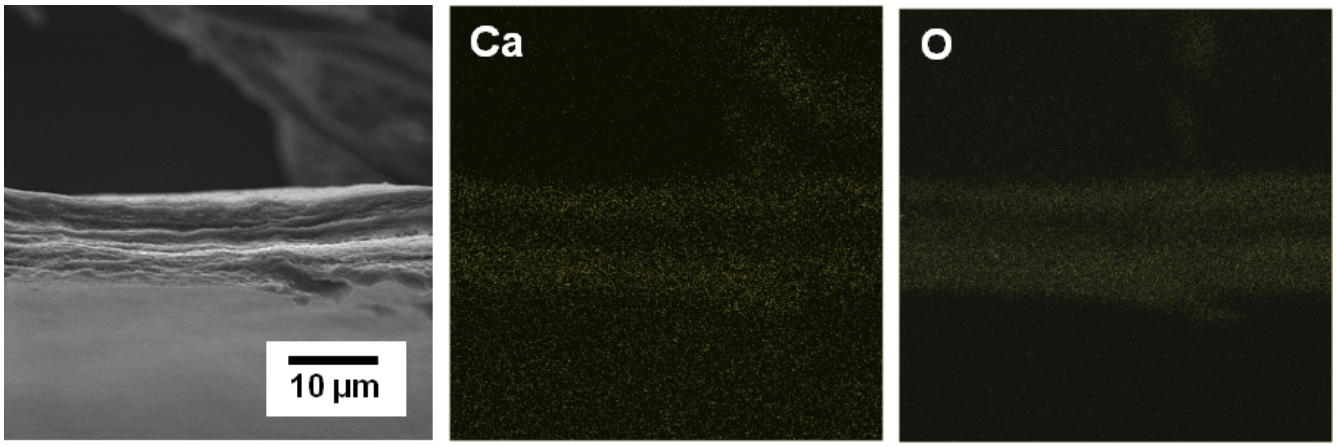

Figure S2. SEM-EDX analysis of the CACell/ACC/PAA composite. Elemental mapping of calcium (from $CaCO_3$) and oxygen (from all the components) on the composite cross-section.

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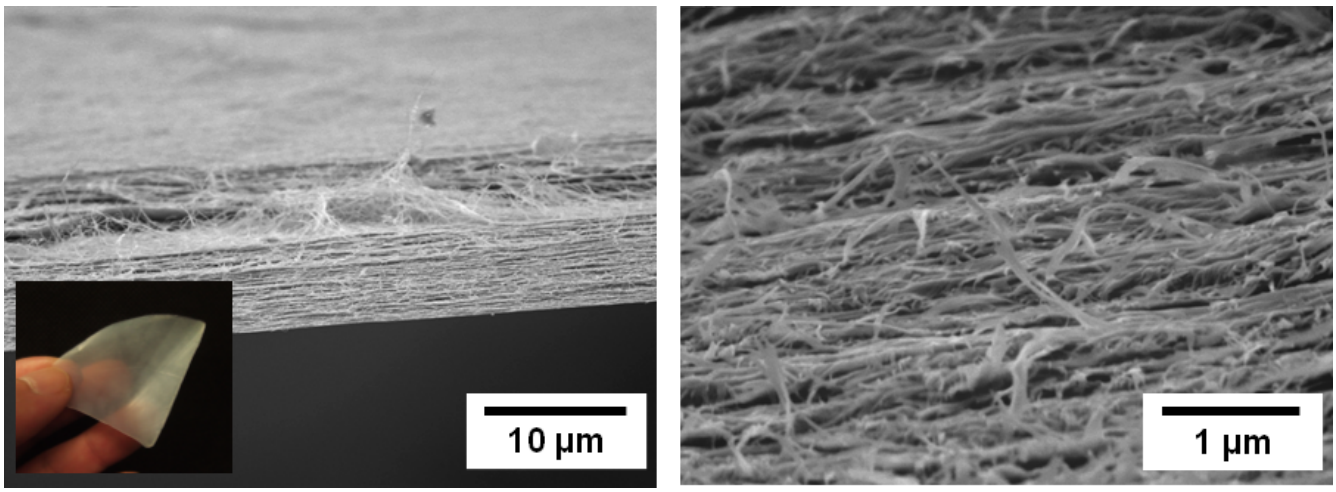

Figure S3. Cross-sectional SEM images of neat CACell films. The films are obtained by drying the CACell hydrogels as shown in Figure S1. The inset shows opacity of the films.

Figure S4. A fresh and wet carapace of *Marsupenaeus japonicus*.

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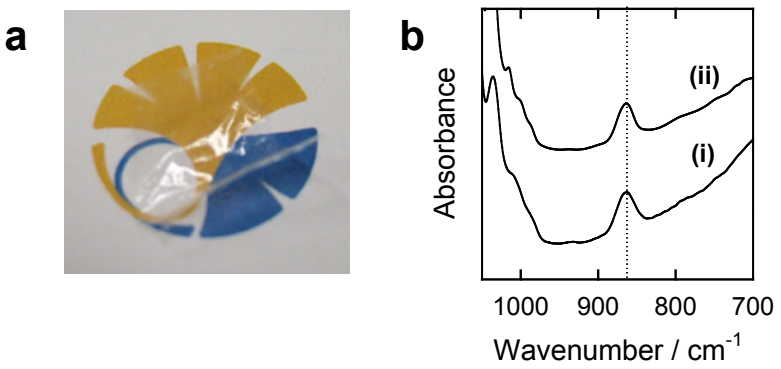

Figure S5. a) The CACell/ACC/PAA composite sample prepared one year ago. b) FTIR spectra of an as-prepared sample (i) and the sample one year later (ii). The broad peak at 866 cm^{-1} is due to ACC.

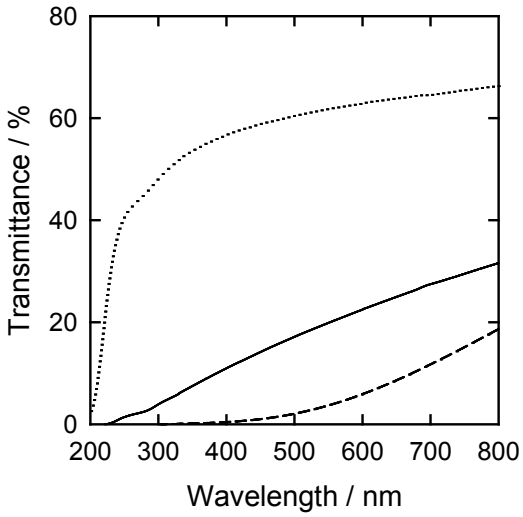

Figure S6. UV-Vis transmittance spectra of the films of noncarboxylated cellulose/ACC/PAA composite (solid line), CACell/ACC/PAA composite (dotted line), and neat CACell (dashed line).

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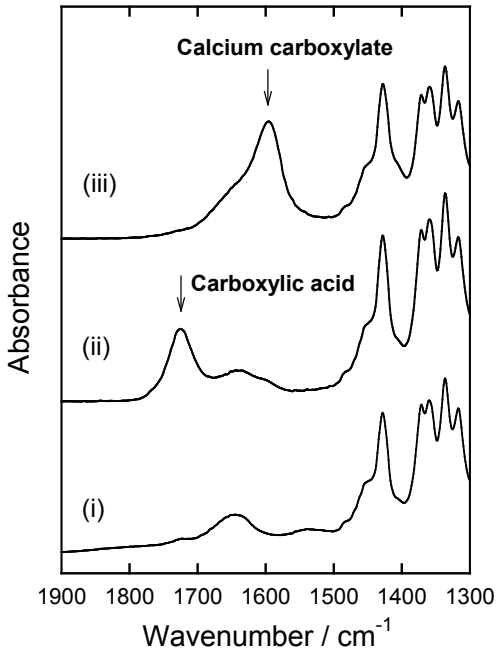

Figure S7. FTIR spectra of the original (i), oxidized (ii), and ion-exchanged (iii) celluloses. Carboxyl groups prepared on the cellulose (1725 cm^{-1}) were ion-exchanged, resulting in forming to calcium carboxylates (1595 cm^{-1}) before making the composites with ACC/PAA.