

Chiral Templating of Alumina Nanofilms by Atomic Layer Deposition Process

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1. ALD process and preparation of alumina nanofilms

The general strategy for the preparation of chiral metal-oxide nanofilms onto chiral fibrous biomolecules (Fig. S1) can be divided into three main stages. In the first step, chiral fibrous biomolecules, cellulose microfibrils, are assembled onto a glass substrate. At the second step, ALD process is performed to deposit nanofilms of alumina. Finally, chemical extraction method is used to remove the biological microfibrils from the alumina nanofilms.

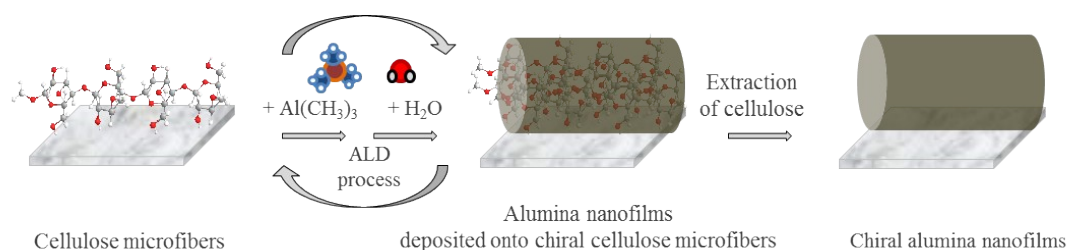


Fig. S1 The overall strategy for the preparation of alumina nanofilms.

The templating chiral fibrous biomolecule was commercial cellulose microfibrils manufactured from 24X20 mesh sterile bandage pad, simply attached to 10X10 cm glass slide using carbon tape at the edges. The bounded cellulose microfibrils were inserted into the ALD system (Cambridge nanotech Fiji F200). The ALD chamber was preheated to 140 °C, while trimethylaluminum (TMA) and H_2O precursors were unheated. The nanofilms were grown under 1×10^{-2} Torr of argon flow. To ensure complete removal of residual reactants and byproducts in each step, a long purge time was used between each precursor pulse. The ALD cycle consisted of 0.06 seconds exposure to the TMA precursor followed by 20 seconds argon purge, and then 0.06 seconds exposure to water followed by 30 seconds of argon purge. The alumina nanofilm with a thickness of 50 ± 3 nm resulted from 600 cycles that have taken about 9 hours.

In order to remove the cellulose microfibrils two methods were used. The first method was calcination at elevated temperature (700 °C) over 4 hours under oxygen environment. The second method was chemical extraction under basic conditions, using a mixture solution of 7 gr of NaOH, 12 gr of urea and 81 ml of water (7:12:81 ratios by weight) that was precooled to -12 °C. Next, the alumina/cellulose composite microfibrils were stirred for 60 minutes, and then washed with water to neutralize.

2. Characterizations of the alumina nanofilms

The morphology and structure of the alumina nanofilms were investigated by transmission electron microscopy (TEM JEOL jem-1400, LaB6, 120 kV) and high-resolution scanning electron microscope (a field-emission, FEI, Helios 600 HR-SEM). The TEM energy-dispersive X-ray analysis (EDAX) was taken with an FEI (model Inspect S) and the HR-SEM EDAX analysis was an 80 mm 2 X-max (Oxford Instruments).

The TEM samples were prepared by dripping a drop of aqueous solution contains nanofilms on a 400 mesh carbon-coated copper grid. The SEM samples were prepared on a carbon tape bounded to stab, and were sputtered with 12 nm layer of Au to overcome charge effects. The EDAX analyses of the alumina nanofilms before and after extraction of cellulose microfibrils are shown in Fig. S2. Fig. S2a demonstrated around 14 At% of carbon, 60 At% oxygen, and 26 At% aluminum in the case of the alumina/cellulose composite. In the case of the nanofilms obtained after extraction of the cellulose, the EDAX analysis revealed a very low At% of carbon, attributed to the carbon tape. After extraction under basic conditions, the ratio between the oxygen and aluminum at the surface was 39 At% oxygen, and 55.5 At% aluminum (Fig. S2b), therefore confirmed that the deposited aluminum-oxide nanofilms is alumina (Al_2O_3). The small amount of sodium is attributed to the basic conditions used for extraction of the cellulose microfibrils. The ratio between the oxygen and aluminum after annealing was 45 At% oxygen, and 52.4 At% aluminum (Fig. S2c), demonstrating an intermediate aluminum-oxide phase of AlO_x .

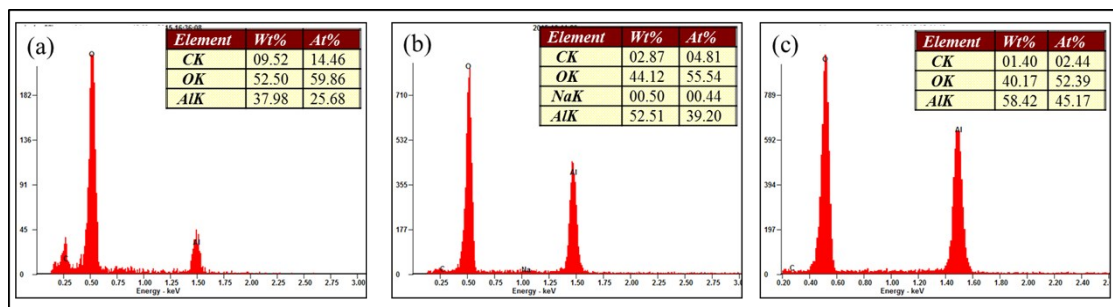


Fig S2 EDAX spectra and elemental analysis of; (a) 50 nm alumina nanofilms deposited onto the cellulose microfibrils; (b) 50 nm alumina nanofilms after cellulose chemical extraction; and (c) after annealing at 700 °C.

The chemical composition of the alumina nanofilms was further studied by EDAX elemental mapping, and proved a total extraction of the cellulose microfibrils. As clearly shown in TEM (Fig. S3a) and SEM (Fig. S3b) mapping, aluminum and oxygen were the only components on the surface of the nanofilms. The carbon identified in the SEM mapping was attributed only to the carbon tape.

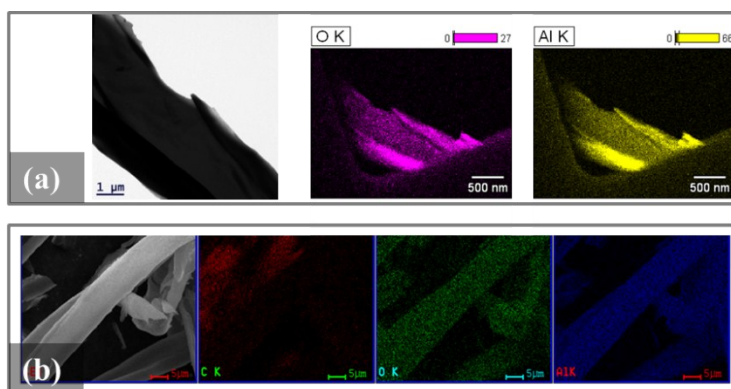


Fig. S3 EDAX elemental mapping 50 nm alumina nanofilms after cellulose extraction.

To support all of the above results, the structure of the fibrous alumina nanofilms obtained after calcination (at 700 °C for 4 hours under oxygen environment) was studied (Fig. S4). Furthermore, the X-ray diffraction (XRD) was used to study the crystallinity of the nanofilms and how they are affected by high temperatures (Fig. S5). The main peaks appear at $2\theta = 16^\circ$, 23° and 34° , corresponding to (1-10), (200), and (004) planes of cellulose, respectively. After calcination at 300 °C, the XRD peaks show only the (1-10) and (200) peaks of cellulose, indicating partial cellulose carbonization. After calcination at 700 °C, no XRD peaks are found, demonstrating total carbonization, as well as the amorphous state of the alumina.

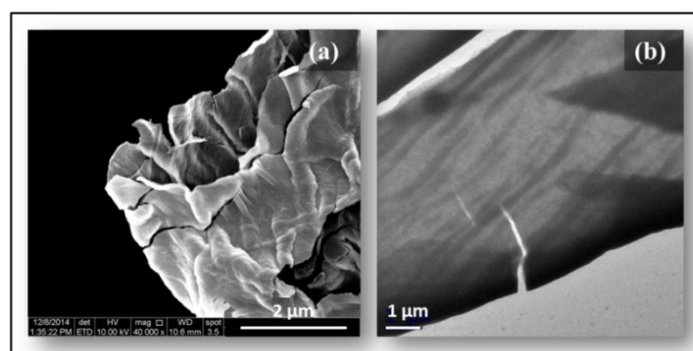


Fig. S4: (a) SEM and (b) TEM images of the alumina nanofilms obtained after calcination at 700 °C.

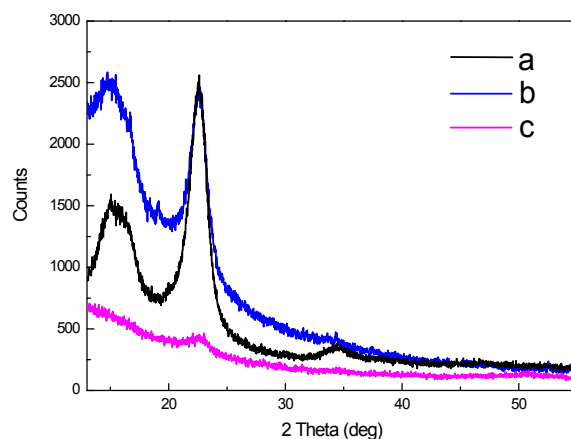


Fig. S5 XRD of (a) unheated alumina/cellulose nanofilms; (b) alumina/cellulose nanofilms after calcination at 300 °C and (c) alumina/cellulose nanofilms after calcination at 700 °C.

3. Chiral study of the alumina nanofilms

3.1. Circular dichroism (CD) spectroscopy

CD spectra were recorded on a Chirascan CD spectrometer (Applied Photophysics) using a bandwidth of 1 nm, from 200 nm to 250 nm. The step size and time were set at 1 nm and 0.5 seconds, respectively. In the chiral adsorption experiments, we have performed selective chiral adsorption measurements of D- and L-phenylalanine (Phe). 3 mg/mL alumina nanofilms were added into 5 mM aqueous solution of D- or L- Phe (41.3 mg in 50 ml water), and the optical activity of each solution was probed after 48 hours of equilibrium. The enantiomeric excess (e.e.%) is calculated as the ratio between the reduction and the addition of L-Phe (36%) and the D-Phe (22%) that adsorbed onto the alumina nanofilms. The calculation of the e.e.% is $\left\{ \frac{(36 - 22)}{(36 + 22)} * 100 \right\}$ to give about 24% of e.e.% for the L-enantiomer of Phe.

3.2. High performance liquid chromatography (HPLC) resolution

The HPLC analysis was carried out by Spectra System HPLC equipped with UV/vis detector (Thermo Scientific, USA) and attached with a reversephase C18 column (75mm×4.6mm, Phenomenex, USA). Astec CHIROBIOTIC™ TAG (Teicoplanin Aglycone) was used as the chiral column for the chiral HPLC resolution. The mobile phase was mixture of acetonitrile:water (20:80 ratio by volume). In the chiral resolution experiments, 3 mg/mL alumina nanofilms were immersed in 25 mM aqueous solution of DL- Phe (4.125 mg/ml) for 48 hours. Next, the solution was injected into the chiral HPLC column using the mobile phase. The resulted chiral resolution of the racemic solution demonstrated enantioselectivity of the alumina nanofilms (Fig. S6). The chiral resolution confirmed enantiomeric excess of 38.08 % for the L- enantiomer of Phe. As control, the bare racemic solution was injected and proved 1:1 peaks ratio after retention time of 7 and 11 minutes, attributed to the L- and D-enantiomers, respectively.

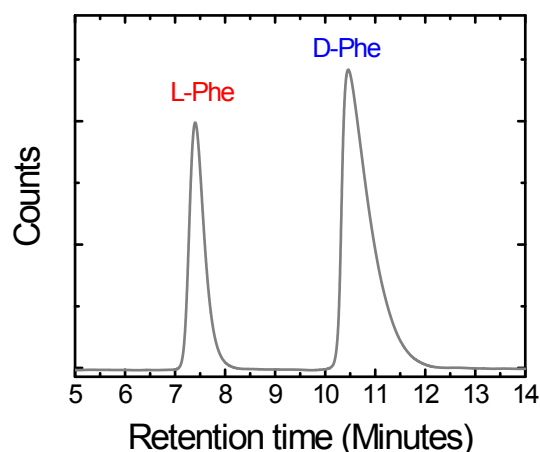


Fig. S6: Chiral HPLC resolution of DL Phe selectively adsorbed onto 50nm alumina nanofilms

3.3. Quartz crystal microbalance (QCM) measurements

5mHz quartz crystal sensor coated by Au was used as sensing substrate in the QCM measurements (QCM-D from Biolin Scientific). The sensitivity factor used for calculations taken the *Saubrey equation* was 56.6 Hz*cm²/ug. Prior to use, the Au crystal was immersed in 10 mM ethanoic solution (2.18 mg/ml) of 11-Mercaptoundecanoic acid over 24 hours in order to form self-assembled monolayer at the crystal surface. After washing with ethanol, the alumina nanofilms were attached onto the assembled surface, and then dried under a soft nitrogen stream. For the QMC adsorption/desorption measurements, the samples were immersed in 20 mM aqueous solutions of the D- or L-enantiomers. The adsorption/desorption profile of L- and D-Phe is shown in Fig. S7.

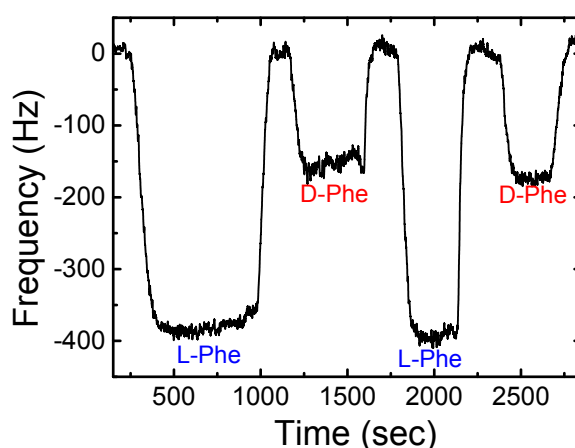


Fig. S7: QCM adsorption/desorption profile of L- and D- phenylalanine and onto 50nm Alumina nanofilms.

3.4. Cyclic voltammetry (CV):

Electrochemical experiments were performed using a Ecochemie Autolab 30 Potentiostat in a standard three-electrode setup, with alumina nanofilms on 200 nm thick Au layer as the working electrode, Pt wire as the counter electrode and Ag/AgCl reference electrode. The CV experiment was performed in 20 mM (60 mg in 20

mL) aqueous solutions of L- and D-tartaric acid, and 0.2 M aqueous solution (568 mg in 20 mL) of Na₂SO₄ as a supporting electrolyte, and at a scan rate of 10 mV/s, and in the voltage range of -0.1V to 0.1V.

4. Materials

Sodium sulfate was purchased from *chem-impex* international, sodium hydroxide (NaOH) was purchased from *Frutarom*, and ethanol was purchased from *Bio-lab*. All of the other chemicals were purchased from *Sigma Aldrich* (DL- phenylalanine, D- phenylalanine, L- phenylalanine, D- tartaric acid, L- tartaric acid, Na₂SO₄, 11-Mercaptoundecanoic Acid (MAU). All of the chemicals were used as-received without further purification.