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Supplementary Info

Design of anticoagulant surfaces based on cellulose nanocrystals

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Materials and Methods

Cellulose nanocrystals (CNC) are prepared from microcrystalline cellulose (MCC, Sigma Aldrich Sigmacell Type 20). In a typical procedure, 10 ml of sulfuric acid (2.0 M; Carl Roth 98 %) are added to 1.0 g MCC. The mixture was stirred at 45 °C for 90 min, followed by subsequent dilution with 100 ml cold water (~4°C) to stop the hydrolysis. The suspension is then subjected to centrifugation (10 min at 11000 rpm). The precipitate is redispersed in doubly distilled water and centrifuged again. This procedure is repeated until the pH of the suspensions reaches a value higher than 4. Purification is done by dialysis against MilliQ-water for one week, whereby the water is exchanged every 24 hours. The dialysis was performed using regenerated cellulose membranes (Carl Roth) with a molecular weight cut-off of 8000 – 10000 Da. After dialysis, the suspensions were freeze dried. For redispersion, the CNC in water the suspension was sonicated using a Vibra-Cell VCX 750 (Sonicator with a high-intensity Ultrasonic Processor) ultrasonication dip for 10 min using 0.5 s pulses with 1.5 s intervals at a fixed amplitude of 30 %.

All QCM-D experiments were performed on a Q-Sense E4 (Q-Sense, Gothenborg, Sweden). The adsorption studies were performed at 21.0±0.1°C. The SiO_2 coated quartz crystals (LOT-Oriel, Germany) used for the measurements were characterized by their Eigen-frequency and dissipation prior to measurements. Firstly, the crystals were equilibrated in water for 15 minutes by rinsing the chambers with a flow rate of 0.1 mL/min at 21 °C. Before the analytes were injected, the frequency and dissipation values were set to zero. All experiments were repeated two times, whereby always at minimum 4 chambers were used to study the adsorption behavior. The viscoelastic Voigt model was applied for calculating the adsorbed mass (Γ_{QCM}), film thickness (h_{f}),viscosity (η_{f}) and elastic shear modulus (μ_{f}) of the PEI and CNC coated layer. In this model, the adsorbed layer was treated as a viscoelastic layer between the quartz crystal and a semi-infinite Newtonian liquid layer. For data evaluation or fitting the different overtones (n = 3, 5, 7, 9 and 11) were used. All calculations were carried out using the software package QTools 3.0.12 (Q-Sense). The fitting parameters used for the modelling are: viscosity, from 1×10^{-3} to 1×10^{-2} N·s·m^{-2};elastic shear modulus, from 1×10^{4} to 1×10^{9} N·m^{-2}; and thickness, from 1×10^{-10} to 1×10^{-6} m. It is important to note that the values of h_f and ρ_f were not independent variables. In order to calculate the effective thickness and adsorbed mass (equation 3), the density ρ_f values was varied between 1000 and 1200 kg m^{-3}. It turned out that a mass change was not observed by changing the density value and therefore the density (ρ_f) of 1025 kg m^{-3} was used for all calculations (eq. 1).

$$\Gamma_{QCM} = h_f \rho_f \quad (1)$$

In the case of the anticoagulative investigations, the SiO_2-crystals were coated *ex-situ* via layer-by layer solution deposition. For this purpose, the surfaces were covered completely with 200 µL PEI for 10 min (Polyethyleneimine, SigmaAldrich high molecular weight and branched, 20 mM in 0.5 M KCl) and immersed into MilliQ water for another 10 min to desorb loosely bound species. Afterwards the crystals were dried gently under a steady nitrogen stream, followed by adsorption of the CNC suspensions (again 200 µL; 0.1 wt%) for 10 min and subsequent immersion into fresh MilliQ-water. The so-coated crystals were dried overnight in a Lami-flow bench with continuous sterile air flow. The determination of anticoagulant properties and clot formation was performed according to literature in an open QCM-D cell made from PTFE which was designed and manufactured in the institute's workshop. Surface modified QCM sensors were mounted in the open measurement cell, and equilibrated to 37.0±0.1°C. Then, citrated normal blood plasma (ORKE 41, 100 □l) was equilibrated to 37.0 °C and placed onto the crystal. After ca. 2 min coagulation was triggered by addition of 100 □l 0.025 M $CaCl_2$ (equilibrated to 37.0 °C). All experiments were performed on three sensors. The frequency and dissipation change as a function of time provides information about the onset time, fibrin deposition rate and total coagulation time. A more detailed description of the test set-up can be found elsewhere. It has to be noted that the formation of bubbles, which occur due to the mixing procedure, must be strictly avoided.

The atomic force microscopy images were obtained using a Solver Pro-M Scanning Probe Microscope (NT-MDT) with rectangular silicon cantilever at room temperature (23 °C), in tapping mode (6µm/s). The data analysis was performed using the freeware Gwyddion 2.26.

The ζ-potential measurements of the layer-by-layer deposited films on glass slides (PEI and CNC on PEI, respectively) were realized using a streaming potential analyser (EKA, A. Paar KG Electrokinetic Analyzer, Graz; Austria) with a titrator (Particle charge detector (ASG, Mütek)). The base line was measured using a 1 mM KCl solution. The pH was adjusted to ~ 9 using 0.1 M NaOH solution. The stepwise titration to a final pH value of ~ 3 was achieved using 0.1 M HCl. These chemicals were purchased from Sigma Aldrich (purity > 98 %) and dissolved/diluted with MilliQ-water. The ζ-potential measurements of the dispersed particles in solution (Figure S3) were performed using the Brookhaven ZetaPALS from Brookhaven Inc in dependence of the pH value.

The sulfur content was determined using inductively coupled plasma mass spectrometry (ICPMS). Aliquots (~50 mg) of the different dried nanocrystalline samples were weighed to 0.1 mg into 12 mL quartz digestion vessels and 5 mL of HNO_3 were added. The samples were heated in the UltraClave IV (EMLS, Leutkirch, Germany) using the following program: Step 1: 5 minutes => 80 °C; step 2: 15 minutes => 150 °C; step 3: 15 minutes => 250 °C; step 4: 30 minutes at 250 °C. For the S-determination an Agilent 7500ce at m/z 34 system was used. The instrument was tuned to give $9.0 \cdot 10^5$ cps at m/z 7 for a 1 µg Li/L, $10 \cdot 10^5$ cps at m/z 89 for a 1 µg Y/L solution, and $7.5 \cdot 10^5$ cps at m/z 205 for a 1 µg Tl/L solution. The oxide ratio 156 CeO / 140

Ce was less than 0.013. As internal standard Be at m/z 9 was used. Data acquisition and evaluation were performed with ICPMS Masshunter B.01.01 (Build 123.10Patch 3) software. The sulfur content was determined to be 5.7 g sulfur in 1.0 kg CNC.

Table S1. Viscoeleastic properties of PEI and CNC coated layers.

Sample	Γ_{QCM} (mg m^{-2})	h_f (nm)	$\eta_f \times 10^{-3}$ (N s m^{-2})	$\mu_f \times 10^6$ (N m^{-2})
PEI	3.1 ± 0.3	3.0 ± 0.4	7.4 ± 0.4	18.6 ± 2.3
CNC	30.4 ± 2.2	30.4 ± 2.1	4.3 ± 0.01	3.8 ± 1.5

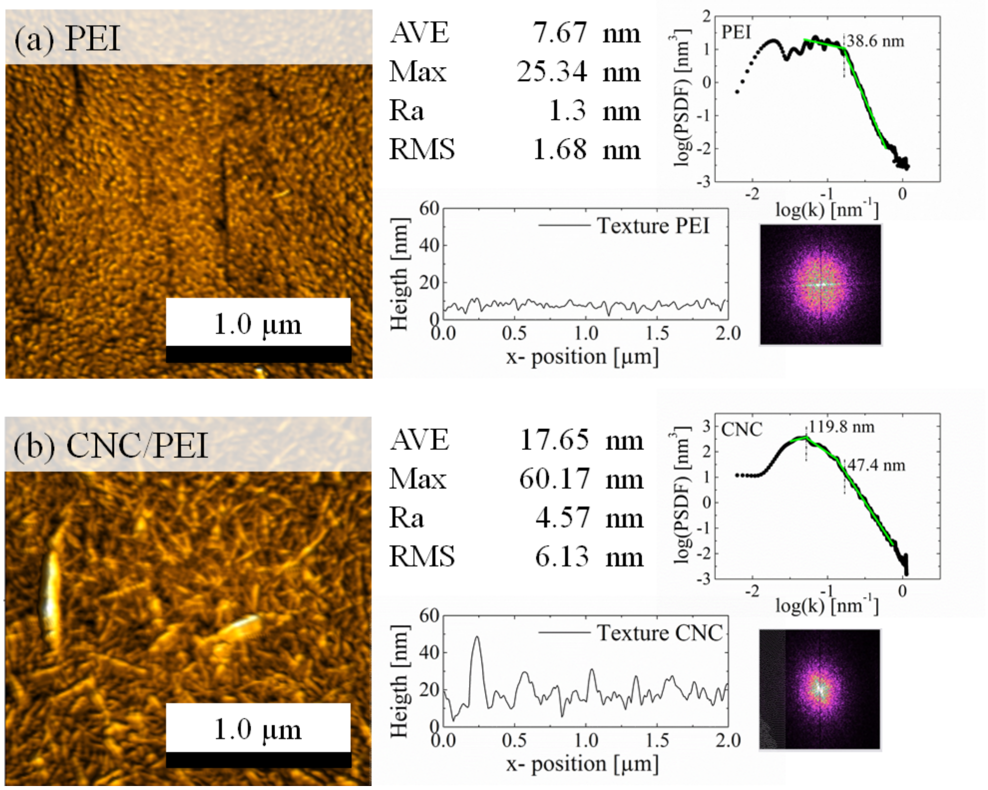

Figure S1. AFM topographies of PEI (a) and CNC (b) after *in situ* adsorption in the QCM (left images); ; texture (middle, bottom) and roughness parameters (middle, top); power spectral density function (PSDF) and double logarithmic plot (right) reveal the most dominant length scale

AVE…average height; Max…maximum height; Ra…average roughness; RMS…root mean square roughness)

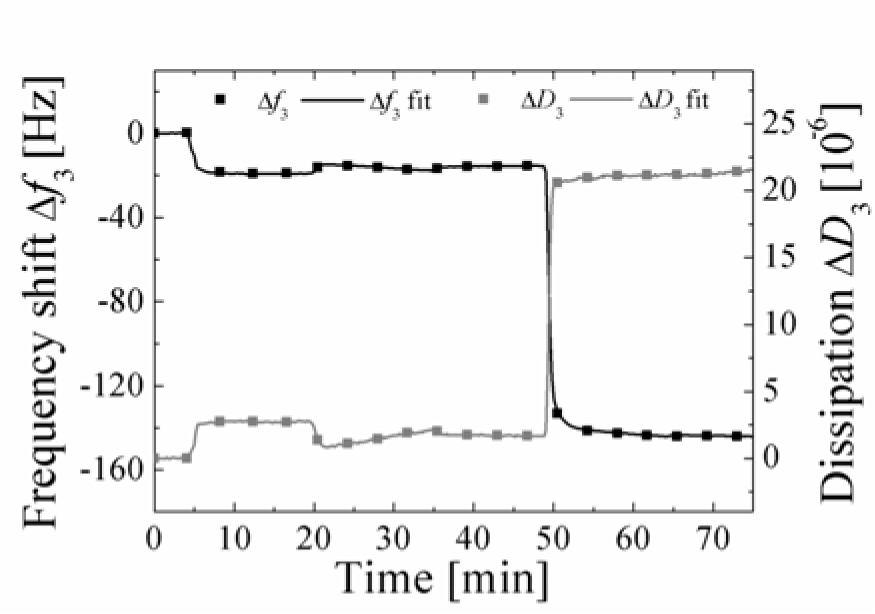

Figure S2 QCM.D adsorption experiment; experimental data (rectangles) and Voigt model fits (full lines) of the frequency (black) and dissipation (grey)

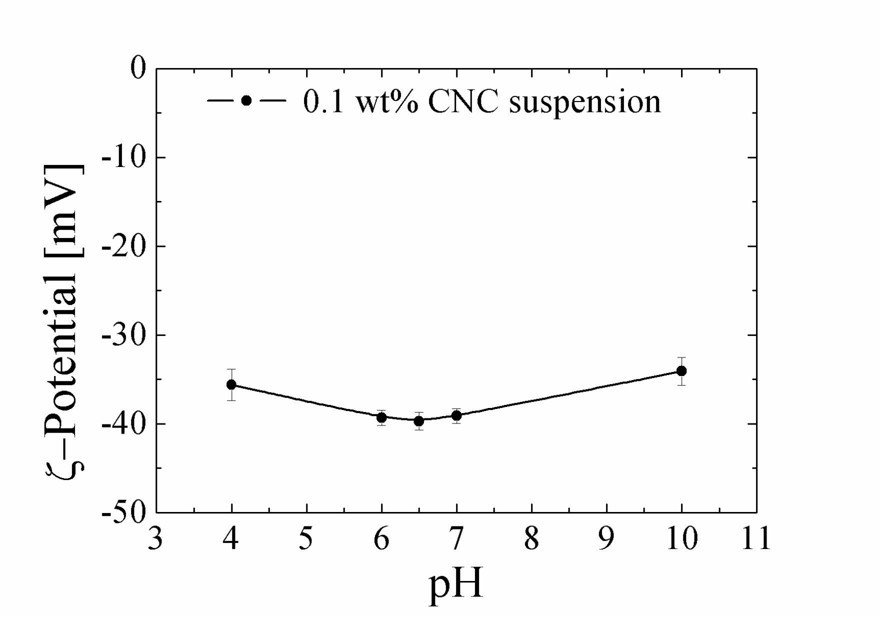

Figure S3. Zeta potential as a function of the pH of 0.1 wt% CNC suspension in water