Supplementary Material (ESI) for Soft Matter

Supplemental Information

In situ quantitative visualization and characterization of chitosan electrodeposition with paired sidewall electrodes

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Neutralization process illustration

Figure S1 is a schematic demonstration of the evolutionary neutralization process which is responsible for the gelation of the chitosan hydrogel on the cathode during electrodeposition.

At the beginning, electrolysis of water leads to the generation of hydroxide ions (OH^-) at the cathode surface. Figure S1a shows initially a basic environment (purple) is created at the surface of cathode (yellow) due to the electrochemical reduction reaction described as:

$$2H_2O + 2e^- \rightarrow H_2 + 2OH^- \quad (1)$$

Consequently, the neutralization of chitosan is caused by the electrochemically generated OH^- ions localized at cathode and their subsequent migration outward from the cathode. Figure S1b shows the expanding of the basic environment area (purple) in chitosan polyelectrolyte (pink) due to the migration of a large number of OH^- ions generated at cathode. The neutralization reaction can be described as:

$$Chit\text{-}NH_3^+ + OH^- \rightarrow Chit\text{-}NH_2 + H_2O \quad (2)$$

Due to the constant consumption of OH^- ions at the neutralization front by protonated chitosan molecules, a sharp pH gradient is created and maintained at the interface of hydrogel and bulk polyelectrolyte solution indicating chitosan solution acts as a strong pH buffer. Figure S1c illustrates the location of the neutralization front (white dotted line) and different neutralization levels within deposited hydrogel. Such a pH gradient defines the boundary of the electrodeposited hydrogel and its propagation determines the size of the hydrogel. A zoomed-in schematic representation of the pH gradient is shown in Figure S1d. As the pH value increases, the charge density of NH_3^+ on chitosan chain decreases accordingly; at the same time, long, straight rod-like polymer chains become entangled and curled with the disappearance of electrostatic repulsion.

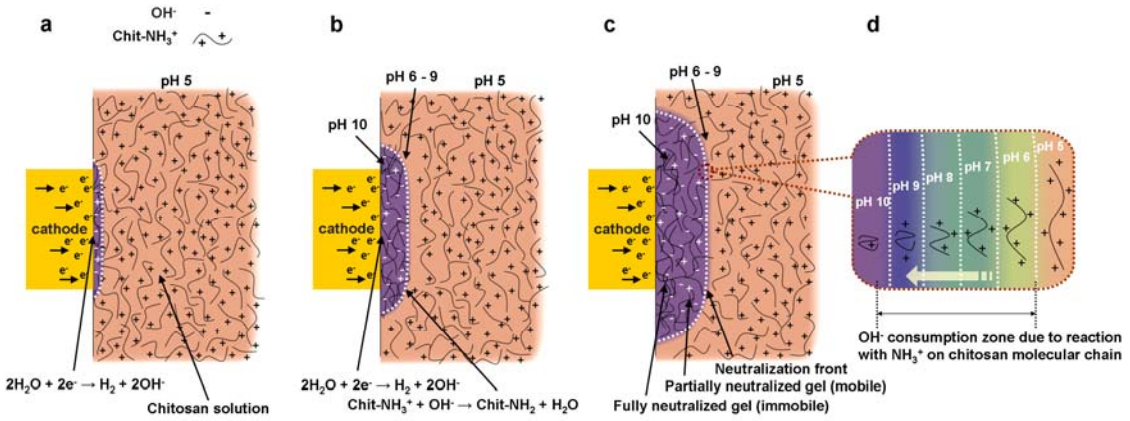

Figure S1. Schematic representation of the evolution of an electrodeposited chitosan hydrogel at the cathode surface with pH indication (by color). (a) At the beginning, electrolysis of water leads to the generation of hydroxide ions (OH^-) at the cathode surface, which leads to a localized increase of pH level at cathode surface as compared with chitosan bulk solution. (b) Electrochemically generated OH^- ions at the cathode surface and their subsequent migration and neutralizing reactions with charged NH_3^+ sites on chitosan chains. (c) Schematic diagram of the neutralization process: from right to left, pH dramatically increases at the neutralization front where chitosan molecules are initially and partially neutralized by OH^- ions and gelation starts; partially neutralized chitosan molecules, still being mobile, migrate towards the cathode and are further neutralized; a fully neutralized, dense hydrogel is formed close to the cathode surface where chitosan molecules become immobile. (d) A zoomed-in representation of the change of the charge state and conformation of the chitosan chain at different pH levels in the neutralization front zone. White dotted line represents the boundary between different pHs.

Calculation of ΔN_e and ΔN_{NH3+}

By comparing the number of electrons passing through cathode (ΔN_e) and the number of NH_3^+ (ΔN_{NH3+}) being neutralized per unit time, we can estimate the ionization state of chitosan in hydrogel. First, the electric current determines the number of electrons flowing per unit time

$$I = J \cdot S = \frac{Q}{t} = \frac{Ne}{t} \tag{3}$$

where I is the current, J the current density, S the active electrode area, Q the total charge going through the electrode, t the time, N the number of electrons, and e the charge of an electron. According to equation (3), we can estimate how many OH^- ions are generated per second at the cathode if all electrons are contributing to the generation of OH^- ions. For example, for the current density at 4 A/m^2, we have an electron generation rate of $\Delta N_e = 2.50 \times 10^{13}$ /s at the cathode, which is the same for the number of OH^- generated per second assuming reaction (1).

Furthermore, with the area growth profile (Figure 2e) we can estimate how many NH_3^+ groups are getting neutralized per second for a continuously growing hydrogel. The concentration of chitosan solution we used is 0.5% (w/v), which is equivalent to a molar concentration of 0.031 M using 161 as the molecular weight of the chitosan monomer. Assuming each monomer chain has one NH_3^+ site and since the chitosan solution has a pH of 5.3 and a pK_a value of 6.3, we can then estimate the percentage of protonated amine groups using the Henderson–Hasselbalch equation

$$pH = pK_a + \log([NH_2]/[NH_3^+]) \tag{4}$$

where pH is the solution pH, pK_a the acid dissociation constant, $[NH_2]$ the concentration of amine groups, and $[NH_3^+]$ the concentration of protonated amine groups. The calculation yielded a value of 91% amine group protonation for the solution we used. Therefore with a deacetylation value of 85%, we can then estimate the NH_3^+ concentration as 0.024 mol/L = 1.44×10^{22} /L. From figure 2e we can estimate the area growth rate at 4 A/m^2 from the slope of the 4 A/m^2 curve which gives a area growth rate of 1.86×10^{-3} mm^2/s. This is equivalent to volumetric growth rate of 1.86×10^{-3} mm^3/s = 1.86×10^{-9} L/s given the channel height as 1mm and assuming a uniform vertical profile. We then can calculate the number of protonated amines being neutralized per second within the hydrogel as $\Delta N_{NH3+} = 2.68 \times 10^{13}$ /s based on the assumption that within the gel all positively charged amine groups will be neutralized. The fact that ΔN_e and ΔN_{NH3+} are on the same order of magnitude indicates a

rational hypothesis of electro-induced chitosan hydrogel growth mechanism. Moreover, the fact that the latter is slightly greater (7%) than the former suggests that the chitosan molecules are not fully neutralized within hydrogel and about 7% of amine sites remain positively charged within the hydrogel.

Nernst-Planck Equation governing the migration of OH^- and positively charged chitosan

The ionic concentration gradient results in a drift of charge carriers into a lower concentration region, increasing the concentration. The flux associated with the drift can be defined by Fick's laws of diffusion

$$j = -|Z|D\frac{d[C]}{dx} \tag{5}$$

where C is the concentration of ions, Z is the valence of the charge carrier and D is the diffusion constant. The constantly generated OH^- ions at the surface of the cathode continue to migrate outward due to the chemical concentration gradient as well as the electrostatic force induced by the electrical field and the negatively charged cathode. The current density associated with the flow of charge within an electric field in a solution can be defined by Ohm's law

$$j = v_d|Z|F[C] = |Z|F[C]\mu E = |Z|F[C]\mu\frac{dV}{dx} \tag{6}$$

where Z is the valence of the charge carrier, F is the Faraday's Constant, C is the concentration of charge carriers, V_d is the drift velocity, μ is the mobility of the charge, E is the electrical field, and V is the electric potential. Combining the diffusive and electrical components of the flux and expanding to three dimentions results in the Nernst-Planck Equation for ion *i*

$$\vec{J}_i = -D_i\nabla C_i - Z_i\mu_i FC_i\nabla V_i \tag{7}$$

The flux of OH^- ions in the electrolyte therefore results in a local increase of pH level and this pH gradient propagates outwards (towards the anode) as the electrochemical reactions continue.

Estimation of hydrogel density

Figure S2a illustrates the dimensional comparison of the hydrogel in wet and dry formats. The wet gel outline (figure S2a, black curve) is replicated from captured bright field image using Carl Zeiss microscopy imaging software AxioVision with to scale dimensions. The topography profile of the dry film was obtained by profilometry. For a better comparison the scale in y direction (horizontal) of the dry film is magnified 20 times. The surface topography of the dry film (figure S2a, red curve) clearly demonstrates an increased thickness at the edges of cathode which suggests more mass at the edge than at the center. The area of the wet gel is determined to be about 1.60×10^5 μm^2 by using the outline tool from the AxioVision software. Integration of the topography scanning curve of the dry film using a profilometer gives an area of 1.27×10^3 μm^2. By assuming a uniform distribution of the thickness throughout the channel height (1mm in z direction in figure S2a) we then have a wet/dry volume ratio of about 126, indicating a dry/wet volume ratio of 0.79%. Assuming a mean density value of 0.8 g/cm^3 between dry chitosan flakes (0.6 g/cm^3) and water (1.0 g/cm^3) for a room-temperature naturally dehydrated film, after 85 seconds of electrodepostion at 4 A/m^2, a chitosan concentration of 0.63% (w/v) is calculated for the wet hydrogel which contains approximately 99% of water. This concentration is slightly smaller than the estimated value 0.72% using transmitted light intensity (TLI) ratio between hydrogel (123 a.u.) at 85 seconds of deposition and solution (177 a.u.) at 0 second of deposition.

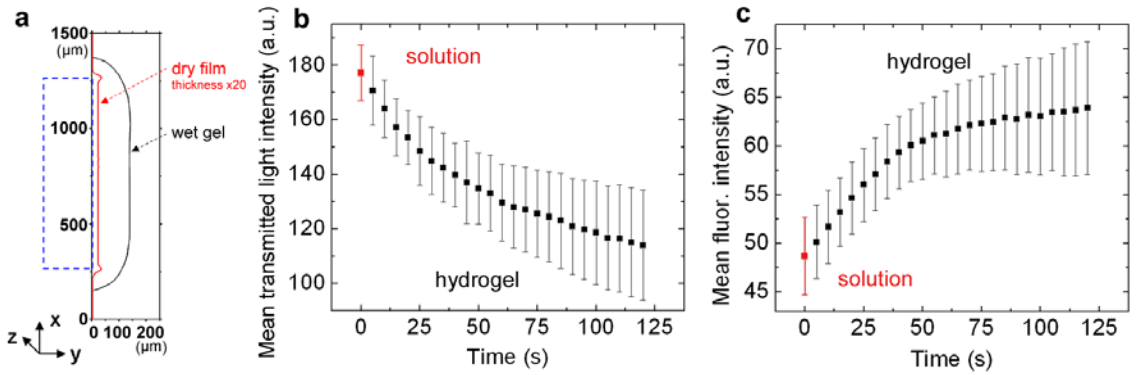

Figure S2. Estimation of hydrogel density. (a) Dimensional comparison of the wet (in solution) and dry (in air) format of hydrogel: Up to scale outline of the wet hyrogel (black curve) after 85 seconds electrodeposition at 4 A/m^2 and subsequent topography of the dry one (red curve, dimension in y direction: × 20). Blue dashed rectangle indicates where the cathode is. (b) Time dependent mean transmitted light intensity (TLI) of the chitosan hydrogel area. The red dot indicates the mean TLI value of the chitosan solution within the fluidic channel before electrodeposition. The black dots indicate the mean TLI value of the deposited hydrogel as a function of electrodeposition time. The error bars represent the standard deviation of the TLI values in the hydrogel area. (c) Time dependent mean fluorescence intensity of the chitosan hydrogel area using NHS-fluorescein tagged chitosan.. The red dot indicates the mean fluorescence intensity value of the chitosan solution within the fluidic channel. The black dots indicate the mean fluorescence intensity value of the deposited hydrogel as a function of electrodeposition time. The error bars represent the standard deviation of the fluorescence intensity values in the hydrogel area.

Figure S2b is the average transmitted light intensity value of chitosan solution (red dot) at time 0 seconds and that of the subsequent hydrogel (black dots) deposited up to 120 seconds. This concentration value is also comparable to the estimated value 0.64% using a fluorescence intensity ratio between the hydrogel (63 a.u.) and bulk chitosan solution (49 a.u.). The good agreement of these estimated density values suggests a reasonable agreement between these experimental measures. Figure S2c shows the average fluorescence intensity value of chitosan solution (red dot) at time 0 seconds and that of the subsequent hydrogel (black dots) deposited up to 120 seconds. Curves in figure S2b and c exhibit opposite evolutionary trend of the intensity change, indicating the similar hydrogel density transformation during the electrodeposition. As expected, the standard deviation increases with time in both cases (figure S2b and c) because as the charged chitosan molecules migrate further, the degree of non-uniformity induced by the electric field becomes more pronounced with time. The relatively steady rise in density during the first 50 seconds is followed by a slower increase afterward. This behavior is possibly due to the decreasing amount of available chitosan molecules in solution and their limited migration/diffusion towards denser hydrogel area.

Experimental details:

Fluidic channel and side wall electrodes fabrication

The sidewall electrodes and leads were defined by angled thermal evaporation of 10 nm Cr and 120 nm Au onto a piranha cleaned, tilted glass slide using bent stainless steel shadow mask with 1 mm wide slits. Two patterned glass slides were then mounted side by side on a piece of thin cured PDMS (Sylgard 184 Silicone Elastomer Kit, Dow Corning) with sidewall electrodes on the side of glass slide and electrodes leads on the top to form a fluidic channel wall with desirable width (normally 1 mm). Another thin piece of cured PDMS was put on the top of a pair of glass slides to form the ceiling of the channel. An open fluidic channel can also be fabricated without the top PDMS layer. The PDMS layers and the patterned glass slides can be permanently sealed by oxygen plasma bonding (pressure: 450 mT, forward power: 20W, Oxygen flow rate: 20 sccm O_2, plasma treatment time: 30 seconds) if a disassembly is not necessary. The channel height is determined by the thickness (about 1.06mm) of the glass slide and the channel width is adjustable. The width of all the fluidic channels reported here is around 1050 μm.

Preparation of Chitosan with pH indicator solution

Universal (pH 4-10) pH indicator solutions (Sigma-Aldrich, Fluka) were separately mixed with 0.1 M solutions of acetic acid (pH 4 – 5), monobasic sodium phosphate (pH 6 – 7), tris-hydroxymethyl-aminomethane (pH 8 – 9),

and bicarbonate (pH 10) buffers and injected into the fluidic channel. Bright field images with transmitted light from bottom were taken using Zeiss LSM-310 Microscope with AxioVision imaging software (color balance setting: 1.67 for cyan/red, 1.09 for magenta/green, 0.23 for yellow/blue) for calibrating the color chart for different pH values. An RGB (red, green, and blue) analysis of the calibrated color chart was performed using Matlab and the pH value was found to be linearly dependent on the Red (pH 4-8) and Green (pH 8-10) channel value (digital 8-bit numbers). Therefore an estimated pH profile could be obtained by analyzing the red and green channel value of an image. The estimated pH line profiles in figure 2d were obtained by using this method. A pH indicator solution working at higher pH (pH 9-13) was used to avoid the out-of-range circumstances and to confirm the accurate pH value of the chitosan. A slightly higher pH (increment less than 1) was observed at the area close to cathode in figure 2c' after 175 s of electrodeposition, indicating a slightly higher OH^- concentration at the cathode surface than in the remainder of the hydrogel.

Preparation of Chitosan Solution and Fluorescently Labeled Chitosan Solutions

Chitosan solutions were prepared by adding chitosan flakes (Sigma Aldrich, 85% deacetylated) to a diluted acidic solution of distilled water and HCl to maintain a pH around 3. After being stirred and mixed overnight, the chitosan solutions were filtered to remove undissolved chitosan flakes and chunks, and the pH of chitosan solution was dropwise titrated to pH 5.3 by addition of NaOH. Fluorescently labeled chitosan facilitated visualization of the time dependent process of electrodeposition. In our experiments, NHS-fluorescein and NHS-rhodamine (Thermo Scientific Pierce Protein Research Products) were separately used to conjugate the chitosan molecules for fluorescence microscopy studies. For fluorescent-labeling of chitosan, 5 mL of 1.6% (w/v) chitosan solution was poured into Petri dishes and dried overnight at 50 °C. The dried thin film was immersed in 1 M NaOH (10 mL for 30 min) for neutralization, followed by thorough washing with distilled water and 0.1 M PBS buffer (pH 7.4). The neutralized film was then immersed into 10 mL of PBS (pH 7.4). 10 mg of NHS-fluorescein (or 11.16 mg NHS-rhodamine) dissolved in 1 mL DMSO was then added dropwise to the neutralized chitosan thin film in the Petri dish (molar ratio of NHS-fluorescein to chitosan macromolecules was about 3.6%). After 2 h of reaction at room temperature with 75 rpm gyratory shaking, the fluorescently labeled chitosan film was extensively rinsed with distilled water. Diluted HCl was then added dropwise while stirring to dissolve the fluorescently labeled chitosan. The concentration and pH of the fluorescently tagged chitosan were then adjusted to 0.5% and 5.3 using distilled water and NaOH solution respectively.

Bright field microscopy and fluorescence microscopy

Bright field optical microscopy and fluorescence microscopy were performed on a Zeiss LSM-310 Laser Scanning Confocal Microscope. The bright field optical microscopic images were obtained with transmitted light from the bottom. The fluorescence microscopic images for NHS-fluorescein (excitation peak: 491 nm, emission peak: 518 nm) labeled chitosan were obtained by using a FITC filter set (480 nm excitation, 535 nm emission and 505 nm dichroic bandpass). A TRITC filter set (535 nm excitation, 610 nm emission, and 565 nm dichroic bandpass) was used to characterized the NHS-rhodamine (excitation peak: 552 nm, emission peak: 575 nm) labeled chitosan.

Supporting video 1

Sequential bright field optical microscopic images of the chitosan solution in the fluidic channel showing the real-time electrodeposition of chitosan hydrogel at 9 A/m^2 current density. Time elasped between each frame is 5 seconds. Anode (top) and cathode (bottom) are in black.

Supporting video 1'

Sequential images of 3D surface plot of transmitted light intensity (z axis) of the selected area of chitosan solution during electrodeposition in fluidic channel. Selected area is highlighted with yellow rectangle in video 1.

Supporting video 2

Sequential fluorescence micrograph of the NHS-rhodamine tagged chitosan solution in the fluidic channel showing the migration and deposition of chitosan at 4 A/m^2 current density. Time elasped between each frame is 5 seconds. Anode (top) and cathode (bottom) are marked with white retangles.

Supporting video 2'

Sequential images of 3D surface plot of fluroescence intensity (z axis) of selected area of chitosan in the fluidic channel. Selected area is highlighted with yellow rectangle in video 2.