

# Tubular Chitosan Hydrogels with Tuneable Lamellar Structure Programmed by Electrical Signals

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## Experimental Section

### *Materials*

Chitosan (200–600 mPa·s) was purchased from Tokyo Chemical Industry Co., Ltd. (TCI, Shanghai). Stainless steel needles and platinum (Pt) electrodes were obtained from commercial resources in China. Hydrochloric acid, hydrogen peroxide (30%), phenolphthalein, ethyl alcohol and sodium chloride were purchased from Sinopharm Chemical Reagent Co., Ltd. (China). All the reagents were used as received without further purification.

### *Electro-assembly of lamellar tubular chitosan hydrogel*

Chitosan powder was dissolved by 1% (v/v) hydrochloric acid to prepare a chitosan solution of 1% (w/v). The final pH of chitosan solution is ~5.5. The chitosan solution was filtered to remove insoluble particles. Hydrogen peroxide (600  $\mu$ l) was then added to 100 ml chitosan solution. Electrodeposition was performed in a two-cell electrolytic cell (Gaoss Union C007-1) and an anion exchange membrane (Selemion, AMV) was added. Chitosan solution with different ionic strength by adding NaCl was added to electrolytic cell and used for electro-assembly. A titanium rod with a diameter of 1.5 mm was partially inserted in chitosan solution in cathode chamber and a Pt wire was inserted in anode chamber used as counter electrode. Electro-assembly was performed by connecting the electrodes to an electrochemical source meter (2450, Keithley) and predetermined electrical signals were applied for a certain time. The lamellar chitosan hydrogel with high mechanical strength can be easily demolded from the titanium rod and ready for further characterizations.

### *Characterization*

Optical pictures of the lamellar chitosan hydrogel were obtained using a LEICA ICC50W microscope. The water content of the electro-assembled hydrogels was determined by the weight difference of the chitosan hydrogels in the wet and dry states. Scanning electron microscopy (SEM) images of freeze-dried chitosan hydrogels were taken with a scanning electron microscope (Zeiss, SIGMA, Germany) operating at an acceleration voltage of 20 kV. Tensile testing of the chitosan hydrogels was performed on a universal tensile compressive instrument (Model 5576, INSTRON Instrument, USA). Samples were stretched to break at a constant speed of 10 mm min<sup>-1</sup>. Polarized optical microscope images were taken using an Axio Scope. A1 (ZEISS, Germany). The 2D-wide-angle X-ray diffraction (WAXD) of chitosan hydrogel was obtained by Debye-Scherrer method in a Bruker Smart Apex-II single-

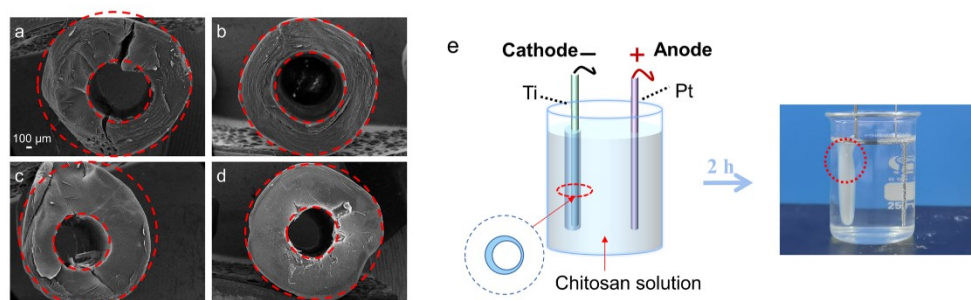
crystal four-circle diffractometer. The radiation source was a copper target ( $\lambda=1.54 \text{ \AA}$ ), and the test voltage and current were 40 kV and 0.65 mA. The X-ray spot was adjusted to the center of the sample, and the Angle was set at  $0^\circ$ . The distance between the sample and the detector and the exposure time were set at 60 mm and 1 min, respectively. The 2D diffraction images were analyzed with Fit2D software from the European Synchronization Radiation Facility. The orientation factor ( $f$ ) of the samples were calculated by equation (1):

$$f = \frac{180^\circ - \psi^\circ}{180^\circ} \quad (1)$$

where  $\psi^\circ$  is the full width at half maximum of the azimuthal distribution. Two-dimensional (2D) small-angle X-ray scattering (SAXS) was tested on a 3.5 m NANOPIX system using high-flux X-ray generator (MM007) with Cu  $K\alpha$  target ( $\lambda= 1.54 \text{ \AA}$ ) as the radiation source, under the operating voltage of 40 kV and current of 30 mA. Scattering images were obtained using HyPix-6000 detectors.

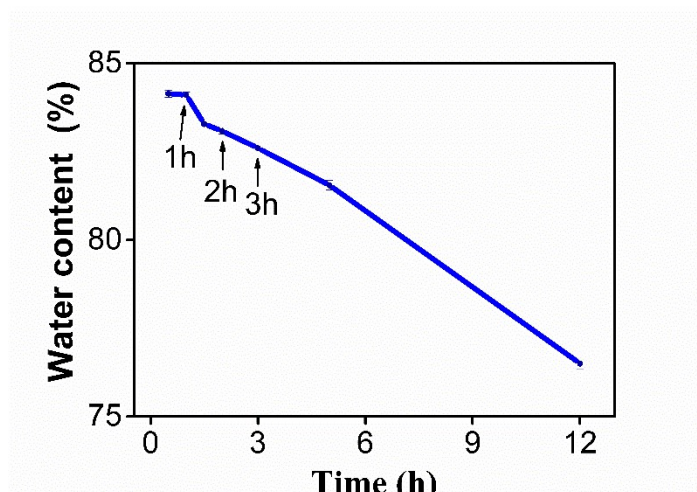
### The electro-assembled chitosan hydrogel on the cathode in a single compartment electrolytic cell

As shown in **Figure S1a, c, e**, under different current densities, chitosan hydrogels deposited by single cell electrolytic cell showed uneven thickness and incomplete lamellar structure, due to the effect of  $H^+$  generated by the anode. The defect can be improved by electrodeposition of hydrogel in a two-compartment electrolytic cell (**Figure S1b, d**).



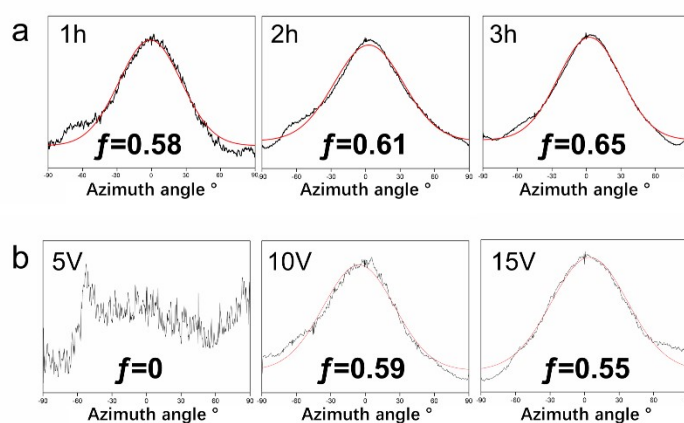
**Figure S1.** SEM image of electro-assembled chitosan hydrogel on the cathode in a single compartment electrolytic cell at  $10 \text{ A/m}^2$ (a),  $20 \text{ A/m}^2$ (c). SEM image of electro-assembled chitosan hydrogel on the cathode in a double compartment electrolytic cell at  $10 \text{ A/m}^2$ (b),  $20 \text{ A/m}^2$ (d). Schematic and optical image of electro-assembled chitosan hydrogel on the cathode in a single compartment electrolytic cell.

### Water content of chitosan lamellar tubes changing with electro-assembly time



**Figure S2.** Water content of chitosan lamellar tubes changing with electro-assembly time

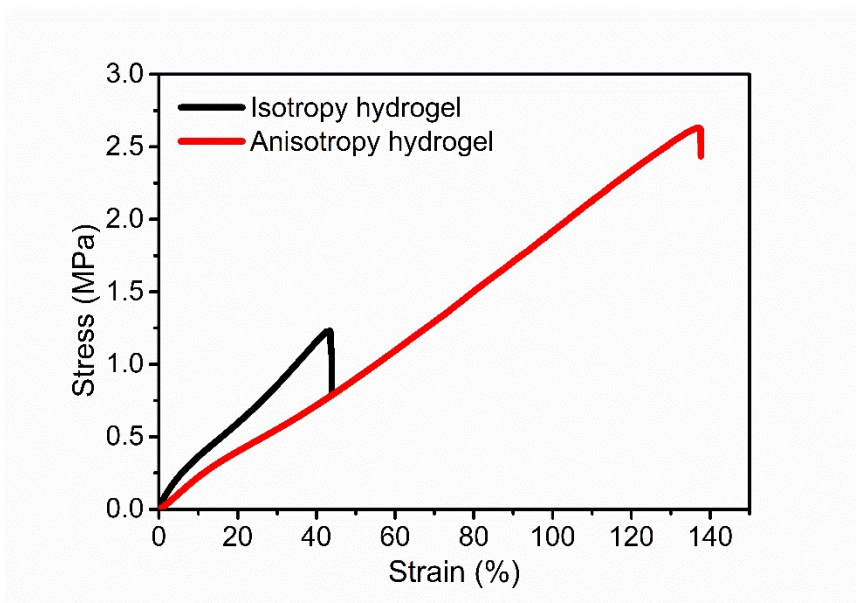
### Orientation degree of electrodeposited chitosan hydrogels



**Figure S3.** Orientation degree of electrodeposited chitosan hydrogels with different (a) deposition times, (b) voltages.

### Comparison of properties of hydrogels with isotropic/anisotropic structures

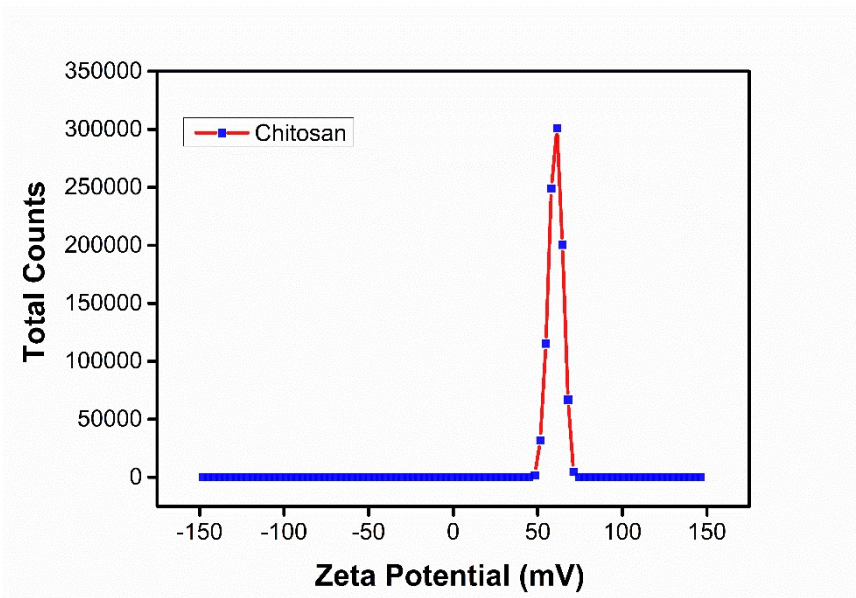
After drying the 1% chitosan solution into a film, it was neutralized in 2% NaOH solution, and then thoroughly cleaned with deionized water. The hydrogel obtained had no ordered structure. Then, the mechanical properties of the anisotropic chitosan hydrogel prepared by electro-assembly at 10V were compared. As can be seen from **Figure S4**, the anisotropic structure significantly improved the tensile strength and elongation at break of the hydrogel.



**Figure S4.** Comparison of mechanical properties of chitosan hydrogels prepared by base neutralization and electro-assembly.

#### Zeta potential of chitosan solution

The zeta potential of chitosan solution was determined by using a Zetasizer 3690 (Malvern, UK) instrument. The result shows a Zeta potential over 50 mV due to the presence of positive charges on chitosan .

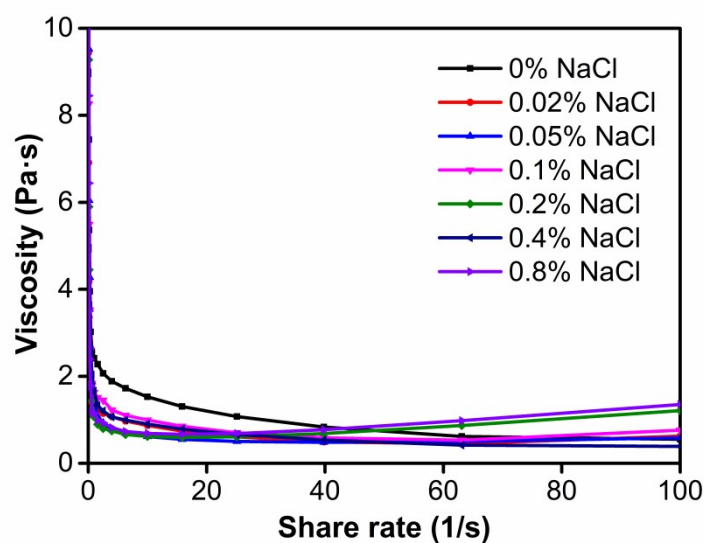


**Figure S5.** Zeta potential of chitosan solution

#### Effect of NaCl concentration on viscosity of chitosan solution

Different concentrations of NaCl (0%, 0.02%, 0.05%, 0.1%, 0.2%, 0.4% and 0.8%, w/v) were added to

1% chitosan solution. The rheological property of the solution was measured by TADHR-2 rheometer (TA, USA).

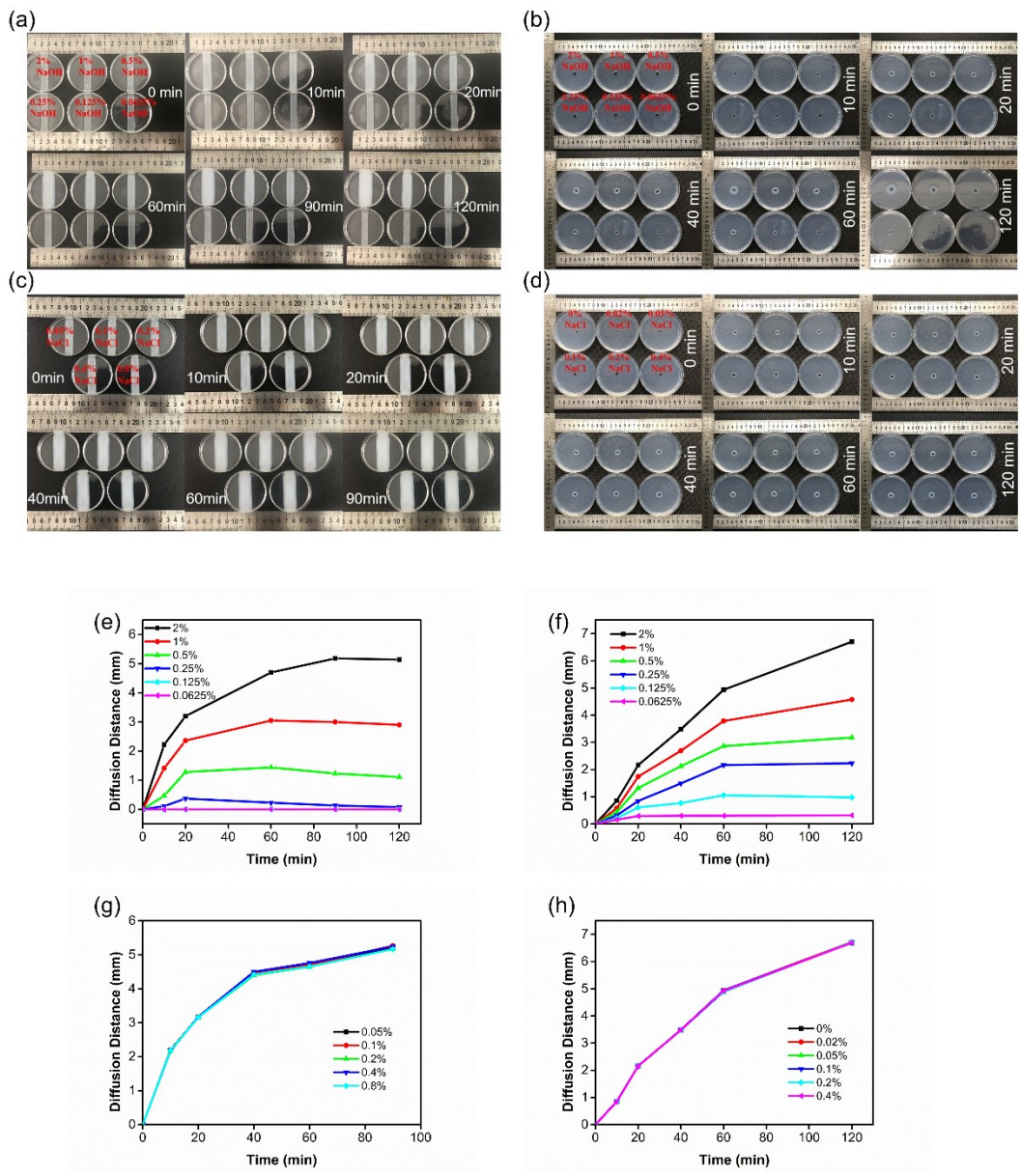


**Figure S6.** Effect of different concentrations of NaCl on viscosity of chitosan solution

#### **Effect of NaCl concentration on the gelation of chitosan in free diffusion system**

We designed two kinds of free diffusion systems, which were in solution state (1% chitosan) (**Figure S7 a, c**) and hydrogel state (**Figure S7 b, d**) (mixing 2% chitosan solution with 2% agar solution in equal volume). In solution state, 1ml of 0.0625%, 0.125%, 0.25%, 0.5%, 1% and 2% NaOH (*w/v*) solutions were added into dialysis bags ( $M_w=3000$ ), and then placed in 1% chitosan solution (**Figure S7 a**) or 1ml 2% NaOH (*w/v*) solution was added into dialysis bag ( $M_w=3000$ ), and then placed in chitosan solutions with different concentrations of NaCl (0.05%, 0.1%, 0.2%, 0.4% and 0.8%, *w/v*) (**Figure S7 c**). In hydrogel state, a hole ( $d=5$  mm) was drilled in chitosan-agar gel. Then 200  $\mu$ l NaOH with different concentrations of 0.0625%, 0.125%, 0.25%, 0.5%, 1% and 2% (*w/v*) was added (**Figure S7 b**) or 200  $\mu$ l 0.5% NaOH (*w/v*) solution was added to the hydrogel with different concentrations of NaCl (0.05%, 0.1%, 0.2%, 0.4% and 0.8%, *w/v*) (**Figure S7 d**). The growth of chitosan gel was recorded by photographing at specific intervals and the diffusion distance was quantified with time (**Figure S7 e, f, g, h**).

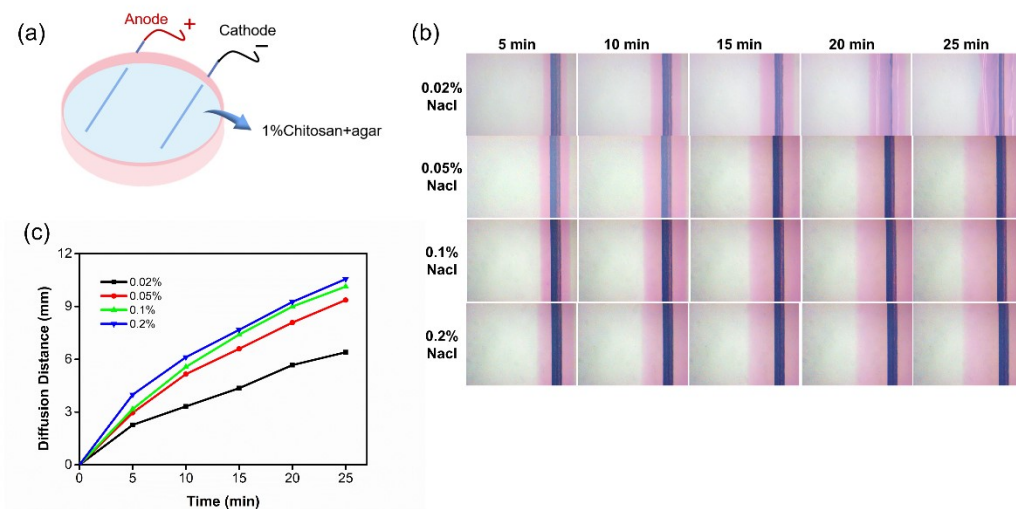




**Figure S7.** The effect of NaCl on the gelation of chitosan in free diffusion system. (a, b, e, f) Effect of different concentration of alkali solution on gelation of chitosan. (c, d, g, h) Effect of different concentration of NaCl on gelation of chitosan.

### Effect of NaCl on the electro-assembled chitosan hydrogel

Chitosan (2%, w/v) and agarose (2%, w/v) were mixed by equal volume and added with NaCl to the concentrations of 0.02%, 0.05%, 0.1% and 0.2% (w/v) respectively. The visualize the diffusion of OH<sup>-</sup>, 200  $\mu$ L 1% phenolphthalein was added and then poured into petri dishes ( $d=10$ cm). After cooling, the chitosan/agar gel was obtained. A platinum wire ( $d=0.8$  mm) and a titanium rod ( $d=0.8$ mm) were inserted into the gel as the anode and cathode, respectively. After applying the electric field, photographs were taken at a set interval to observe the diffusion of OH<sup>-</sup>.

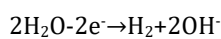


**Figure S8.** The effect of NaCl on the electro-assembled chitosan hydrogel. (a) Schematic and (b-c) The OH<sup>-</sup> diffusion rate of electrodeposition at different concentration of NaCl chitosan solution.

#### Effect of NaCl on the current efficiency

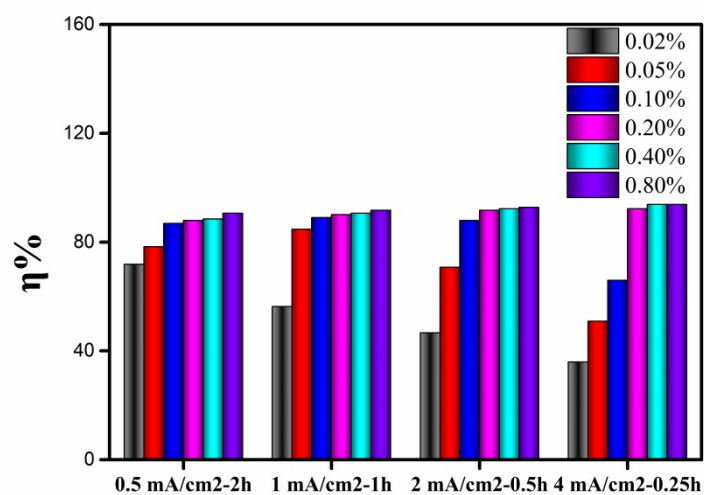
A two-compartment electrolytic cell (containing anion exchange membrane) was used to electrolyze NaCl solution under different current densities. Ensure that the total amount of transferred charge is the same in each group. After the electrolysis, adding 100  $\mu$ l 1% phenolphthalein to the cathodic compartment. The amount of OH<sup>-</sup> generated was titrated with 0.002M HCl and the current efficiency ( $\eta$ ) was calculated according to the following formula:

$$\eta = \frac{N_{HCl}}{N_{total}} \times 100\%$$



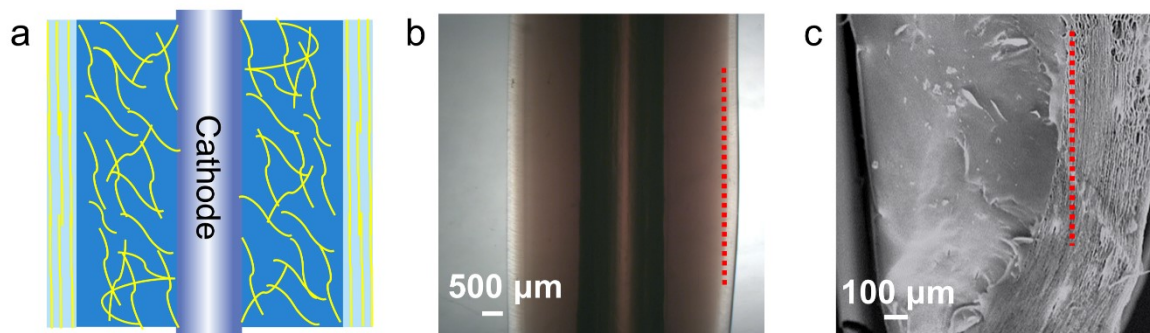
$$N_{total} = Q/F, N_{HCl} = N_{OH^-}$$

Where  $Q$  is total transferred charge,  $F$  is Faraday constant.



**Figure S9.** The effect of different concentration of NaCl on the current efficiency of electrodeposition

**Chitosan hydrogels with different structures (ordered/disordered)**



**Figure S10.** (a) Schematic diagram (b) Microscope and (c) SEM images of the assembled chitosan hydrogels deposited in NaCl chitosan ( $w/v=0.05\%$ ).