

Supplemental 1

Synthesis of CSD functional polymer

CSD functional polymer was synthesized through a modified EDC/NHS coupling reaction³⁶. Briefly, 0.2g of chondroitin sulfate sodium salt (CS, from bovine trachea) was added into 15ml of PBS solution under stirring (50°C, 400rpm) until the CS was totally dissolved. After the pH value was adjusted to 5.5, 0.2g of NHS and 0.5g of EDC were added into the reaction system dropwise after dissolved in 1ml of DI water respectively. The activating reaction was continued for 60mins under Argon (Ar) protection and 250rpm stirring. After that, 1g of dopamine hydrochloride (dissolved in 3ml DI water) was added into the reaction system by dropping. The pH value of the reaction system was then maintained at 5.5~6.0 for another 6hrs. After the reaction, the resultant mixture of the reaction was then dialyzed against DI water for 2 days. CSD functional polymers was collected by lyophilization thereafter, and then kept in 4°C for further studies.

Characterization of CSD functional polymer

Chemical structure of CSD functional polymer was confirmed with ¹H NMR and FT-IR spectroscopies.

For ¹H NMR tests, the CSD functional polymer, the NHS-activated CS (CS-NHS, withdrew from the reaction system before addition of dopamine hydrochloride), the pure dopamine hydrochloride, and the pure CS were analyzed with a Bruker Advance II 300MHz NMR (Bruker, Germany) after dissolved in deuterium oxide (D₂O) respectively (1%, w/v). For FT-IR analysis, a small amount of CS, dopamine hydrochloride, and CSD powders were mixed with pre-dried potassium bromide (KBr) respectively to be made into slice samples under pressure. The slice samples were then analyzed with a Bruker MPA NearIR (Bruker, Germany).

For the microstructure evaluation of the crosslinked CSD hydrogel. The gel samples were freshly prepared (8-mm-diameter) with 80μl of CSD functional polymer solution (20%, w/v), 16μl of Fe³⁺ solution(200mM), and 4μl of NaOH solution (1M). After freeze-drying and coating, the sample was processed to a JSM6700ESEM microscope for the following evaluation.

Rheological properties evaluation

The rheological properties of the CDS hydrogel under different gelation conditions were tested on a rheometer (TA Instrument, Model AR2000ex). 25-mm-diameter parallel plate was used, and the testing temperature were maintained at 25°C by the temperature controller equipped in the substrate

base plate of the rheometer.

The rheological properties of the CSD hydrogel at equilibrium under different conditions, namely as-prepared CSD macromer solution (20% w/v, CSD), CSD gluing hydrogel in natural condition (CSD-Fe³⁺), and CSD gluing hydrogel in alkaline environment (CSD-Fe³⁺-OH), were measured by frequency-sweep experiments (0.1Hz to 100Hz) with a constant strain at 5%. Briefly, 100μl of freshly prepared CSD macromer solution (20% w/v) was added onto the substrate base plate in the test of the CSD macromer solution. The CSD hydrogels under different environmental pH, namely CSD-Fe³⁺ and CSD-Fe³⁺-OH, were formed in situ by addition of corresponding crosslinkers, namely Fe³⁺ (20μl) and NaOH (10μl) solutions according to different gelation conditions, to the CSD macromer solutions on the substrate plate. All the test conditions had undergone 2 mins' equilibrium period before the storage modulus (G') and viscous modulus (G'') were recorded corresponding to the frequency.

For evaluation of the gelation time of the CSD adhesive hydrogel under different additions, time-sweep experiments were performed both under natural and alkaline crosslinking environments. Briefly, in the test of gelation time of CSD gel under natural environment (CSD-Fe³⁺), 100μl of CSD macromer solution was firstly added onto the substrate plate of the rheometer. Right before recording of the modulus, Fe³⁺ was added into the macromer solution. In the test of CSD's gelation time under alkaline environment (CSD-Fe³⁺-OH), firstly CSD macromer solution and Fe³⁺ will be applied in the same way as the gelation time evaluation of CSD-Fe³⁺, and NaOH will be added onto the substrate plate by needle at the end of first minute. All of the time-sweep evaluations were performed for 30min to study the gelation behaviors of CSD adhesive hydrogel.

Study of in vitro swelling and degradation behaviors of crosslinked CSD

Crosslinked CSD hydrogels were freshly prepared in a cylindrical mold (8-mm-diameter) with 80μl of CSD macromer solution (20% w/v), 16μl of Fe³⁺ crosslinker (200mM), and 4μl of NaOH solution (1M). All of the as-prepared CSD hydrogels were weighed and recorded their initial mass (W_0).

For evaluation of CSD hydrogel's swelling kinetics, each of the as-prepared CSD hydrogels was immersed in 10ml of PBS solution in a 50ml centrifuge tube. All of the samples were incubated in 37°C on an orbital shaker (100rpm). At each pre-determined timepoints, 3 samples were taken out from PBS solution. The residual solution on the surface of the gels was roughly wiped, and the samples were weighed again (W_t). The swelling ratio of CSD hydrogels at timepoint t was determined by Equation.1:

$$\text{Swelling ratio (\%)} = \frac{W_t}{W_0} \times 100\% \quad (\text{Equation.1})$$

For studies of the in vitro degradability of crosslinked CSD hydrogel, PBS degradation was conducted. Before degradation studies, all the as-prepared CSD samples were placed in PBS solution for 24 hours to let them reach their swelling equilibrium. After that, CSD samples were immersed in 10ml of PBS solution. The samples were incubated on the abovementioned orbital shaker (100rpm) and the corresponding PBS solution was replaced every 24 hours. 3 samples were taken out from the degradation solution at each pre-determined timepoint. The samples were weighed after thoroughly wiping the residual solutions (W_{dt}). The remaining weight (%) at each timepoints (t) were determined by Equation.2:

$$\text{Remaining weight (\%)} = \frac{W_{dt}}{W_0} \times 100\% \quad (\text{Equation.2})$$

In vitro adhesive properties evaluation

The in vitro adhesive abilities of the CSD hydrogel were assessed by in vitro lap shear tests using porcine skin slices. All the lap shear tests were conducted on a tensile meter (Instron mechanical tester, Model 5543) equipped with 100N load cell and all the tensile tests were conducted at the tensile rate of 1mm/min. Fresh porcine skin bought from local market was prepared into 4cmx1cm rectangle slices following previous studies³⁸. 40µl of freshly prepared CSD macromer solution (20%, w/v) was firstly added to the inner surface of a skin slice. The CSD solution was confined in a 1cmx1cm area to ensure the consistency between different samples. After addition of gluing macromer solution, Fe³⁺ crosslinker and NaOH solution were added onto the CSD-spread area according to different test conditions, followed by the immediate overlapping with another piece of skin slice. After 10s of slight pressure with fingers to enhance the adhesion between the two skin slices, the samples were placed in a 37°C water bath for pre-determined durations before undergoing tensile tests.

To figure out the best CSD to Fe³⁺ ratio, CSD macromer solution, Fe³⁺ crosslinker, and NaOH solution were dosed according to **Supplemental Table.1**. After 2 hours' incubation time, samples were proceeded to the tensile meter for adhesive tests.

Supplemental Table 1 Dosages of CSD components in studies of CSD-Fe³⁺ ratio

CSD-Fe ³⁺ ratio	1-1	3-1	5-1	7-1
CSD solution volume (µl)	20	30	35	35

FeCl₃ solution volume (μl)	20	10	7	5
NaOH solution volume (μl)	4	4	4	4

To study the influence of the alkaline gelation environment of the CSD adhesive hydrogel to its overall adhesive strengths, addition of 2μl of NaOH was taken as another variable during the abovementioned study of CSD to Fe³⁺ ratios. The incubation time was also set as 2 hours.

Taking incubation time as a variable, the adhesion kinetics of the CSD adhesive hydrogel was also studied. 40μl of CSD macromer solution, 8μl of Fe³⁺ crosslinker, and 2μl of NaOH solution were added to each of the samples. The samples were incubated for 2min, 5min, 10min, 30min, 60min, and 120min, respectively before proceeded to tensile tests.

Cytocompatibility evaluation of CSD

The sol-content of crosslinked CSD hydrogel was prepared as previous studies³⁶. Briefly, one as-prepared CSD hydrogel was immersed in 10ml of cell culture medium and incubated in 37°C for 24h to make 100X CSD sol-content medium. 50X, 25X, and 10X CSD sol-content mediums were made by diluting the 100X sol-content medium with Normal cell culture medium. Normal cell culture medium was taken as control medium.

3T3s and hMSCs were seeded onto a 96-well-plate in density of 5x10³ per well. After all the cells were cultured with normal cell culture mediums for 24h, the mediums were replaced by 200μl of corresponding sol-content mediums or control medium based on different culture conditions. Cytotoxicity tests were conducted by means of WST-1 cell viability and proliferation assay on day-1, -4, and -7 after mediums replacement. All the tests were conducted three repeats.

To assess the viability of the cells cultured with CSD gels visually, 3T3s was cultured with 50X CSD sol-content medium. On day-1, -3, and -5, the cells were stained with Live/Dead viability kit and the cell viability was checked with fluorescence microscope.

To further test the viability of the cells on direct contact with CSD gels, one crosslinked CSD hydrogel was put in a well of 24-well plate seeded with 3T3s (2x10⁴ per well), Live/Dead viability tests were also performed on day-1, -3, and -5.

In vivo seroma prevention ability of CSD adhesive

Twenty-four SD rats were divided into 3 groups randomly (n=8), namely CSD group, Saline group (negative control), and TISSEL group (commercial fibrin glue, positive control). All of the rats received mastectomy surgeries according to the protocols from previous studies³⁶. Briefly, a midline incision was firstly

processed from jugular notch to the xiphoid. After raising up of the left-side skin flap, all the visible muscular tissues, including the pectoral major, the pectoral minor, and most of the subcutaneous fatty tissue were excised successively. To create lymphovascular disruptions, the inner surface of the left-side skin flap was then scratched with a surgical scalpel for 50 times.

Before closing up of the incisions, the rats from CSD group received 160µl of CSD macromer solution, 32µl of Fe³⁺ crosslinker solution, and 8µl of NaOH solution; the rats from Saline group received 200µl of saline solution; while those from TISSEEL group received 100µl of solution A and 100µl of solution B provided by TISSEEL kit. After addition of the corresponding reagents to the surgical site, the skin flap was properly put back and the air inside the incision was eliminated subsequently. The incisions were sealed up by interrupted sutures.

All the animals were monitored daily during post-operation days for wound dehiscence or infection. On day-1, day-3, and day-7, seroma fluid was quantitatively aspirated with an 18G needle (Sterican, B.Braun, Singapore) percutaneously. Seroma volumes of each rats at each aspiration timepoint were recorded.

Micronuclei test

On post-surgical day-2 and day-7, 100µl of tail vein blood was drawn from each of the SD rat. Each of the blood samples was then transferred into a heparin (100U/ml)-containing microtube, respectively. The blood samples were then proceeded to be stained with Acridine Orange (AO) according to previous protocols^{36, 38}. After that, the samples were tested by flow cytometric analysis. Briefly, 10000 cells were analyzed for each sample. Before analyzing, one unstained sample was analyzed first for the sake of gating the low signal area (Fig.6c), and AO-stained DNA and RNA were detected and counted by using FITC (530nm) and PI (630nm) channels. The normochromatic erythrocyte (NCE) and the polychromatic erythrocyte (PCE) populations were thereby gated out and the ratio of PCE/NCE of all the samples were calculated.

Histopathological evaluation

On day-7 postoperatively, after all of the SD rats were sacrificed, full-thickness left-side chest wall were cut off from three randomly picked rats from each group, and fixed in formalin solution. The chest wall samples were then sectioned into 10µm-thick slices and were stained with hematoxylin and eosin (H&E) and Masson trichrome. The stained slices were proceeded to a pathologist for semiquantitative histopathological examination. Briefly, the histological samples were scored based on Eser's scoring criteria according to the severity of fibrosis, inflammation, and the level of cell filtration³⁹.

Hemostatic ability study of CSD adhesive

To test the hemostatic ability of the CSD adhesive hydrogel, a rat hemorrhaging liver model was utilized. Nineteen SD rats (female, 8 weeks, weighing 200~250g) were randomized into 3 groups, namely CSD group (n=8), Control group (n=8), and Blank group (n=3). After hemorrhaging liver models were built according to previous protocols with a 20G needle, 80 μ l of CSD macromer solution, 16 μ l of Fe³⁺ crosslinker solution, and 4 μ l of NaOH solution were immediately added onto the bleeding sites of each of the rats from CSD group, while no treatment was given to those from Control group. After 3min, the weight of the filter papers from CSD group and Control group (W_f), and the weight of those from Blank group (W_b) were recorded, and the weight of the unused filter papers were also recorded as (W_0). The amount of bleeding of CSD and Control groups were calculated by Equation.4 and Equation.5, respectively:

$$\text{Amount of Bleeding (CSD group)} = W_f - W_b - W_0 \quad (\text{Equation.4})$$

$$\text{Amount of Bleeding (Control group)} = W_f - W_0 \quad (\text{Equation.5})$$

Supplemental 2

Supplemental Table 2 Data of in vitro Tensile tests

Fe ³⁺ to CSD ratio	w OH (kPa)	wo OH (kPa)
1-1	74.72 \pm 20.96	18.81 \pm 7.76
3-1	23.21 \pm 0.36	12.99 \pm 3.72
5-1	16.13 \pm 4.22	10.01 \pm 0.83
7-1	9.71 \pm 2.35	6.82 \pm 1.85

Supplemental Table 3 Modulus (G' and G'') of frequent-sweep evaluations

	G' (Pa)	G'' (Pa)
CSD macromer solution	54.12 \pm 9.41	62.61 \pm 44.00
CSD-Fe ³⁺	447.97 \pm 53.10	95.99 \pm 37.23
CSD-Fe ³⁺ -OH	6760.71 \pm 99.12	787.40 \pm 45.45