Supporting information

Multifaceted nature of catechol chemistry: bioinspired pH-initiated hyaluronic acid hydrogels with tunable cohesive and adhesive properties

Zhongwei Guo1,2, Shengli Mi2*, Wei Sun1,2,3,4*

1 Precision Medicine and Healthcare Research Center, Tsinghua-Berkeley Shenzhen Institute, Shenzhen, China.

2 Biomanufacturing Engineering Laboratory, Graduate School at Shenzhen, Tsinghua University, Shenzhen, China.

3 Department of Mechanical Engineering and Mechanics, Tsinghua University, Beijing, China.

4 Department of Mechanical Engineering, Drexel University, Philadelphia, PA, USA.

*Email - weisun@mail.tsinghua.edu.cn

KEYWORDS: mussel-inspired chemistry, pH switch, catechol, cohesive and adhesive, hydrogel

Materials and reagents

Hyaluronic acid (HA, MW 100-200 kDa) was purchased from Lifecore

Biomedical (Chaska, MN, USA). 1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), 1-hydroxybenzotriazole hydrate (HOBt), dopamine hydrochloride, sodium periodate ($NaIO_4$), EDTA disodium salt dihydrate, dithiothreitol (DTT), ethylenediamine, ferric chloride hexahydrate, and cysteine were purchased from Sigma-Aldrich (St. Louis, MO, USA). Other analytical grade chemicals were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All chemicals were of analytical grade and were used without further purification. Porcine skins were purchased from a local grocery store. Ultrapure water was obtained using a NANOpure Infinity® system (Barnstead Thermolyne, Sigma-Aldrich). The degree of substitution of HA was calculated using ^{1}H-nuclear magnetic resonance (^{1}H-NMR, Bruker, Germany: AVANCE III 400) spectroscopy.

1.1 Polymer synthesis and chemical characterization

1.1.1 Synthesis of Catechol-modified HA (HA-Cat)

Catechol-modified hyaluronic acid (HA-Cat) was synthesized by an EDC/NHS coupling reaction, which conjugates the carboxylic acid group of HA to the amine group of dopamine. Briefly, HA was fully dissolved in ultrapure water at a concentration of 1% (w/v). EDC and NHS were then added to the HA solution, which was then stirred for 30 min at pH 5.0-5.5. Dopamine hydrochloride was added to the solution, which was then stirred overnight at room temperature while maintaining a pH of 5.0-5.5 using 1 M hydrochloride or sodium hydroxide. The solution was then

dialyzed (MW cutoff of 8-10 kDa, SpectraPor) against a pH 5.0-5.5 aqueous solution adjusted with 1 M HCl for 2 days and dialyzed again with ultrapure water for 1 day to fully remove unreacted reagents and salts. The final product was lyophilized and stored at 4 °C before use. The molar ratio of reactants was HA:EDC:NHS:dopamine = 1:1:1:1, 1:2:2:2 and 1:3:3:3, which resulted in corresponding catechol-modification ratios of HA-Cat of 26%, 35% and 43%, respectively. The percentage of catechol groups conjugated to the HA backbone was calculated from ^{1}H-NMR (Bruker, Germany: AVANCE III 400) data.

1.1.2 Synthesis of Thiol-modified HA (HA-SH).

Thiol-modified HA (HA-SH) was synthesized by a simple two-step reaction according to a previously reported method with modifications. Briefly, HA (1 g, MW 100-200 kDa) was fully dissolved in ultrapure water at a concentration of 1% (w/v). EDC (0.58 g) and HOBt (0.46 g) were reacted with HA for 30 min at pH 5-5.5, and then cystamine (0.68 g) was added, followed by stirring for 12 h. The reaction mixture was then dialyzed against ultrapure water for 2 days. Subsequently, DTT was added to the solution to reduce the disulfide bonds of HA-conjugated cystamine, and the pH was raised to 8.5. After the mixture was stirred for 12 h, the solution pH was dropped to 3.5. Later, the mixture was precipitated by excess EtOH and redissolved, followed by dialysis against ultrapure water for 2 days. The final product was lyophilized and stored at -20 °C before use. The percentage of thiol groups conjugated to the HA backbone was calculated from ^{1}H-NMR (Bruker, Germany: AVANCE III

400) data. The thiol-modification ratio of HA-SH was approximately 21%, as determined from ^{1}H-NMR data.

1.1.3 Synthesis of Amine-modified HA (HA-NH_2)

Amine-modified hyaluronic acid (HA-NH_2) was synthesized by an EDC/HOBt coupling reaction, which conjugates the carboxylic acid groups of HA to the amine groups of ethylenediamine. Briefly, HA (1 g, MW 100-200 kDa) was fully dissolved in ultrapure water at a concentration of 1% (w/v). EDC (0.58 g) and HOBt (0.46 g) were reacted with HA for 30 min at pH 5-5.5, and then ethylenediamine (0.18 g) was added, followed by stirring for 12 h. Later, the solution was precipitated by adding excess EtOH and redissolved, followed by dialysis against ultrapure water for 2 days. The final product was lyophilized and stored at 4°C before use. The percentage of amine groups conjugated to the HA backbone was calculated from ^{1}H-NMR (Bruker, Germany: AVANCE III 400) data. The amine-modification ratio of HA-NH_2 was approximately 27%, as determined from the ^{1}H-NMR data.

1.2 Preparation of hydrogels

1.2.1 Fe^{3+}-induced HA-Cat hydrogels

The lyophilized HA-Cat (43% substitution) obtained as described in the previous section was used for all the Fe^{3+}-induced HA-Cat hydrogel formation processes. HA-Cat was fully dissolved in ultrapure water at 3% (w/v). To study the effect of the Fe^{3+}:catechol ratio on the gel properties, stock solutions of $FeCl_3$ (50 mM, 100 mM,

150 mM; solvent: 5% acetic acid solution (v/v)) and HA-Cat (catechol concentration: 9 mM, 18 mM, 27 mM; solvent: ultrapure water) were prepared. A typical Fe^{3+}-induced HA-Cat hydrogel was made as follows: 1) The appropriate HA-Cat solution (100 μL) was mixed with 18.2 μL of the corresponding $FeCl_3$ stock solution. 2) The gel mixture was physically mixed until a homogenous color and physical state were established. Specifically, Fe^{3+}:catechol ratios of 1:1, 1:2, and 1:3 were obtained by the addition of 18.2 μL of 50 mM $FeCl_3$ solution into 100 μL of HA-Cat solution with catechol concentrations of 9 mM, 18 mM, and 27 mM, respectively. Fe^{3+}:catechol ratios of 2:3 and 3:3 were obtained by mixing 100 μL of HA-Cat solution (catechol concentration: 27 mM) with 18.2 μL of 100 mM or 150 mM $FeCl_3$ solution, respectively.

The pH regulation of the Fe^{3+}-induced HA-Cat hydrogels was performed by immediately increasing or decreasing the pH of the mixture by adding an appropriate volume of 0.5 M NaOH or 0.5 M HCl, followed by sufficient mixing. Different gelation processes and color variations developed according to the Fe^{3+}:catechol ratio and pH value. The end of gel formation was identified by inverting the gel at various times. The gelation time was calculated by the inversion method and the oscillatory time sweep test.

1.2.2 IO_4^--induced HA-Cat hydrogels

The lyophilized HA-Cat (43% substitution) obtained as described in the previous section was used for all the IO_4^--induced HA-Cat hydrogel formation processes. The

protocol described in the previous section for the preparation of the Fe^{3+}-induced HA-Cat hydrogels was followed for the preparation of the IO_4^--induced HA-Cat hydrogels, substituting $FeCl_3$ with $NaIO_4$.

1.2.3 HA-Cat & HA-SH hydrogels

The lyophilized HA-Cat (43% substitution) and HA-SH (21% substitution) obtained as described in the previous section were used for all the HA-Cat & HA-SH hydrogel formation processes. HA-Cat and HA-SH were fully dissolved in ultrapure water at 3% (w/v) each. A typical HA-Cat & HA-SH hydrogel was made as follows: 1) The appropriate HA-Cat solution (100 μL) was mixed with 200 μL of the corresponding HA-SH stock solution. 2) The gel mixture was physically mixed until a homogenous color and physical state were established. The effect of the catechol:thiol group ratio (1:0.5, 1:1, and 0.5:1) on the gel properties was investigated. The pH regulation of HA-Cat & HA-SH hydrogels was performed by immediately increasing or decreasing the pH of the mixture by adding an appropriate volume of 0.5 M NaOH or 0.5 M HCl, followed by sufficient mixing. Different gelation processes and color variations developed according to the Fe^{3+}:catechol ratio and pH value. The end of gel formation was identified by inverting the gel at various times. The gelation time was calculated by the inversion method and the oscillatory time sweep test.

1.2.4 HA-Cat and HA-NH_2 hydrogels

The lyophilized HA-Cat (43% substitution) and HA-NH_2 (27% substitution) obtained as described in the previous section were used for all the HA-Cat & HA-NH_2

hydrogel formation processes. The protocol described in the previous section for the preparation of the HA-Cat & HA-SH hydrogels was followed for the preparation of the HA-Cat & HA-NH_2 hydrogels, substituting HA-SH solution (200 μL) with HA-NH_2 solution (150 μL), due to the difference in the substitution degree of HA-NH_2 and HA-SH.

1.3 Rheology of the hydrogels

The mechanical properties of the hydrogels were tested using a rheometer (HAAKE MARS Ⅲ, Thermo Scientific Instruments) with parallel plate geometry (20 mm diameter rotating top plate) at 25 °C. All tests were performed immediately after transferring the gel sample onto the sample stage, except for the oscillatory time sweep tests. Time tests were performed at 5% strain and 1 Hz. The crossover point of G′ and G″ was considered the gelation point. Frequency sweeps were performed at 5% strain from 0.1-10 Hz. Self-healing tests were performed by performing strain sweeps under increasing strain from 0.1% to 100% and then allowing the gel to heal at 0% strain for 100 s. Later, the gel recovery ability was identified by the time sweep tests after the recovery time span. All the tests were performed within the linear viscoelastic region.

1.4 UV-Vis spectroscopy

UV-Vis data were recorded on a UV-visible spectrophotometer (Agilent Cary 5000) using a quartz cuvette with a path length of 1 cm. The final solution concentration was a 20x dilution of the corresponding pre-gel solution. The final

solution was pipetted several times until it was well mixed.

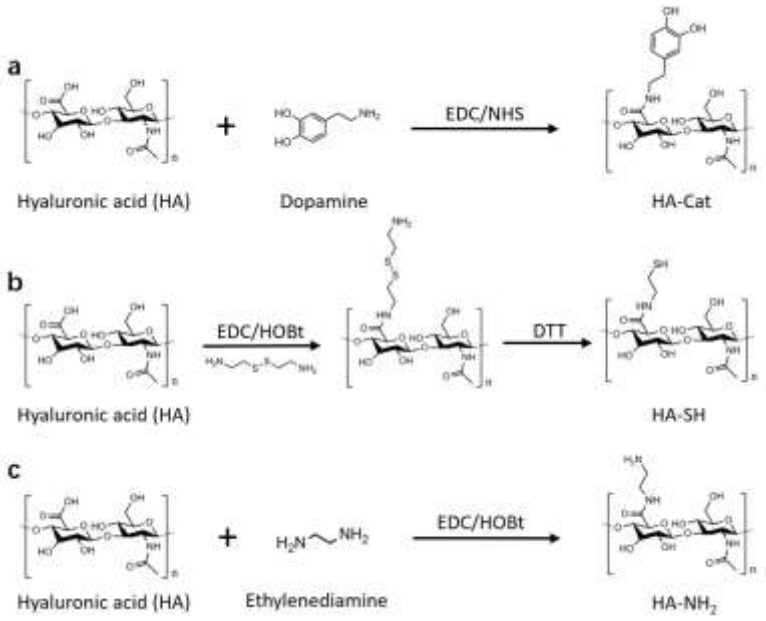
1.5 Morphology of the hydrogels

To elucidate the interior cross-linking network morphology of the hydrogels, freeze-dried hydrogels were observed using a Hitachi SU8010 scanning electron microscope. All specimens were coated with a conductive layer of sputtered gold. All experiments were performed at 25°C.

1.6 Bulk adhesion strength test

Lap shear tests were conducted to measure the bulk adhesive strength using a universal test machine (Instron E3000) with a crosshead of 1 mm/min. Various materials with different properties should be tested to acquire more complete knowledge about the adhesion mechanism. Commercial glass, SiO_2, Al, Ti, stainless steel (SS), PTFE, PU, PE, PC, PS and porcine skin (PS) slides with a size of 75 mm × 25 mm × 1 mm were used as the adherends. The gel samples were prepared in situ between the test materials, and the overlap area was 25 mm × 25 mm × 1 mm. Finally, the adhesion strength was calculated by dividing the maximum load (force) by the overlapping contact area using the following equation: $Pa = N/m^2$. Three samples for each group (n = 3) were used in the lap shear test. The tests were repeated three times to confirm the reproducibility of the adhesion strength data.

Results and discussions



Scheme S1. The synthesis route of HA-Cat (a); HA-SH (b); HA-NH_2 (c).

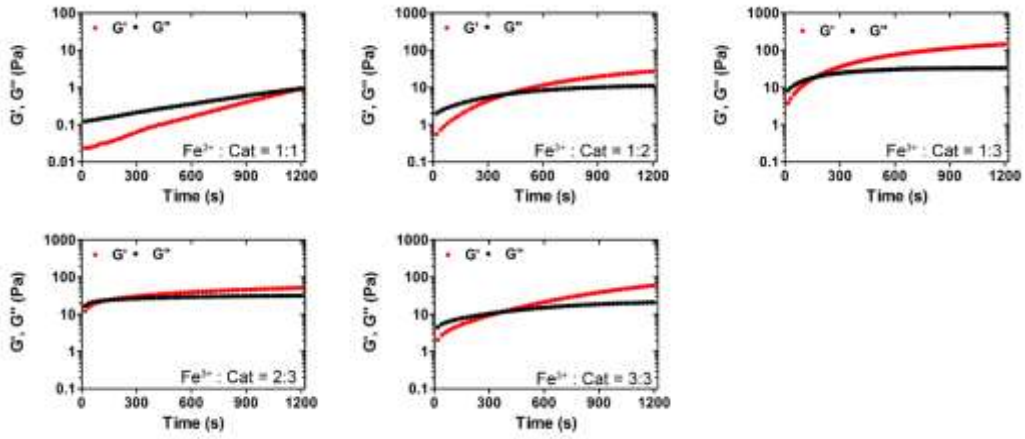


Figure S1. Oscillatory time sweep of Fe^{3+}-induced HA-Cat gels at different molar ratios between Fe^{3+} : Cat (Time sweep performed at a strain of 5% and frequency of 1Hz).

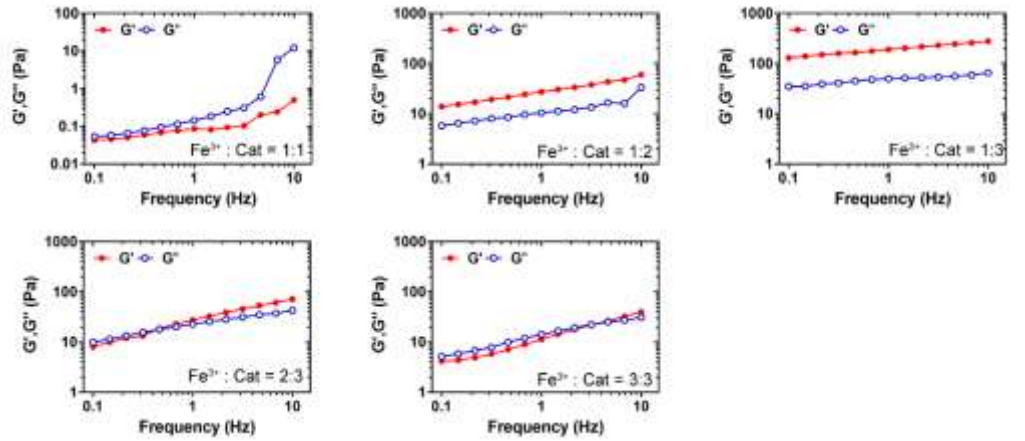


Figure S2. Oscillatory frequency sweep of Fe^{3+}-induced HA-Cat gels at different molar ratios between Fe^{3+} : Cat (Frequency sweep performed at 5% strain).

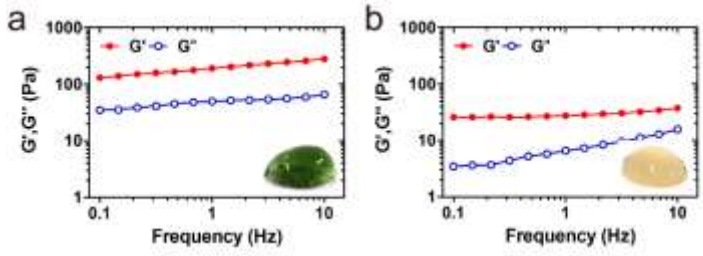


Figure S3. Oscillatory frequency sweep of Fe^{3+}-induced HA-Cat gels before (a) and after (b) EDTA treatment (Fe^{3+}:catechol molar ratio of 1:3, frequency sweep performed at 5% strain, pH: 3.5).

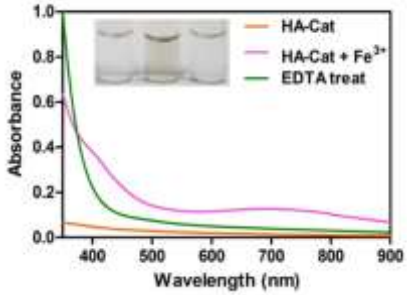


Figure S4. UV-Vis spectra of Fe^{3+}-induced HA-Cat solution (pH: 3.5) before and after EDTA treatment.

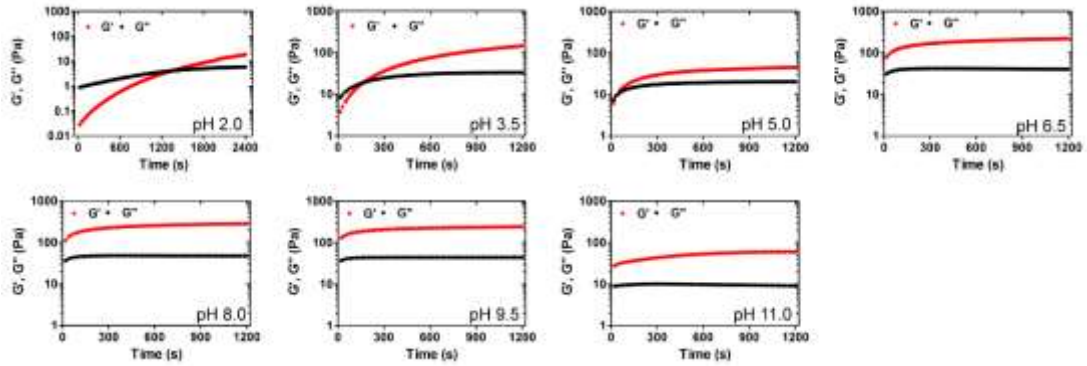


Figure S5. Oscillatory time sweep of Fe^{3+}-induced HA-Cat gels at different pH values (Fe^{3+}:catechol molar ratio of 1:3, time sweep performed at a strain of 5% and frequency of 1Hz).

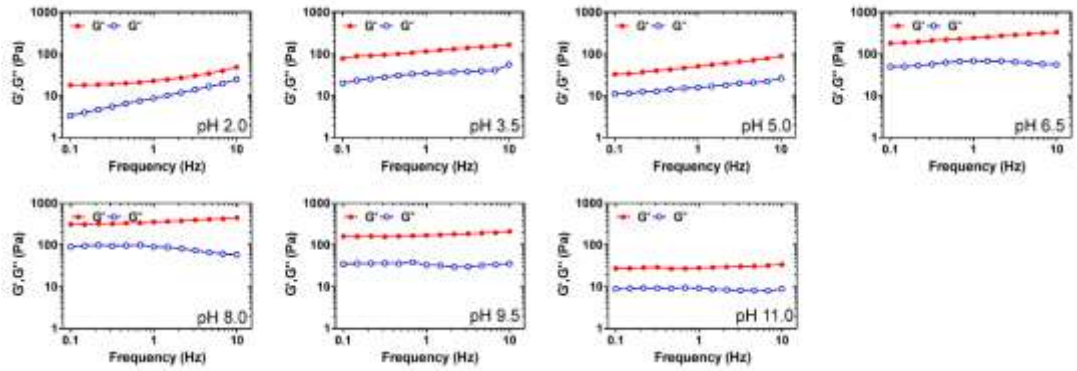


Figure S6. Oscillatory frequency sweep of Fe^{3+}-induced HA-Cat gels at different pH values (Fe^{3+}:catechol molar ratio of 1:3, frequency sweep performed at 5% strain).

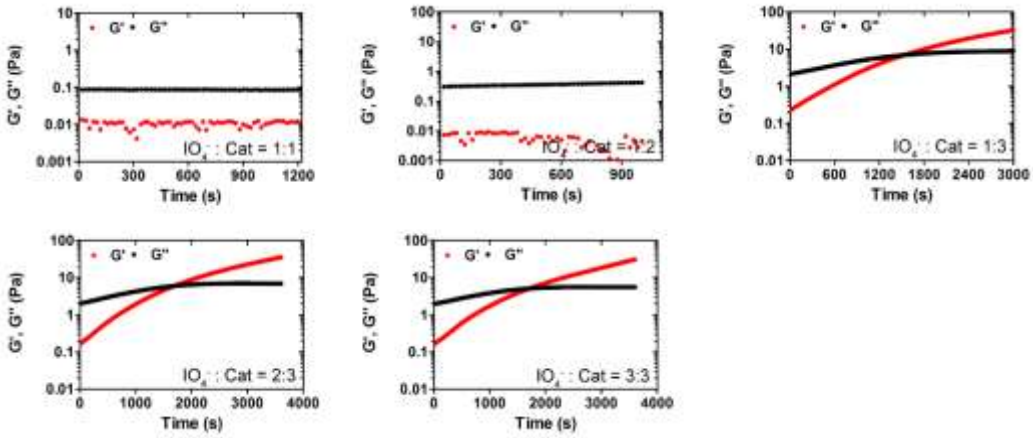


Figure S7. Oscillatory time sweep of $NaIO_4$-induced HA-Cat gels at different molar ratios between IO_4^- : Cat (Time sweep performed at a strain of 5% and frequency of 1Hz).

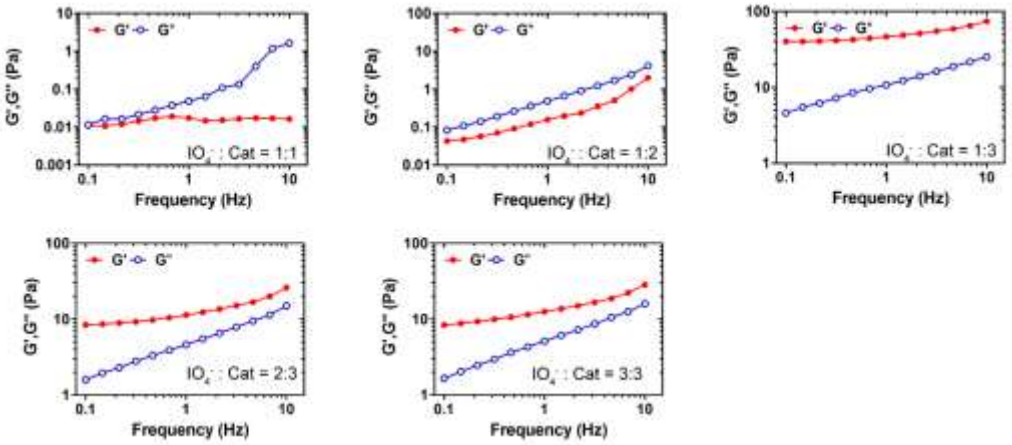


Figure S8. Oscillatory frequency sweep of $NaIO_4$-induced HA-Cat gels at different molar ratios between IO_4^- : Cat (Frequency sweep performed at 5% strain).

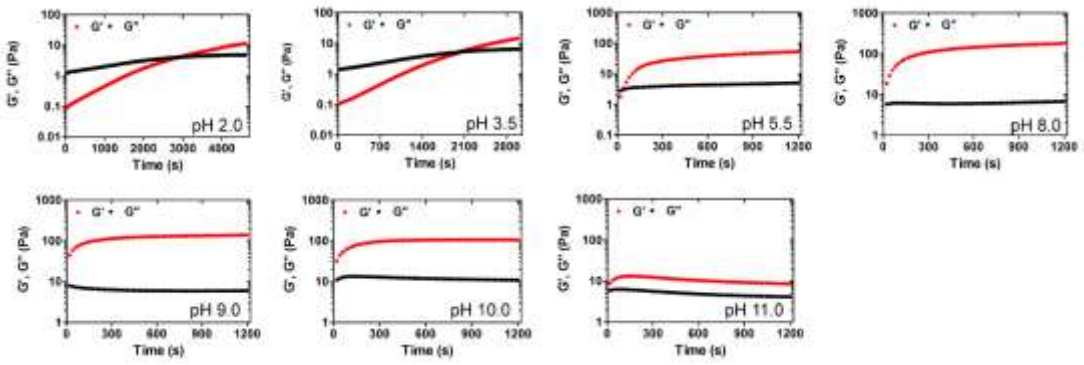


Figure S9. Oscillatory time sweep of $NaIO_4$-induced HA-Cat gels at different pH values

(IO_4^-:catechol molar ratio of 1:3, time sweep performed at a strain of 5% and frequency of 1Hz).

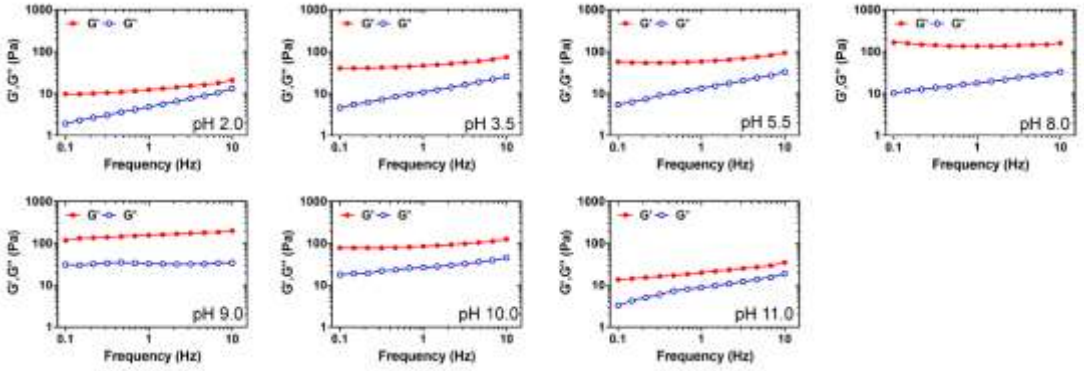


Figure S10. Oscillatory frequency sweep of $NaIO_4$-induced HA-Cat gels at different pH values

(IO_4^-:catechol molar ratio of 1:3, frequency sweep performed at 5% strain).

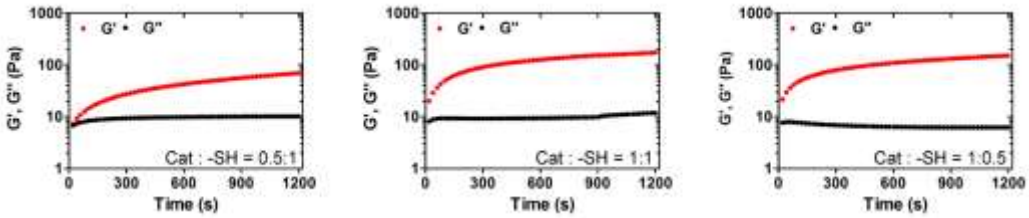


Figure S11. Oscillatory time sweep of HA-Cat & HA-SH gels at different molar ratios between Cat : -SH (Time sweep performed at a strain of 5% and frequency of 1Hz).

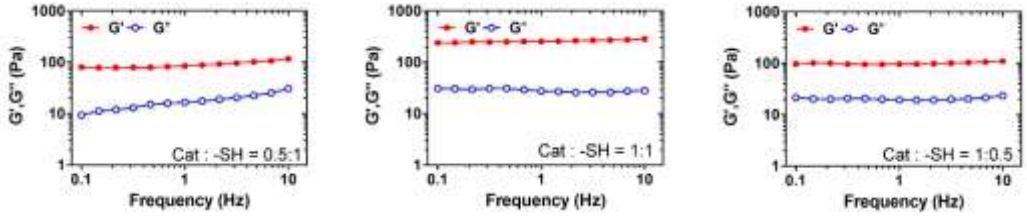


Figure S12. Oscillatory frequency sweep of HA-Cat & HA-SH gels at different molar ratios

between Cat : -SH (Frequency sweep performed at 5% strain).

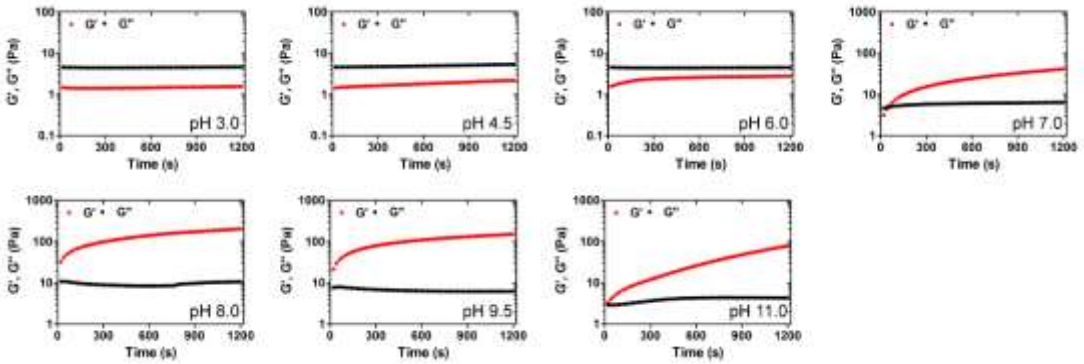


Figure S13. Oscillatory time sweep of HA-Cat & HA-SH gels at different pH values

(Catechol:thiol molar ratio of 1:1, time sweep performed at a strain of 5% and frequency of 1Hz).

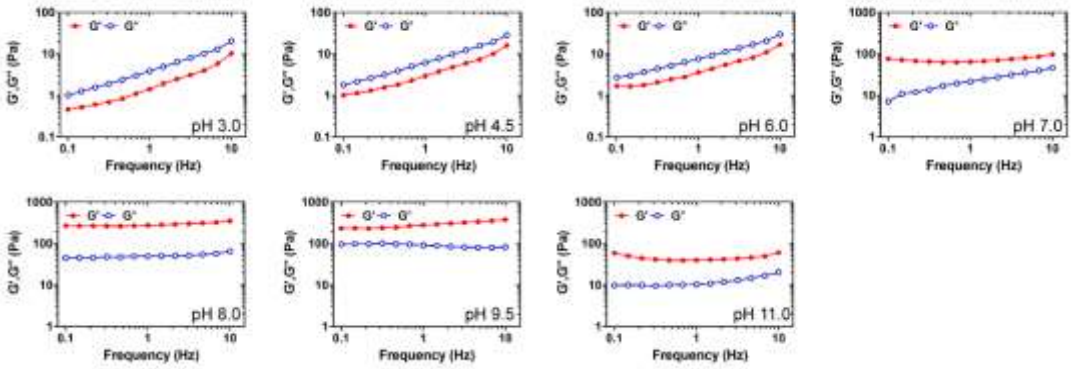


Figure S14. Oscillatory frequency sweep of HA-Cat & HA-SH gels at different pH values

(Catechol:thiol molar ratio of 1:1, frequency sweep performed at 5% strain).

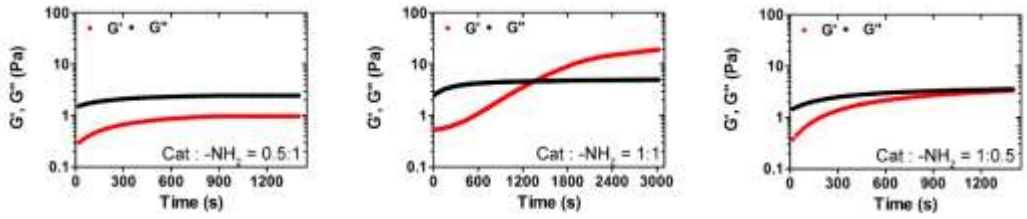


Figure S15. Oscillatory time sweep of HA-Cat & HA-NH_2 gels at different molar ratios between Cat : -NH_2 (Time sweep performed at a strain of 5% and frequency of 1Hz).

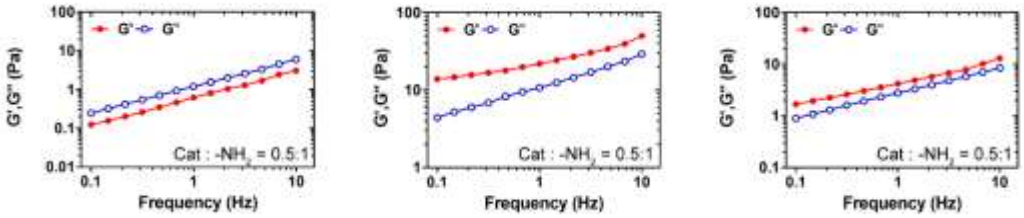


Figure S16. Oscillatory frequency sweep of HA-Cat & HA-NH_2 gels at different molar ratios between Cat : -NH_2 (Frequency sweep performed at 5% strain).

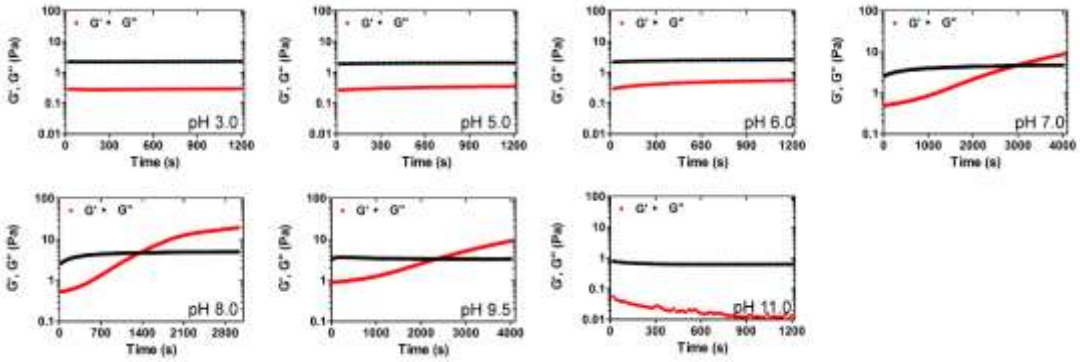


Figure S17. Oscillatory time sweep of HA-Cat & HA-NH_2 gels at different pH values (Catechol:amine molar ratio of 1:1, time sweep performed at a strain of 5% and frequency of 1Hz).

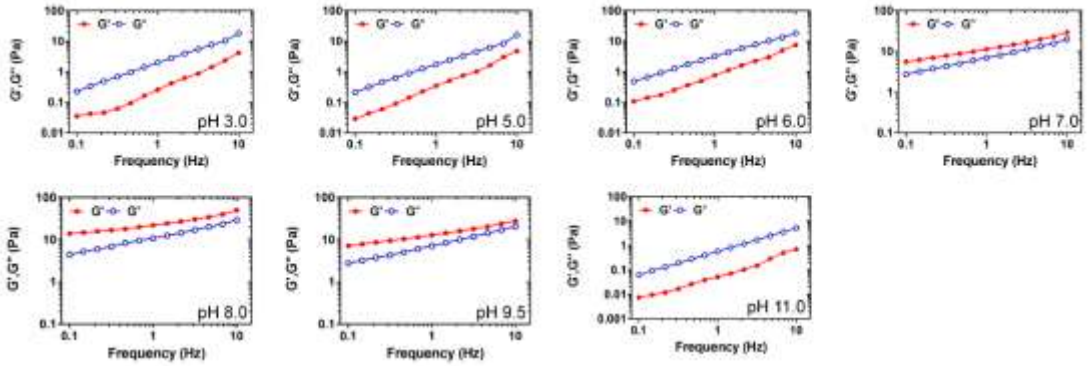


Figure S18. Oscillatory frequency sweep of HA-Cat & HA-NH_2 gels at different pH values (Catechol:amine molar ratio of 1:1, frequency sweep performed at 5% strain).

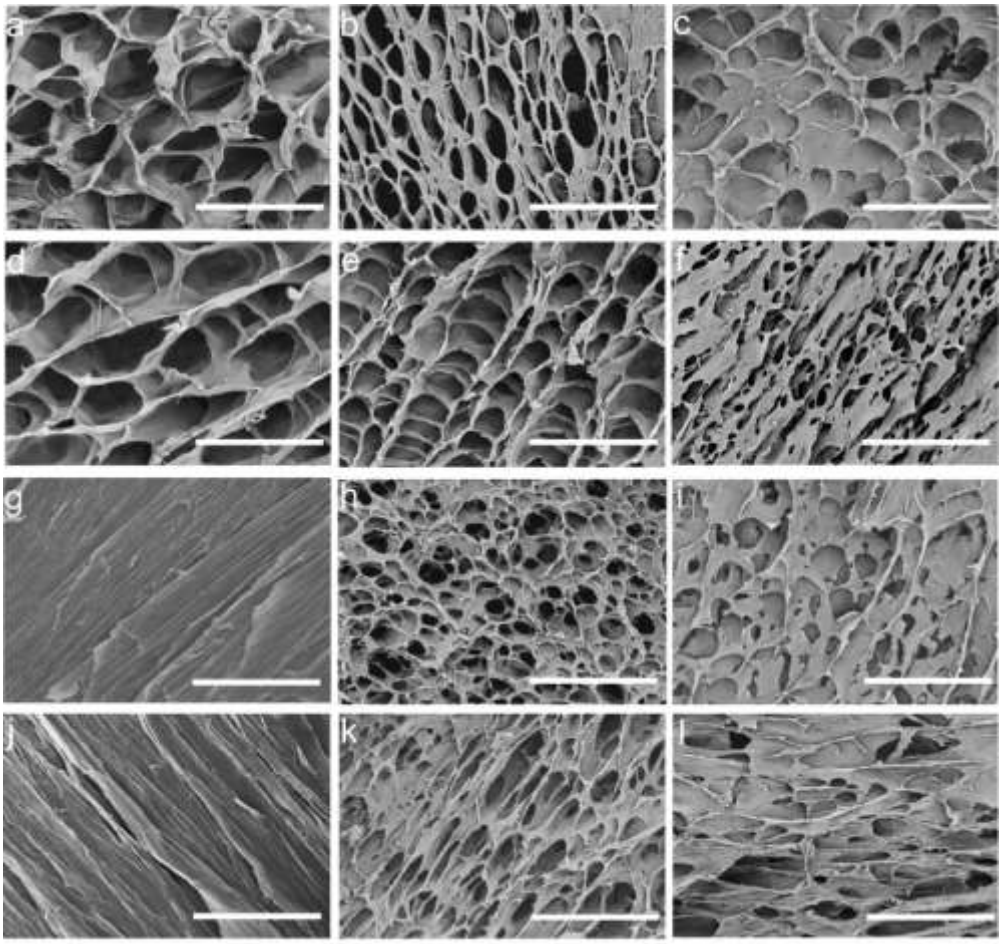


Figure S19. SEM images of hyaluronic acid (HA) hydrogels (scale bars: 100 μm). Fe^{3+}-induced HA-Cat gels at pH 3.5 (a), pH 8.0 (b), pH 10.0 (c); $NaIO_4$-induced HA-Cat gels at pH 3.5 (d), pH 8.0 (e), pH 10.0 (f); HA-Cat & HA-SH gels at pH 3.5 (g), pH 8.0 (h), pH 10.0 (i); HA-Cat & $HA\text{-}NH_2$ gels at pH 3.5 (j), pH 8.0 (k), pH 10.0 (l).

Figure S20. Representative image of lap shear test.