Supporting Information

Shear-thinning hyaluronan-based fluid hydrogels to modulate viscoelastic properties of osteoarthritis synovial fluids

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Experimental section

FT-IR spectroscopy

Fourier-transform infrared (FT-IR) spectra were recorded with a Perkin Elmer Spectrum 100 FT-IR spectrometer (Perkin Elmer, USA) in the range of 4000 to 400 cm^{-1} using the KBr−disk method. HA, HA-VS, and freeze-dried HA-VS/SH-2-PEG gel were mixed with the KBr powder. The mixed powder were compressed into disks for measurements.

NMR spectroscopy

The ^{1}H-NMR spectra of HA and HA-VS were recorded on an AVANCE III 400 spectrometer (Bruker, Switzerland) at room temperature in D_2O. The ^{1}H chemical shifts were referred to the solvent peak (δ = 4.8 ppm for HOD) used as an internal standard. The DS of HA-VS was also determined using ^{1}H-NMR spectroscopy. The ^{1}H-NMR spectra of HA hydrogel were recorded on the same instrument using tetramethylsilane (TMS) as internal reference and (D_2O/D_2SO_4 = 3:1) as solvent. Briefly, the freeze-dried HA-VS/SH-2-PEG gel was introduced into D_2O/D_2SO_4 (3:1) and then heated at 80°C for about 10 min. A portion of this solution was filtered directly into an NMR tube for measurement. All data processing was carried out with MestReNova software.

Swelling equilibrium of hydrogels

The as-obtained freeze-dried samples were immersed in PBS buffer (10 mM, pH=7.4) at 37°C in sealed vials. The hydrogel samples were taken periodically from the solution, and hydrogel weight was measured after removal of excess surface water with filter paper; the hydrogel samples were then returned to the vial until they had insufficient physical integrity to handle. The experiment was performed in triplicate. The swelling ratio of S_w can be calculated by measuring hydrogel weight in the swollen state and its corresponding dry weight (after lyophilization) by the following equation:

$$\text{Swelling ratio } (S_w) = (W_s - W_d)/W_d \times 100\%, \quad (1)$$

where W_s and W_d are the swollen weight and dry weight, respectively.

Surface morphology

The surface morphology and structure of freeze-dried HA-VS/SH-2-PEG samples were obtained by Nova NanoSEM 450 scanning electron microscopy (FEI, Netherlands). The sample was cross-sectioned and mounted on aluminum stubs with carbon tape, and then observed at 20 kV of electron acceleration under a low vacuum.

Injectability

The viscosupplement (HV-3) and hydrogel were loaded into syringes (1 mL), respectively, for injection tests. The injection forces of the HV-3 viscosupplement and the hydrogel were measured by unconfined, uniaxial compression tests using a TA-XT plus Texture Analyzer in compression mode (Stable Micro System Co., Britain). The maximum force during the testing was used as the injection force.

Optical microscopy of viscosupplements

Optical microscopy observations were made with a Leica DMLP polarizing optical microscope (Leica Microsystems, Germany). The samples were sandwiched between precleaned glasses during observation.

Degradation of viscosupplements

The degradation kinetics of the viscosupplements in the presence or absence of hyaluronidase (100 U/mL) were evaluated by comparing the evolution of the storage modulus (G′) as a function of time at 37°C on AR-G2. Briefly, at specified time intervals, the viscosupplements loaded on the geometry through a syringe needle were analyzed for G′ of each sample. The measurements were stopped when complete degradation occurred or the study terminated.

In vitro drug release from viscosupplements

TA is employed as a glucocorticoid to inhibit joint inflammation and pain in patients with osteoarthritis. TA-loaded HA-VS/SH-2-PEG hydrogel was prepared following the same procedures as described in "Formation of HA-Based Hydrogel (HA-VS/SH-2-PEG)". In brief, the HA-VS derivative was dissolved in TA acetate injection (2.5 mL at 10 mg/mL) to obtain an 8 mg/mL HA-VS solution, and the solution was neutralized to pH=7.4 by addition of NaOH solution (1 M). TA-loaded hydrogel was initiated by adding the SH-2-PEG compound to the hydrogel precursor solution (HA-VS solution). The TA-loaded hydrogel was broken up and passed through a sieve (1 mm) covered with a nylon cloth of 200 mesh. The microgels containing TA were then immersed in 0.8% HA solution at 37°C with continuous agitation (50 rpm) in the absence or presence of hyaluronidase (100 U/mL). At desired intervals, 200 μL of the solution was taken out to quantify the release and at the same time replaced with an equal volume of fresh HA solution. Subsequently, 200 μL of the solution was diluted by methanol/water (1:1) to form 3 mL of solution. The percentage of TA released was quantitatively analyzed using UV−vis spectroscopy and was calculated by interpolation from a standard calibration curve constructed from known concentrations of TA (methanol: water = 1:1). Three replicates were performed for each time point.

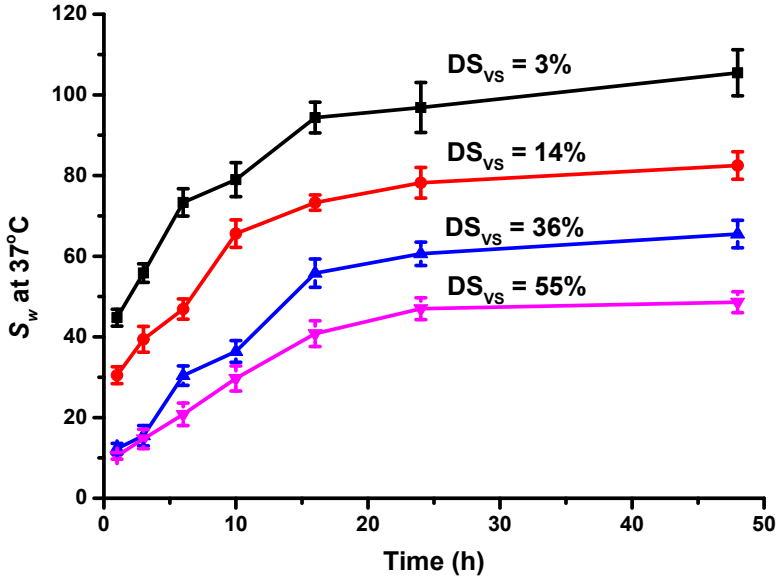

Figure S1 Swelling equilibrium experiments of hydrogels in PBS as a function of DS_{VS}.

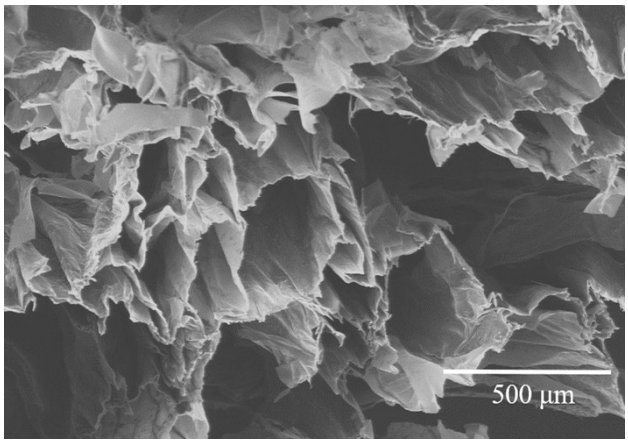

Figure S2. Typical SEM photographs (surface morphologies and cross-sectional morphologies) of freeze-dried HA-VS/SH-2-PEG hydrogel (DS_{VS} = 3%; M_w (SH-2-PEG) = 3400).

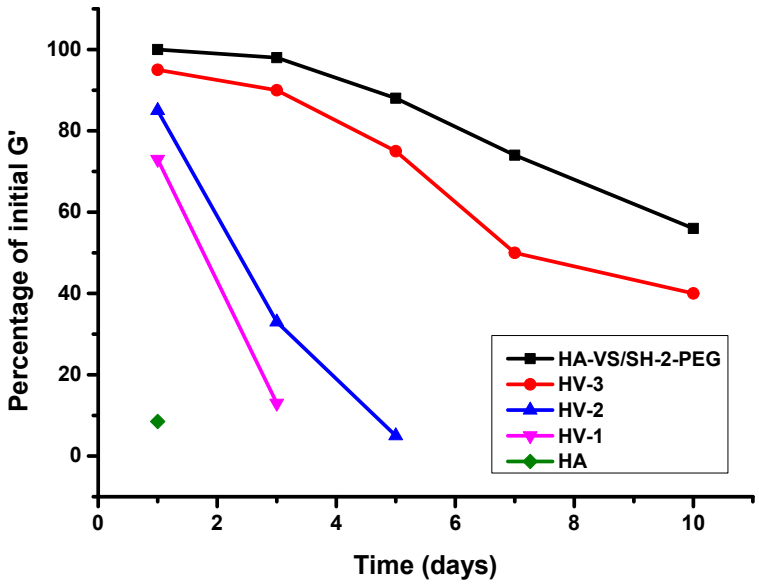

Figure S3 Degradation time dependence of G' of HA, viscosupplements, and hydrogel at 37 °C

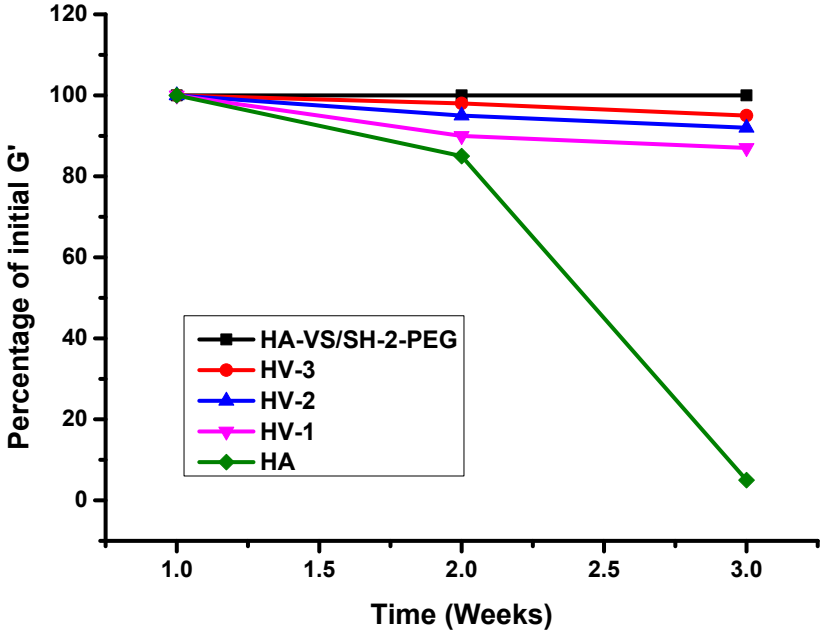

Figure S4 Degradation time dependence of G' of HA, viscosupplements, and hydrogel at 37 °C in the presence of hyaluronidase.

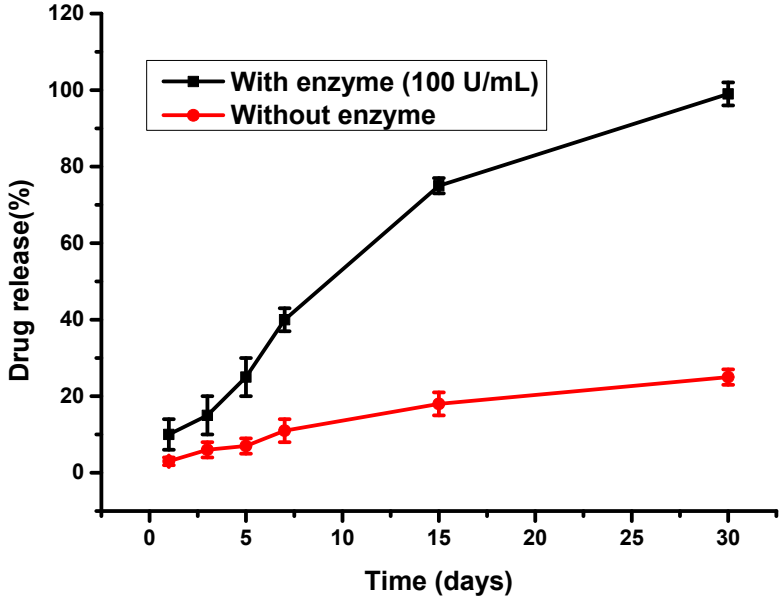

Figure S5 *In vitro* release test of TA from microgels (HV-1) in the absence or presence of hyaluronidase.