Supplementary Information

Migration of endothelial cells and mesenchymal stem cells into hyaluronic acid hydrogels with different modulus under induction of pro-inflammatory macrophages

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Calculation of hydrogels mesh size

The hydrogels network mesh sizes were calculated from swelling ratio. The average molecular weight of the effective chain of polymers ($\overline{Mc}$) could be calculated from the following equation [1].

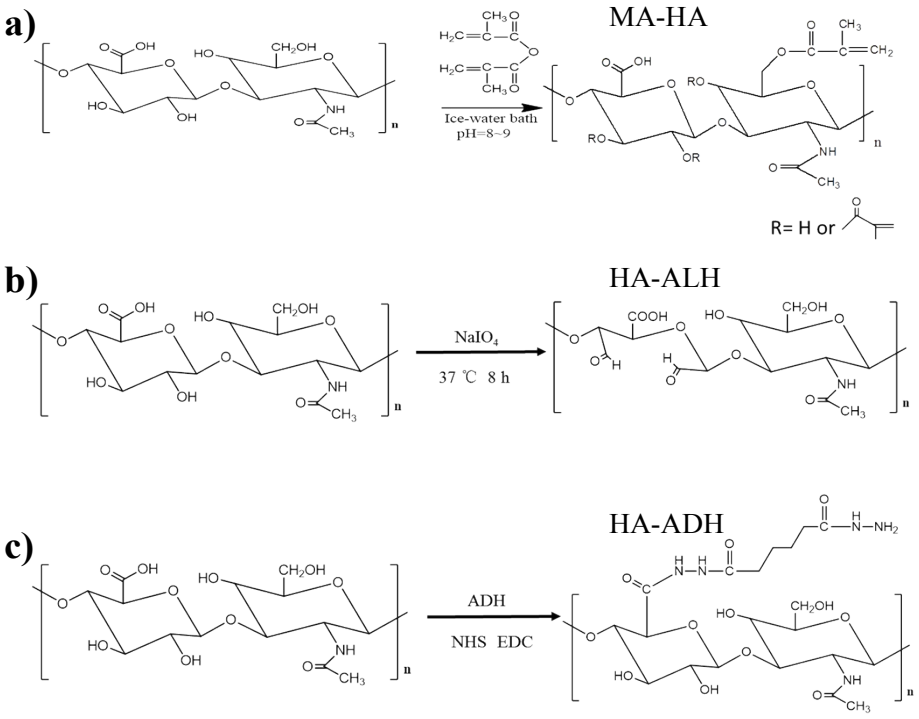
$$\overline{Mc} = \frac{Q^{5/3} V_1}{|0.5 - \chi_1| \bar{v}}$$

Where $\bar{v}$ is the specific volume of the polymer chain, Q is the volumetric swelling ratio of the hydrogel which is linearly related with weight swelling ratio (Q_w), V_1 is the molar volume of water (18 cm^3/mol), χ_1 is the Huggins coefficients of MA-HA. Typical values of Huggins coefficients for polymers with a coil structure, as linear polysaccharides, for instance, are near 0.6 according to the hydrodynamic theory of Riseman and Ullmann [2].

The average mesh size (ξ) can be approximately calculated by the following formula with the value of $\overline{Mc}$ [3]:

$$\xi = Q^{1/3} \times l \times \left(C_n \frac{2\overline{Mc}}{\overline{Mr}}\right)^{1/2}$$

Where C_n is the Flory characteristic ratio (4 for a concentrated polysaccharide solution [4]), l is the bond length along the polymer backbone (0.153 nm [5]), and Mr is the molecular weight of the repeating polymer unit (515 g/mol).



Scheme S1. Schematic illustration of the synthesis pathways for (a) MA-HA, (b) HA-ALH, and (c) HA-ADH.

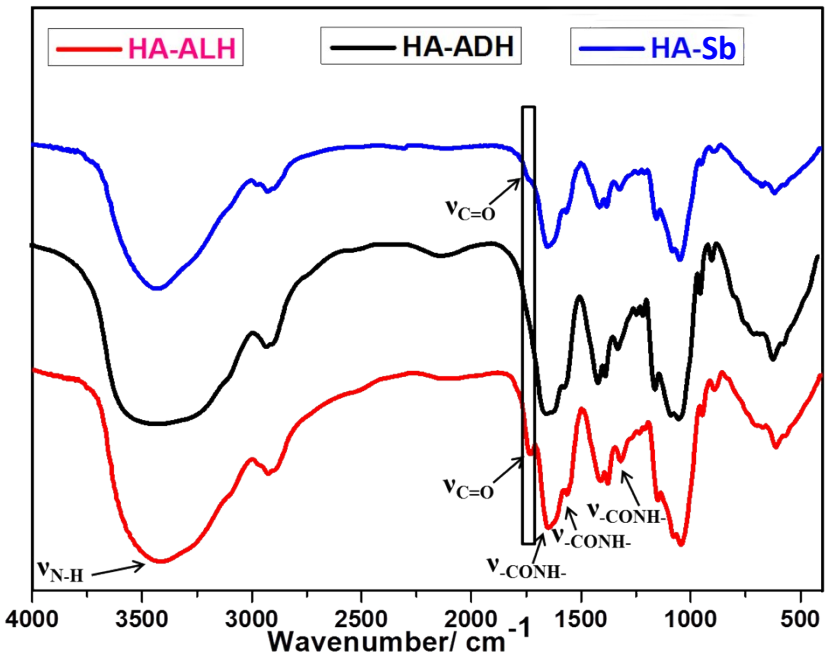


Figure S1. FTIR spectra of HA-ALH, HA-ADH and HA-Sb.

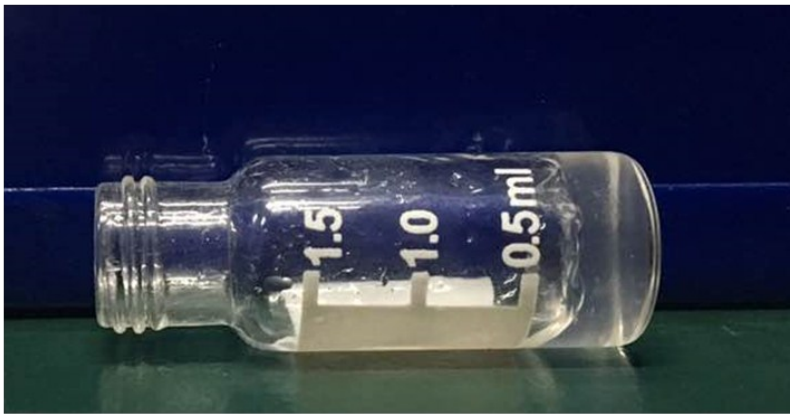


Figure S2. The picture of HA-Sb hydrogel formed in 5 M NaCl solution.

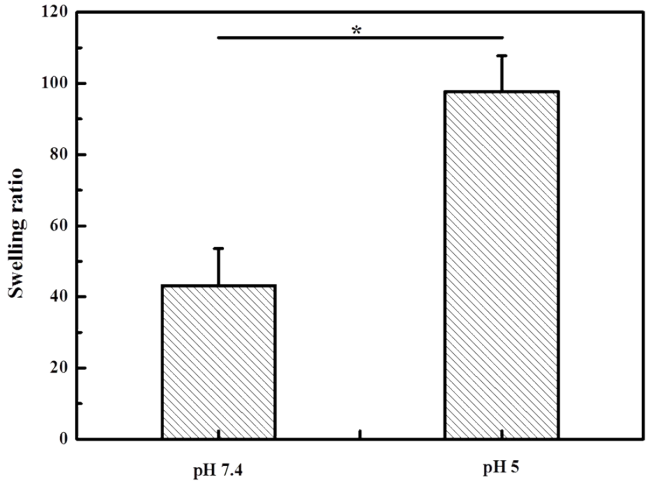


Figure S3. Swelling ratio of HA-Sb hydrogels being treated with pH 7.4 and pH 5 buffers, respectively. n=3, *p<0.05.

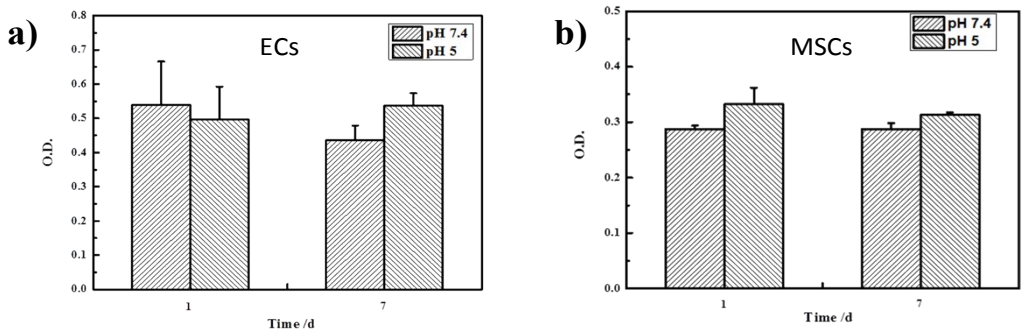


Figure S4. Viability of ECs and MSCs after being cultured for 1 d and 7 d on pH-responsive hydrogels being pretreated with pH 7.4 and pH 5 buffers, respectively. n=3.

For viability assay, 6.25×10^4/cm^2 ECs and MSCs cultured for 1 day and 7 days on the hydrogels were incubated in 1 mL complete DMEM medium containing 100 μL 5 mg mL^{-1} MTT solution at 37 ^{o}C for 4 h. The hydrogels were then cut into small pieces and placed into centrifugation tubes, into which 1 mL dimethyl sulphoxide (DMSO) was added to dissolve the formed formazan crystals for about 15 min. The solution was centrifuged at 1000 rpm for 5 min, and the absorbance of the supernatant, which was diluted twice, was measured at 565 nm by a microplate reader (M200 PRO, Tecan, Switzerland).

Table S1. Gene name, NCBI accession number and primer sequences for MSCs used in this study.

Rat Gene	NCBI Accession No.	Primer sequences
18s	NR_046237.1	Forward 5'-CCTTCGCTATCACTGCCATTA-3' Reverse 5'-GCTATACTTCCCATCCTTCACG-3'
Cdc42	XM_008764286.2	Forward 5'- GCTGTCAAGTATGTGGAGTGTT-3' Reverse 5'- GGCTCTGGAGATGCGTTCA-3'
Rac1	NM_031653.2	Forward 5'- CTGCCTGCTCATCAGTTACAC-3' Reverse 5'- CGTCTGTTTGCGGGTAGGA-3'
RhoA	NM_001314068.1	Forward 5'- TTCGGAGTCGTCGTCTTGAG-3' Reverse 5'- CCATCACCAACAATCACCAGTT-3'
Vinculin	NM_001107248.1	Forward 5'-CCGTGTGATGCTGGTGAAC-3' Reverse 5'-TGGCTTCAGTGTCCTTGCT-3'
Integrin β1	NM_017022.2	Forward 5'- TTACTTCAGACTTCCGCATTGG-3' Reverse 5'- CAGCAGTCGTGTTACATTCCT-3'
NM II	NM_001305877.1	Forward 5'-AAGAAGGTGAAGGTGAACAAGG-3' Reverse 5'-TGTCTGTGATGGCATAGATGTG-3'
HYAL 2	NM_172040.2	Forward 5'-CCTCAGAACGGTAGCCTCT-3' Reverse 5'-ACACTGGTCGCCATTCCT-3'
CD44	XM_006234627.3	Forward 5'-ACAACACAGAGTCAAGAGGATG-3' Reverse 5'-GCTAGATGGCAGAACAGAAGTT-3'

Table S2. Gene name, NCBI accession number and primer sequences for ECs used in this study.

Human Gene	NCBI Accession No.	Primer sequences
18s	NM_022551	Forward 5'-ATCACCATTATGCAGAATCCACG-3'
		Reverse 5'-GACCTGGCTGTATTTTCCATCC-3'
Cdc42	NM_044472	Forward 5'- CCATCGGAATATGTACCGACTG-3'
		Reverse 5'- CTCAGCGGTCGTAATCTGTCA-3'
Rac1	NM_018890	Forward 5'- ATGTCCGTGCAAAGTGGTATC-3'
		Reverse 5'- CTCGGATCGCTTCGTCAAACA-3'
RhoA	NM_001664	Forward 5'- AGCCTGTGGAAAGACATGCTT-3'
		Reverse 5'- TCAAACACTGTGGGCACATAC-3'
Vinculin	NM_003373	Forward 5'- CTCGTCCGGGTTGGAAAAGAG-3'
		Reverse 5'- AGTAAGGGTCTGACTGAAGCAT-3'
Integrin β1	NM_002211.4	Forward 5'-CCTACTTCTGCACGATGTGATG-3'
		Reverse 5'- CCTTTGCTACGGTTGGTTACATT-3'
NM II	NM_002473.5	Forward 5'-CAGCAAGCTGCCGATAAGTAT-3'
		Reverse 5'- CTTGTCGGAAGGCACCCAT-3'
HYAL 2	NM_033158	Forward 5'-GAGCACTACATTCGGACACAG-3'
		Reverse 5'- GATAACCGGCGATACACATCTT-3'
CD44	NM_000610.3	Forward 5'-TACAGCATCTCTCGGACGGA-3'
		Reverse 5'- CACCCCTGTGTTGTTTGCTG-3'

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