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 (\( \overline{M_c} \)) could be calculated from the following equation \(^1\).

\[
\overline{M_c} = \frac{Q^{5/3} V_1}{|0.5 - \chi_1|^\nu}
\]

Where \( \bar{\theta} \) 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), \( \chi_1 \) 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 \( (\xi) \) can be approximately calculated by the following formula with the value of \( \overline{M_c} \) \(^3\):

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

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 °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>
<thead>
<tr>
<th>Rat Gene</th>
<th>NCBI Accession No.</th>
<th>Primer sequences</th>
</tr>
</thead>
</table>
| **18s** | NR_046237.1       | Forward 5'-CCTTCGCTATCACTGCCATTA-3’  
Reverse 5'-GCTATACTCCCCATCTTTCAGC-3’ |
| **Cdc42** | XM_008764286.2    | Forward 5'-GCTGTCAGATATGTGGAGTGTT-3’  
Reverse 5'-GGCTCTGGAGATGCGTTCA-3’ |
| **Rac1** | NM_031653.2       | Forward 5'-GTCCTGTCTCATCAGTTACAC-3’  
Reverse 5'-GTGCTGCTGGGATGCGTTCA-3’ |
| **RhoA** | NM_001314068.1    | Forward 5'-TTGGAGATGCGTTCA-3’  
Reverse 5'-CCATCAACCAAAATCACCAGTT-3’ |
| **Vinculin** | NM_001107248.1   | Forward 5'-CCGTGTGATGCTGGTGTAAC-3’  
Reverse 5'-TGGCTTCAGTGTCCTTGCT-3’ |
| **Integrin β1** | NM_017022.2       | Forward 5'-TTCAGGATAGGCTGGTGTAAC-3’  
Reverse 5'-CAGCAGTCTTGTGCTTACCTCCT-3’ |
| **NM II** | NM_001305877.1    | Forward 5'-TTCAGGATAGGCTGGTGTAAC-3’  
Reverse 5'-TGGCTTCAGTGTCCTTGCT-3’ |
| **HYAL 2** | NM_172040.2       | Forward 5'-ACACTGGTGGCCATCTTCC-3’  
Reverse 5'-ACACTGGTGGCCATCTTCC-3’ |
| **CD44** | XM_006234627.3    | Forward 5'-ACAACACAGAGTCAAGAGGT-3’  
Reverse 5'-GCTAGATGGCCAGAAGAGGT-3’ |

Table S1. Gene name, NCBI accession number and primer sequences for MSCs used in this study.
Table S2. Gene name, NCBI accession number and primer sequences for ECs used in this study.

<table>
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<th>Human Gene</th>
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<th>Primer sequences</th>
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| 18s        | NM_022551          | Forward 5’-ATCACCTATTATGCAGAATCCACG-3’  
Reverse 5’-GACCTGGCTGTATTTTCATCC-3’ |
| Cdc42      | NM_044472          | Forward 5’-CCATCGGAATATGTACCGACTG-3’  
Reverse 5’-CTCAGCGGTCGTAATCTGTCA-3’ |
| Rac1       | NM_018890          | Forward 5’-ATGTCCTGTGCAAGATGTCTT-3’  
Reverse 5’-CTCGGATCGCTTCGTAATAACA-3’ |
| RhoA       | NM_001664          | Forward 5’-AGCCTGTGGAAAGACATGCTT-3’  
Reverse 5’-CTCAGCGGTCGTAATCTGTCA-3’ |
| Vinculin   | NM_003373          | Forward 5’-AGTAAGGGTTGGAGACATGCTT-3’  
Reverse 5’-CTCAGCGGTCGTAATCTGTCA-3’ |
| Integrin β1| NM_002211.4        | Forward 5’-CCTACTTCTGACAGATGTGATG-3’  
Reverse 5’-CCTTTGCTACGTTGTTACATT-3’ |
| NM II      | NM_002473.5        | Forward 5’-CAGCAAGCTGGCCTAGTGATG-3’  
Reverse 5’-CTTGTCGGAAGGCACCCAT-3’ |
| HYAL 2     | NM_033158          | Forward 5’-GAGCACTACATTCGGAACAGCAG-3’  
Reverse 5’-GAATACGCGGATACACATCTT-3’ |
| CD44       | NM_000610.3        | Forward 5’-TACAGCATCTTCGGAACAGCAG-3’  
Reverse 5’-CACCCTGTTGTGTTTGTCTG-3’ |
References:


