Supporting Information

Fabrication of Cell-Compatible Hyaluronan Hydrogels with a Wide Range of Biophysical Properties Through High Tyramine Functionalization

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Synthesis of HA-Tyr conjugates by conventional carbodiimide chemistry

HA-Tyr was prepared by amidation of the carboxylic acid groups of HA with the amine groups of Tyr as described previously. HANa (500 mg, 1.25 mmol carboxyl groups) was hydrated in MES Buffer (100 mM, pH 5.5, adjusted with NaOH 5 M) for 24 h at a final concentration of 1% (w/v). 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and N-hydroxysuccinimide (NHS) (1/1 to HA) were added and stirred for 1 h at RT. Tyr (1/1 to HA) was dissolved in MES-buffer (100 mM, pH 5.5; 0.1% w/v) and added drop-wise to the HA-solution. The reaction was maintained at RT for 24 h under continuous stirring. Products were precipitated by 96% ethanol. For two-step HA-Tyr conjugation, the isolated HA-Tyr product was isolated and rehydrated in 100 mM MES-buffer (pH 5.5) and the conjugation repeated. Note, that the product after 48 h was highly viscous when dissolved in PBS (1% w/v). This may be caused by a high percentage of auto-crosslinked EDC-Tyr adducts, as shown by improved solubility in alkaline conditions (pH 8).

Figure S1. 1H NMR spectrum of the physical mixture MES, DMTMM and Tyr (0.025 mmol each, without additional chemical modifications) in D2O. Measurements were taken after 24 h incubation at RT.
Figure S2. $^1$H NMR spectrum of the conjugate HA-Tyr synthesized in 100 mM NaCl at 37 °C for 24 h.
**Figure S3.** Swelling of HA-Tyr hydrogels with different DS as a function of H$_2$O$_2$ concentration. Hydrogels were formed with 0.5 U/ml HRP. Data shown as mean ± SD for n = 3 constructs, **p<0.01 for DS 6.5% compared to DS 16 and 21%.

**Figure S4.** Quantification of cell viability of MSCs (5x10$^6$/ml) encapsulated in HA-Tyr hydrogels crosslinked with (a) 0.68 mM H$_2$O$_2$ and (b) 1.1 mM H$_2$O$_2$, after 2, 4 and 6 days of culture in basal medium (DMEM plus 10% FBS), mean + SD of four randomly chosen locations per hydrogel. One representative donor shown with mean + SD of n = 4 randomly chosen locations within the constructs.
Figure S5. Representative images of hMSCs encapsulated in HA-Tyr hydrogels (5x10^6/ml) crosslinked with 0.68 mM and 1.1 mM H_2O_2. Calcein (5 μM, green) /Ethidium homodimer (8 μM, red) staining after 6 days of culture. Images are shown as 3D rendered z-stacks with a thickness of 200 μm, Scale bar 200 μm.

Figure S6. Representative images of encapsulated hMSCs in HA-Tyr (DS 16, 0.68 mM H_2O_2) after 6 days of culture without FBS and staining with Calcein (5 μM, green) /Ethidium homodimer (8 μM, red). Images are shown as 3D rendered z-stacks with a thickness of 200 μm. Scale bar 200 μm.
Figure S7. Representative images of encapsulated hMSCs in HA-Tyr \textit{in-situ} modified with 500 μM RGD-Tyrosine (Genscript) after 6 days of culture and staining with Calcein (5 μM, green) /Ethidium homodimer (8 μM, red). Images are shown as 3D rendered z-stacks with a thickness of 200 μm. Scale bar 200 μm.

Figure S8. (a) Quantification of CD44 expression intensity and (b) representative images of hMSC after incubation with anti-CD44 antibody (abcam 10-44-2). 5x10^6 cells/ml, encapsulated in DS 16% HA-Tyr hydrogels crosslinked with 1 U/ml HRP and 0.68 mM H_2O_2. Cell-laden hydrogels were fixed 30 min after encapsulation with 4% paraformaldehyde (overnight at 4 °C) and washed three times prior incubation with anti-CD44-Allophycocyanin (APC) antibody (Miltenyi Biotec 130-095-177). Confocal images were taken using a Zeiss LSM510 (10x objective). Scale bar 200 μm.
Figure S9. Quantification of cell viability of encapsulated hMSCs (5x10^6/ml, 0.68 mM H_2O_2), blocked with CD44 antibody prior encapsulation compared to un-treated MSCs (control), after (a) 2 days, (b) 4 days and (c) 6 days of culture in basal medium (DMEM plus 10% FBS). One representative donor shown with mean + SD of n = 4 randomly chosen locations within the construct.
Figure S10. (a) Representative images of encapsulated hMSCs treated with CD44 antibody prior encapsulation and stained with Calcein (5 μM, green) /Ethidium homodimer (8 μM, red) after 6 days of culture. Images are shown as 3D rendered z-stacks with a thickness of 200 μm. Dashed box shows a zoom-in of the corresponding area, scale bars are 200 μm. (b) Quantification of cell aspect ratio of encapsulated hMSCs (5x10⁶/ml, 0.68 mM H₂O₂), blocked with CD44 antibody prior encapsulation compared to un-treated MSCs (control), after 6 days of culture in basal medium (DMEM plus 10% FBS). One representative donor shown with mean + SD of n = 4 randomly chosen locations within the construct (≥50 cells per image), *p<0.05.

REFERENCES