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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 previous.[1] 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).

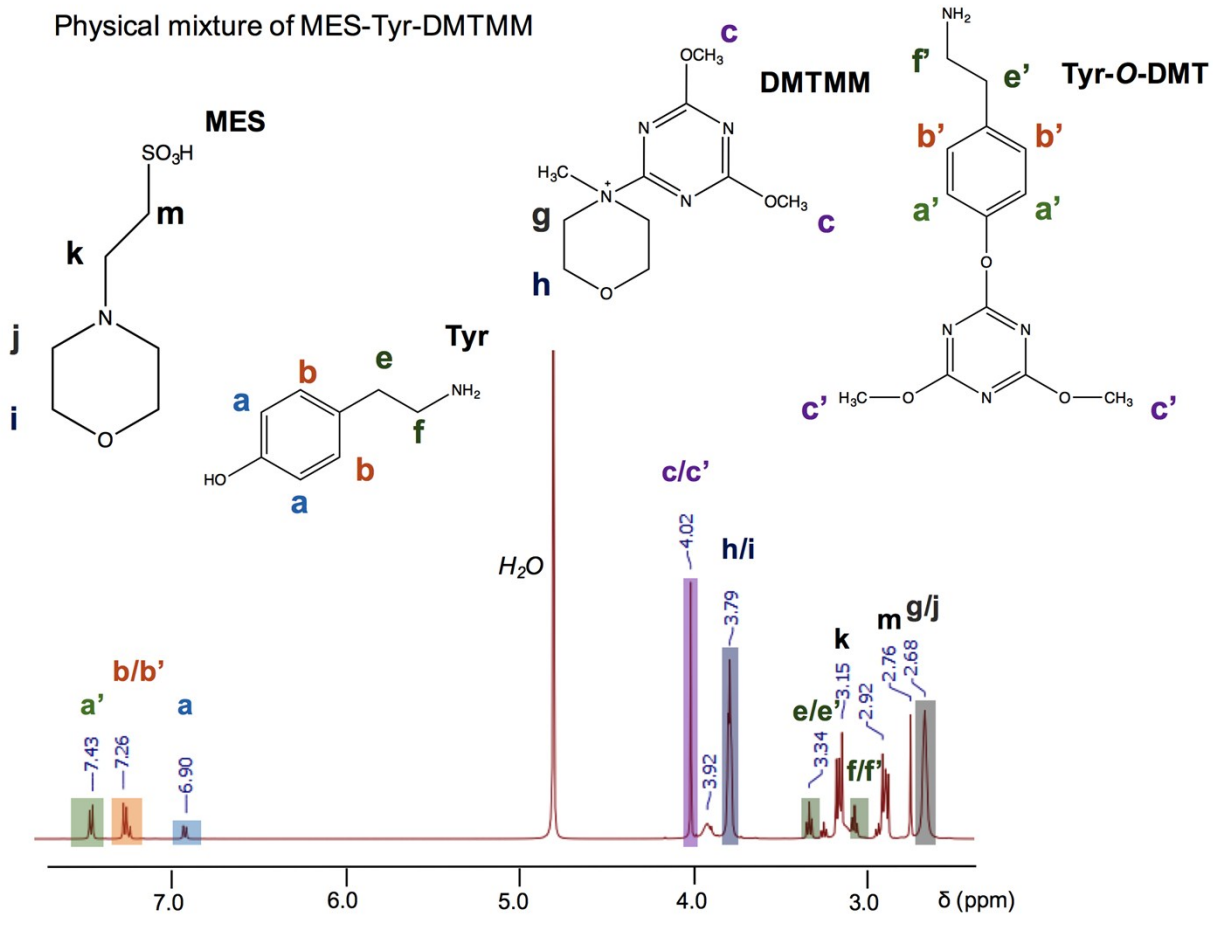

Figure S1. ^{1}H NMR spectrum of the physical mixture MES, DMTMM and Tyr (0.025 mmol each, without additional chemical modifications) in D_2O. Measurements were taken after 24 h incubation at RT.

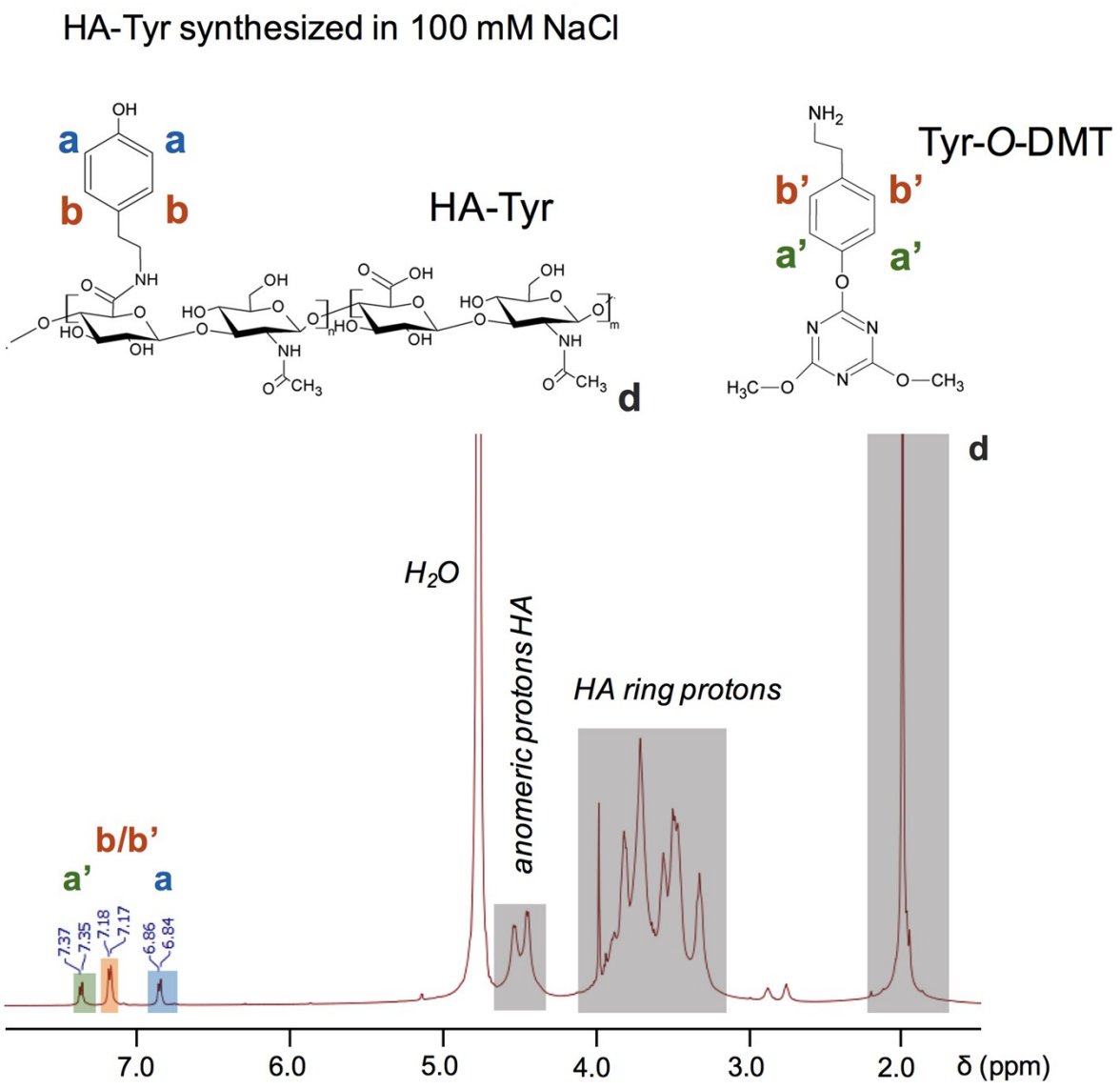

Figure S2. ^{1}H NMR spectrum of the conjugate HA-Tyr synthesized in 100 mM NaCl at 37 °C for 24 h.

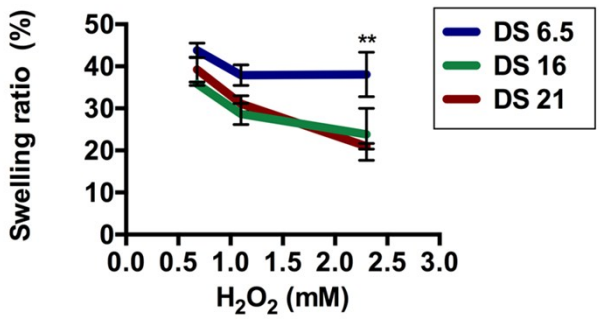

Figure S3. Swelling of HA-Tyr hydrogels with different DS as a function of H_2O_2 concentration. Hydrogels were formed with 0.5 U/ml HRP. Data shown as mean ± SD for n = 3 constructs, **p<0.01 for DS 6.5% compared to DS 16 and 21%.

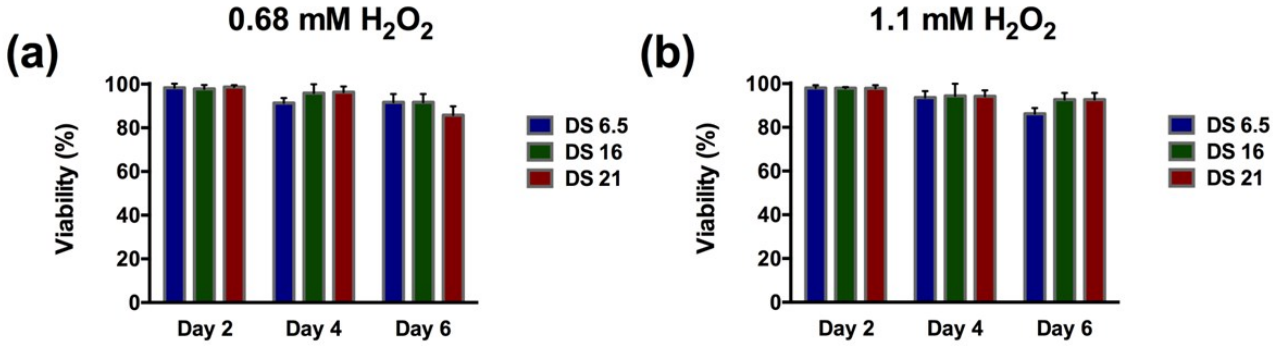

Figure S4. Quantification of cell viability of MSCs ($5x10^6$/ml) encapsulated in HA-Tyr hydrogels crosslinked with **(a)** 0.68 mM H_2O_2 and **(b)** 1.1 mM H_2O_2, after 2, 4 and 6 days of culture in basal medium (DMEM plus 10% FBS), mean + SD of four randomly chosen locations per hydrogel. One representative donor shown with mean + SD of n = 4 randomly chosen locations within the constructs.

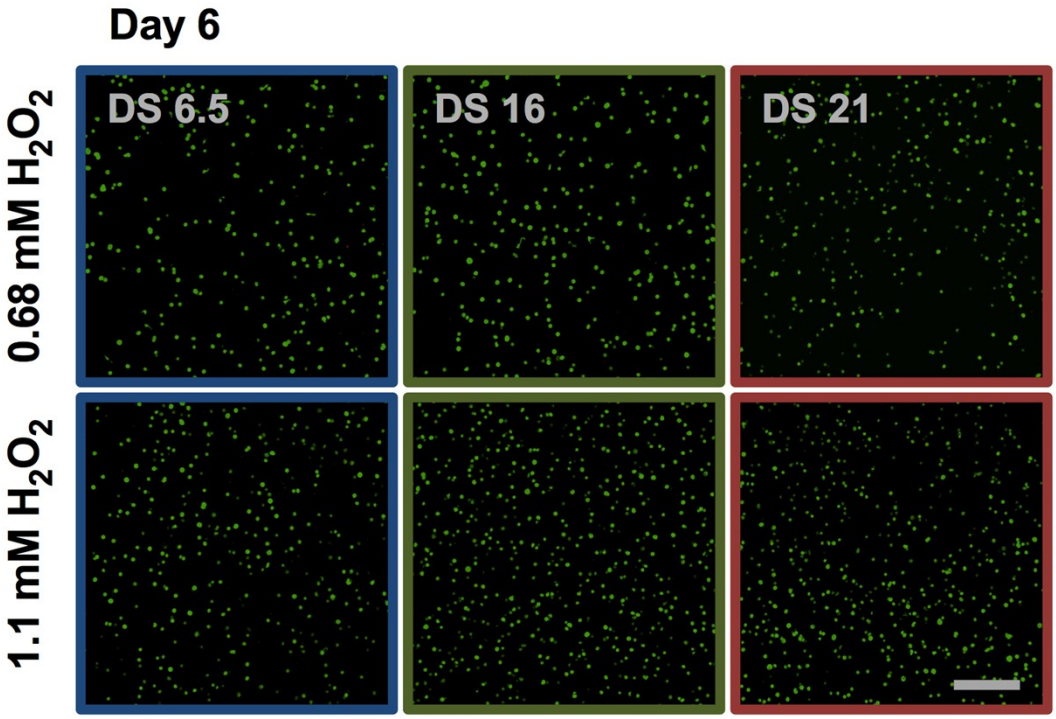

Figure S5. Representative images of hMSCs encapsulated in HA-Tyr hydrogels ($5x10^6$/ml) crosslinked with 0.68 mM and 1.1 mM H_2O_2 . Calcein (5 µM, green) /Ethidium homodimer (8 µM, red) staining after 6 days of culture. Images are shown as 3D rendered z-stacks with a thickness of 200 µm, Scale bar 200 µm.

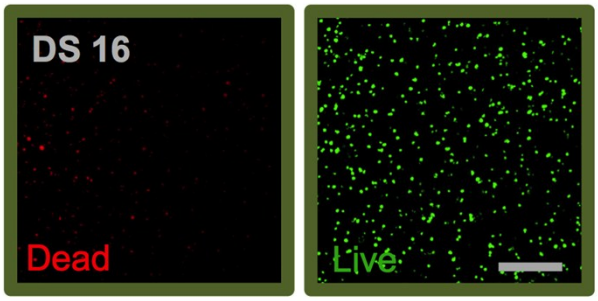

Figure S6. Representative images of encapsulated hMSCs in HA-Tyr (DS 16, 0.68 mM H_2O_2) after 6 days of culture without FBS and staining with Calcein (5 µM, green) /Ethidium homodimer (8 µM, red). Images are shown as 3D rendered z-stacks with a thickness of 200 µm. Scale bar 200 µm.

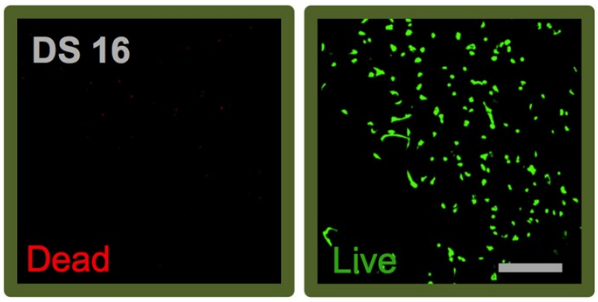

Figure S7. Representative images of encapsulated hMSCs in HA-Tyr *in-situ* modified with 500 µM RGD-Tyrosine (Genscript) after 6 days of culture and staining with Calcein (5 µM, green) /Ethidium homodimer (8 µM, red). Images are shown as 3D rendered z-stacks with a thickness of 200 µm. Scale bar 200 µm.

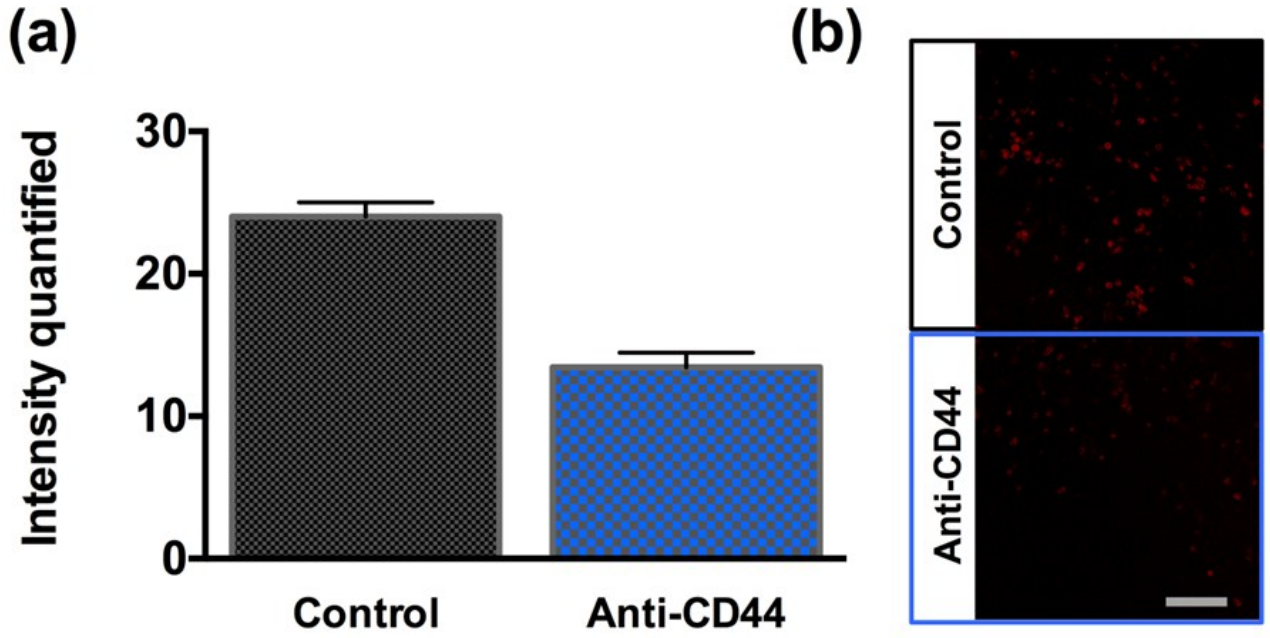

Figure S8. (a) Quantification of CD44 expression intensity and **(b)** representative images of hMCS after incubation with anti-CD44 antibody (abcam 10-44-2). 5x10^{6}cells/ml, encapsulated in DS 16% HA-Tyr hydrogels crosslinked with 1 U/ml HRP and 0.68 mM H_2O_2. Cell-laden hydrogels were fixed 30 min 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.

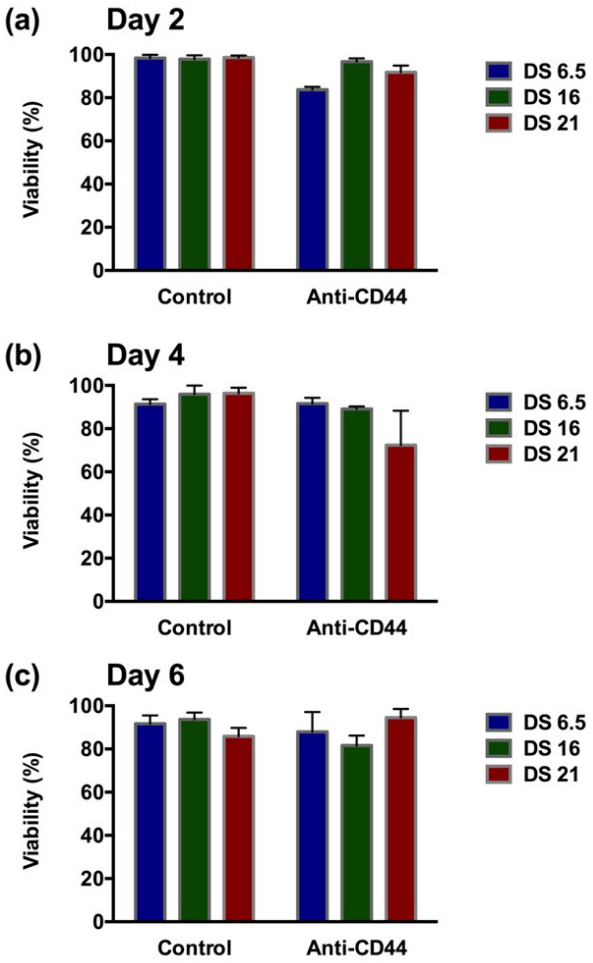

Figure S9. Quantification of cell viability of encapsulated hMSCs ($5x10^6$/ml, 0.68 mM H_2O_2), blocked with CD44 antibody prior encapsulation compared to un-treated MSCs (control), after **(a)** 2 days, **(b)** 4 days and **(c)** 6 days of culture in basal medium (DMEM plus 10% FBS). One representative donor shown with mean + SD of n = 4 randomly chosen locations within the construct.

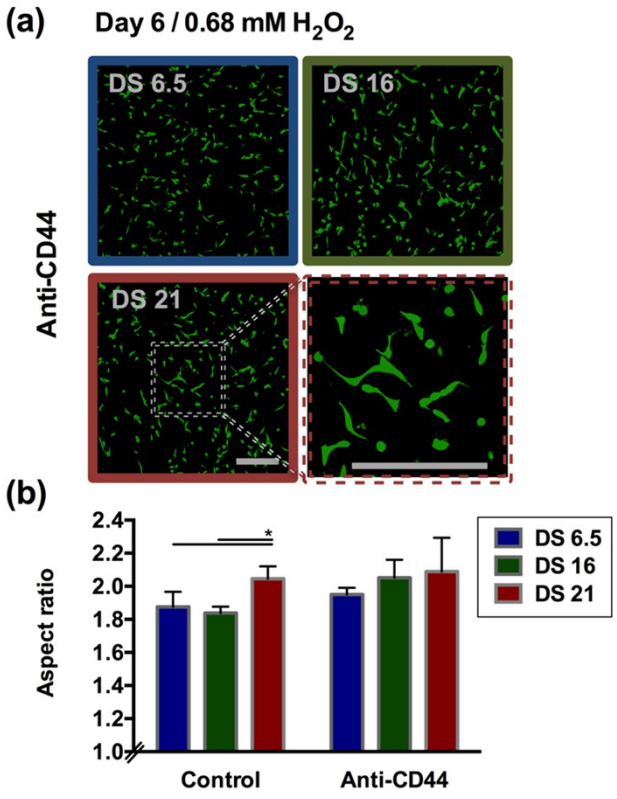

Figure S10. (a) Representative images of encapsulated hMSCs treated with CD44 antibody prior encapsulation and stained with Calcein (5 μM, green) /Ethidium homodimer (8 μM, red) after 6 days of culture. Images are shown as 3D rendered z-stacks with a thickness of 200 μm. Dashed box shows a zoom-in of the corresponding area, scale bars are 200 μm. **(b)** Quantification of cell aspect ratio of encapsulated hMSCs ($5x10^6$/ml, 0.68 mM H_2O_2), 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

1. C. Loebel, M. D'Este, M. Alini, M. Zenobi-Wong and D. Eglin, *Carbohydr Polym*, 2015, **115**, 325-333.