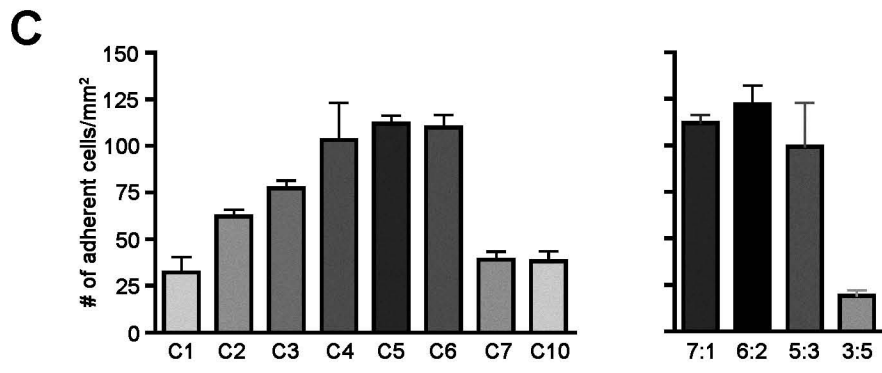
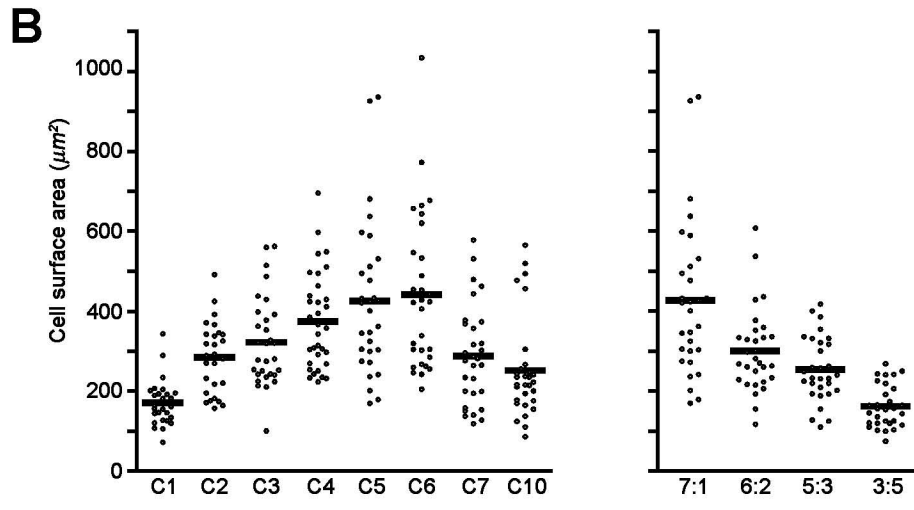
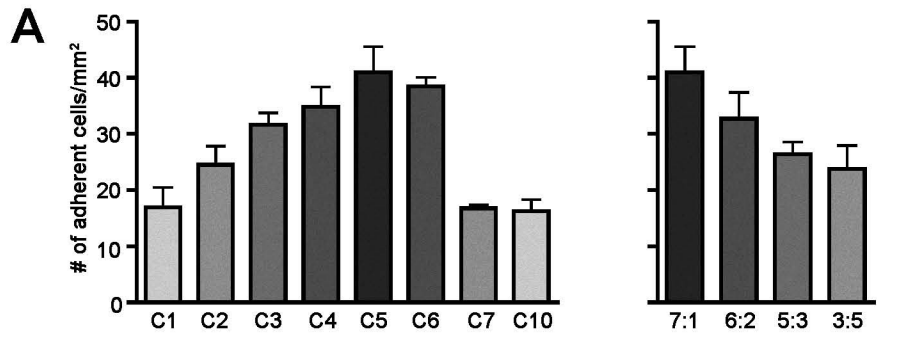
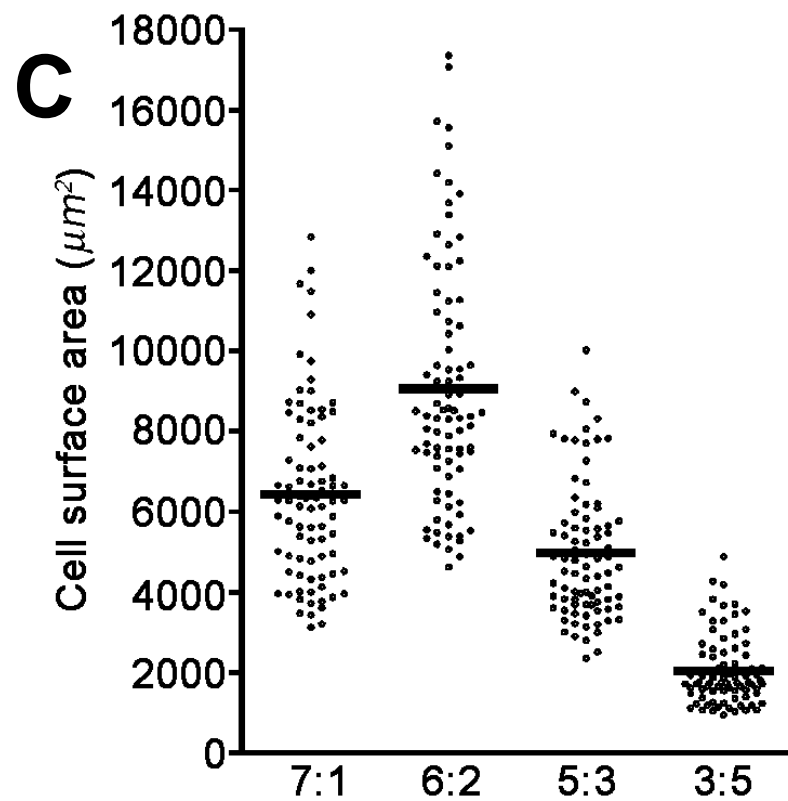
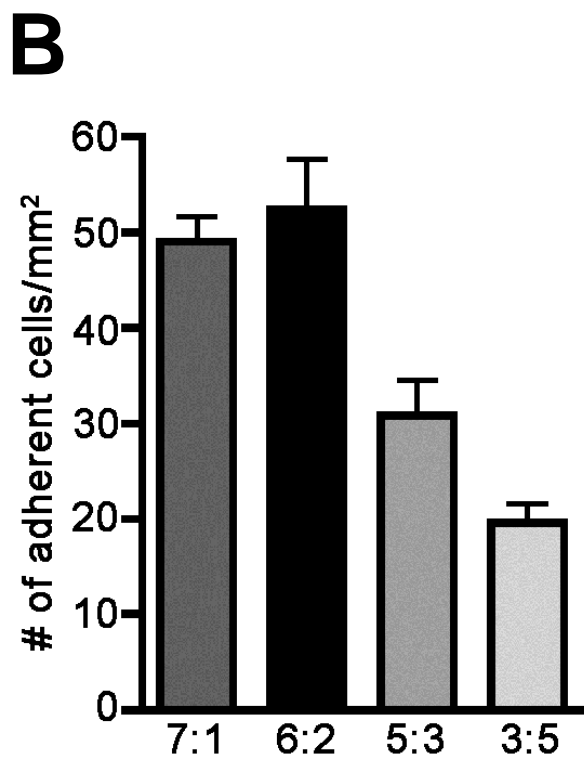
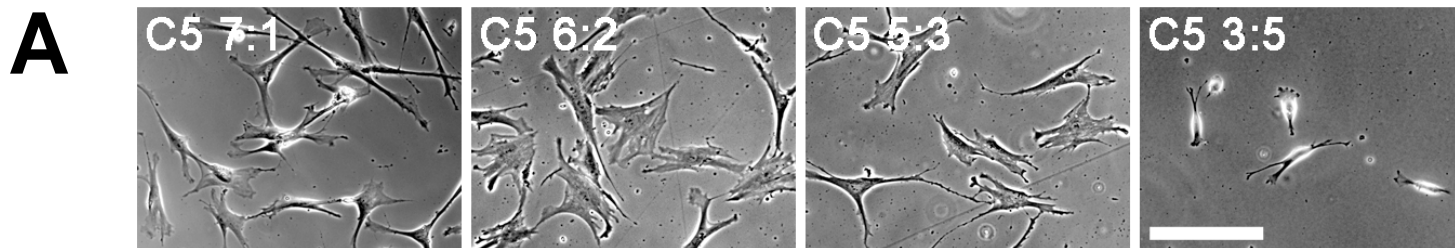
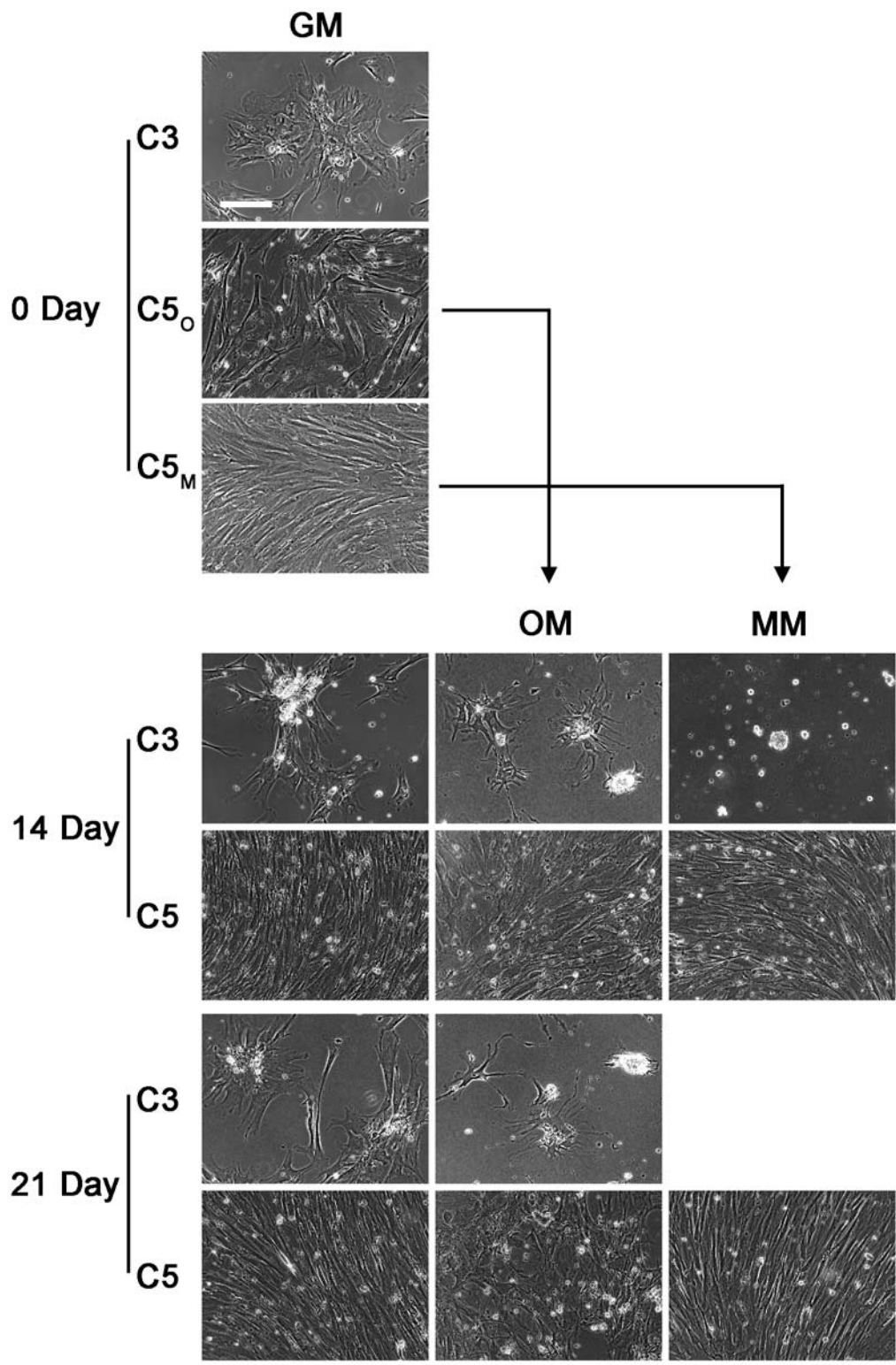


(B)

Scan Area	C1	C3	C5	C7	C10
80x80 nm	0.15 ± 0.007	0.13 ± 0.005	0.15 ± 0.004	0.14 ± 0.008	0.12 ± 0.003
400x400 nm	0.17 ± 0.003	0.16 ± 0.011	0.16 ± 0.001	0.15 ± 0.026	0.17 ± 0.006
800x800 nm	0.25 ± 0.010	0.25 ± 0.021	0.23 ± 0.016	0.20 ± 0.014	0.22 ± 0.006
4x4 μm	0.45 ± 0.042	0.48 ± 0.075	0.42 ± 0.010	0.50 ± 0.045	0.57 ± 0.154
8x8 μm	0.55 ± 0.019	0.55 ± 0.055	0.57 ± 0.029	0.66 ± 0.001	0.57 ± 0.035



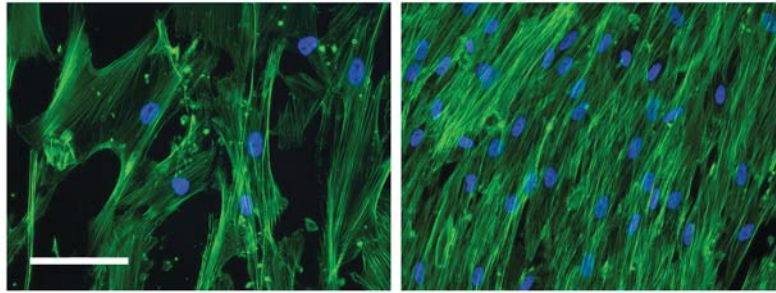




0 Day

C5_O

C5_M

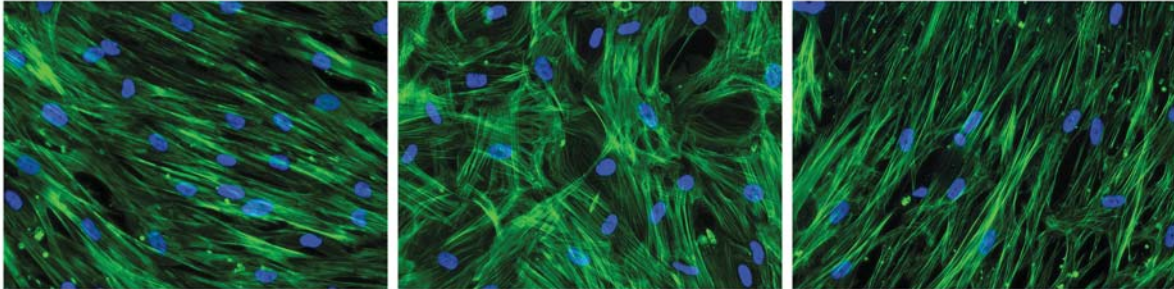


14 Day

GM

OM

MM

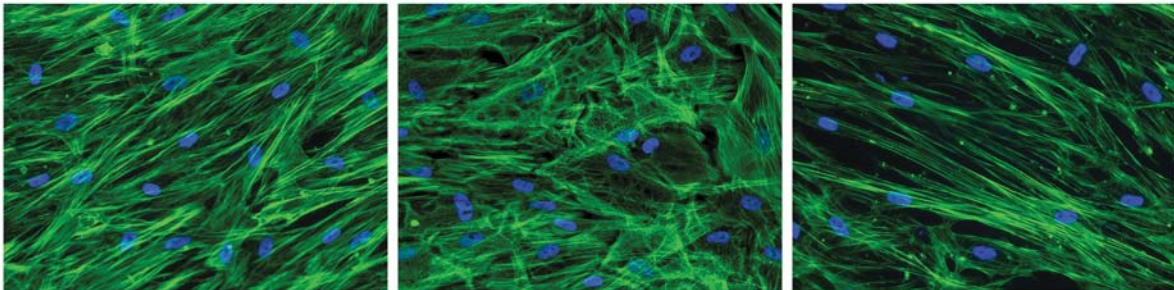


21 Day

GM

OM

MM



Supplementary Figures

Figure S1. Synthesis and characterization of acryloyl amino acid (AA) monomers. (A) Reaction scheme for synthesis of AA monomers. (B) Nuclear magnetic resonance (NMR) analysis of AA monomers. a'-e' represent the position of protons and carbon molecules.

Figure S2. Synthesis of hydrogels. (A) Reaction scheme for Am-co-AA hydrogel synthesis. AA, acryloyl amino acid; Am, acrylamide; Bis-Am, bis-acrylamide. (Left, carbon-13 NMR analysis of AA hydrogels at a 7:1 Am to AA ratio. Right, carbon NMR results of C5 hydrogels at various Am:AA mole ratios. a'-c' represent the position of the carbon atoms.

Figure S3. Surface characterization of acryloyl amino acid based hydrogels. (A) $4 \times 4 \mu\text{m}^2$ AFM images of the hydrogels display flat surfaces with low roughness values. All images were acquired in PBS buffer using contact mode with triangular cantilevers of 0.06 N/m nominal spring constant. () Table showing the RMS roughness values in nm at different scan sizes. Roughness values are given as mean values of four independent measurements and the standard error of the mean.

Figure S4. Analysis of hMSC adhesion and spreading on hydrogels at 2 hours and 4 days post plating. A

Error bars indicate standard deviation. $P < 0.0001$, $n = 9$. Right, quantification of cells

adhered onto hydrogels of varying C5 content two hours after plating. $P = 0.0476$, $n = 9$. () Left, quantification of cell spreading on the hydrogels two hours after plating. Plot graphs display individual cell area measurements. Bars indicate the mean area for all measured cells. $P < 0.0001$, $n = 90$. Right, quantification of cell spreading on hydrogels of varying C5 content two hours after plating. $P < 0.0001$, $n = 90$. () Left, quantification of cells adhered onto the hydrogels 4 days after plating. $P < 0.0001$, $n = 9$. Right, quantification of cells adhered on to C5 hydrogels of varying mole ratios 4 days after plating. $P = 0.0019$, $n = 9$.

5. hMSC cell adhesion and spreading on hydrogels containing varying amounts of C5. **A** **B)** 24 hours after plating on C5 hydrogels of varying Am:AA ratio, 7:1, 6:2, 5:3, 3:5. Error bars indicate standard deviation $P = 0.0005$, $n = 9$. () Quantification of cell spreading on different C5 hydrogels. $P < 0.0001$, $n = 90$. Scale bars in = 400 μm .

Figure S6. Brightfield images of hMSCs under differentiation conditions. hMSCs were cultured on C3 or C5 hydrogels in growth media (GM) before switching (0 day) to osteogenic (OM) or myogenic (MM) media. Arrows indicate that low-density cultures on C5 hydrogels ($C5_o$) were used for osteogenic differentiation, while high-density cultures ($C5_M$) were used for myogenic differentiation. Cells on C3 hydrogels under myogenic conditions die by 10 days. Scale bar = 200 μm .

Figure S7. Actin immunofluorescent staining of hMSCs cultured on C5 hydrogels under differentiation conditions. C5_o, low density cultures of hMSCs on a C5 hydrogel before switching to osteogenic media (OM). C5_m, high density cultures of hMSCs on a C5 hydrogel before switching to myogenic media (MM). GM, growth media. Scale bar = 100 μm .

Supplementary Movies

Movie S1. Time-lapse video of hMSCs on a C1 hydrogel.

Movie S2. Time-lapse video of hMSCs on a C3 hydrogel.

Movie S3. Time-lapse video of hMSCs on a C5 hydrogel.

Supplementary Table

Table S1. PCR primer sequences.