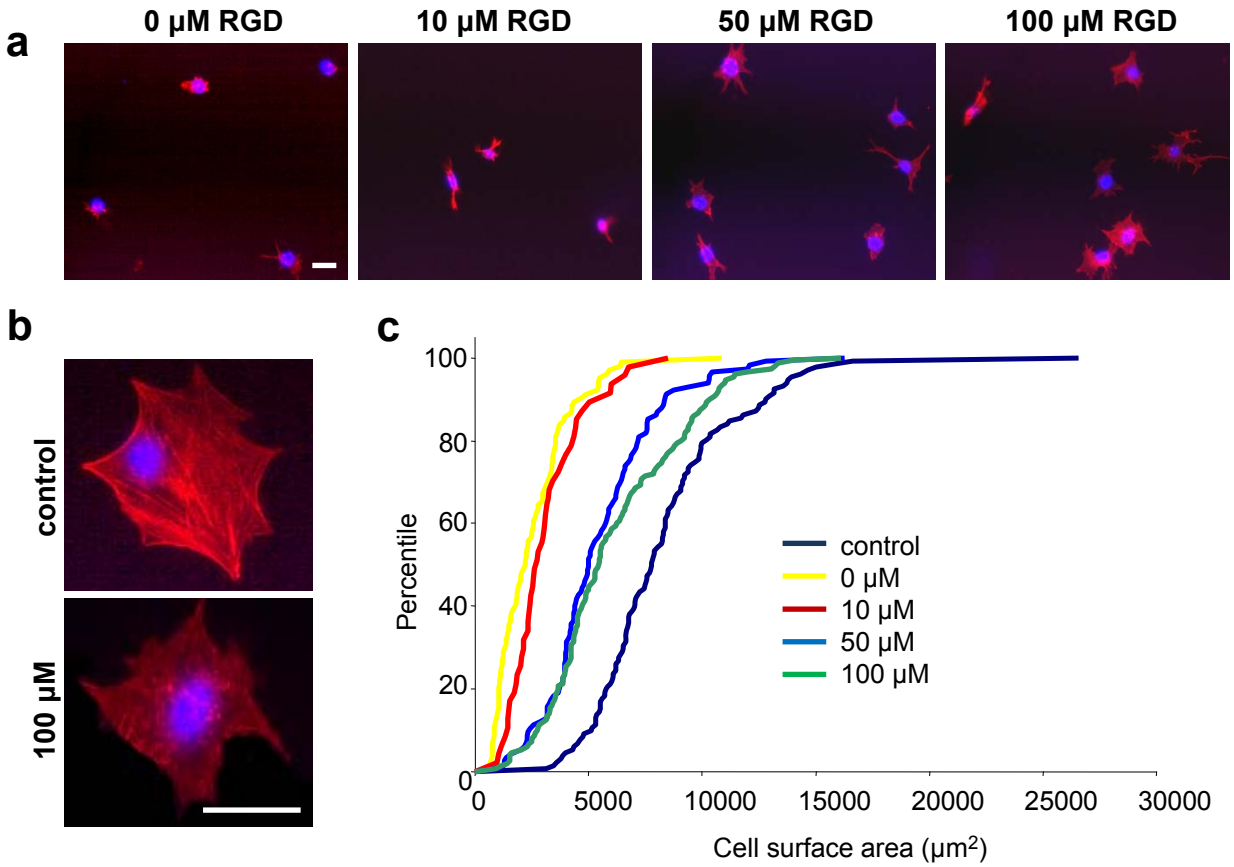
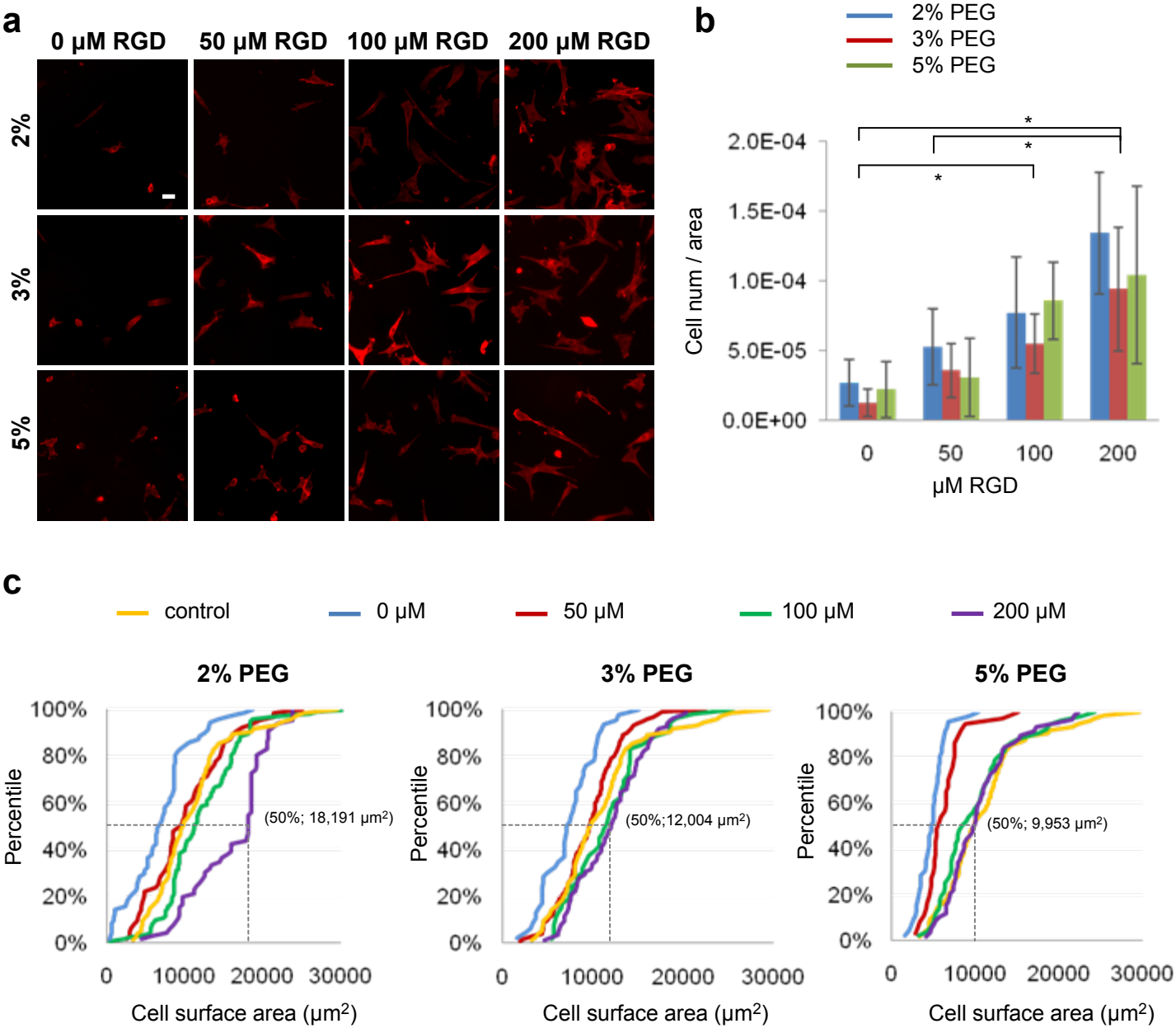
Supplementary Figure 1



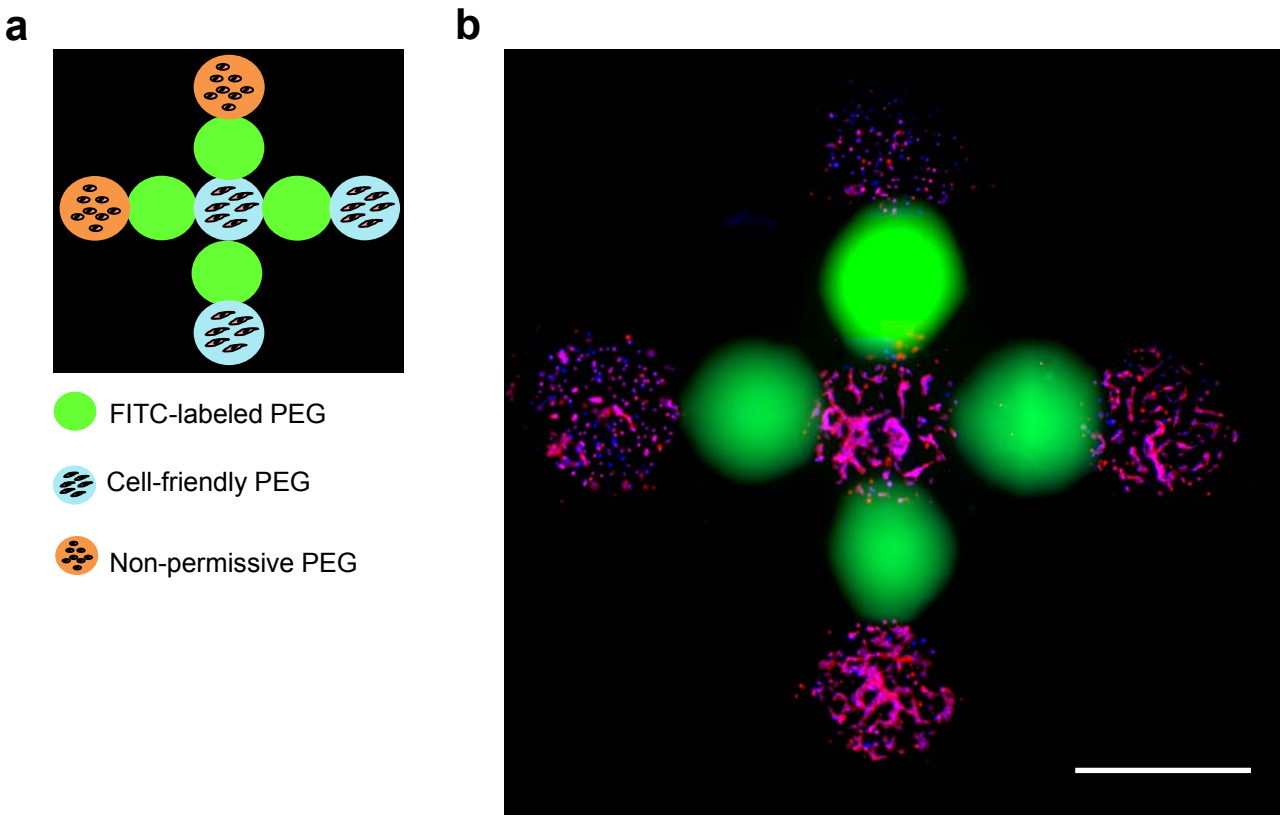
Supplementary Figure 1 | Spreading of MC3T3-E1 on RGD-modified PEG hydrogels. Preosteoblastic MC3T3-E1 were seeded on TCP (control) or on 5% PEG gels which contained 0, 10, 50 and 100 µM RGD. After 4 hours cells were fixed and double stained for their nuclei with DAPI (blue) and their actin cytoskeleton with rhodamine phallodidin (red). (a,b) Representative epifluorescence images (scale bar 100 µm). (c) Cell surface areas as quantified with the help of ImageJ software demonstrate that MC3T3 spreading is dependent on the RGD-adhesive ligand concentration.

Supplementary Figure 2



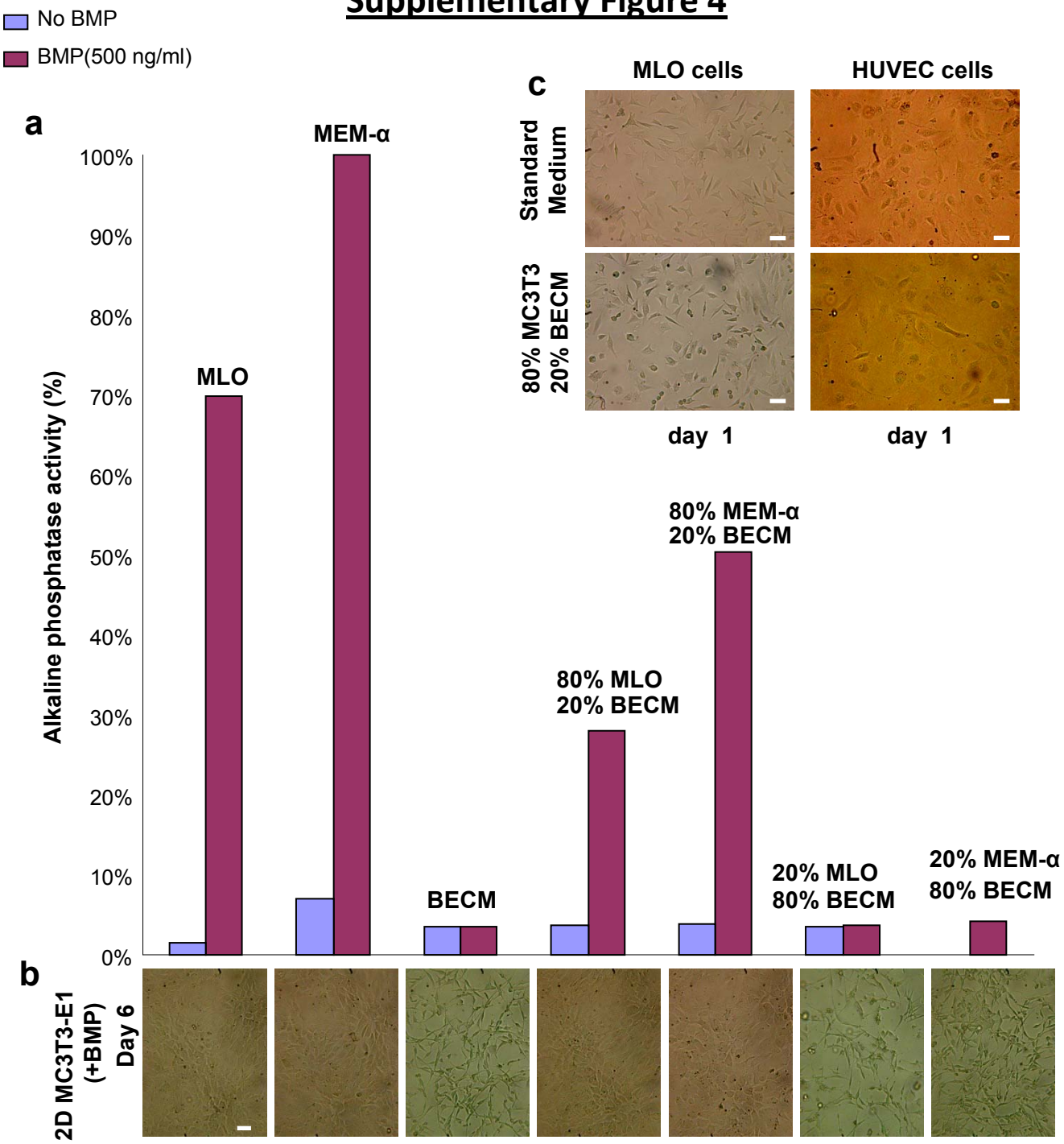
Supplementary Figure 2 | Adhesion and spreading of C2C12 cells on RGD-modified PEG hydrogels.
C2C12 cells were seeded on PDMS wells previously coated with PEG gels (2%, 3% and 5%) which contained 0, 50, 100 and 200 µM RGD (control; PDMS). After 4 hours cells were fixed and double stained for their nuclei with DAPI (not shown) and their actin cytoskeleton with rhodamine phallodidin (red). Both cell adhesion and cell spreading is dependent on the RGD concentration. (a) Representative epifluorescence images (scale bar 100 µm). (b) Cell adhesion was quantified by the number of adhesive cells and (c) cell surface areas was quantified with the help of ImageJ software. Statistical analysis; dunn test n≥30.

Supplementary Figure 3



Supplementary Figure 3 | Robotic pipetting.
(a) Scheme of the printing pattern. (b) Different compositions of hydrogel depots in presence or absence of single dispersed MC3T3-E1 cells were positioned on top of a non-labeled layer of PEG by using a robotic pipetting workstation (Hamilton Microlab StarPlus with Nanopipettor head robot by dispensing a volume of 0.1 µl per drop). A spot to spot distance of 900 µm with a lateral precision of ca. 100 µm was performed. Subsequently, to impair cell spreading out of the spotted areas the spotted experimental set up was sandwiched with a second layer of non-permissive hydrogel. (b) After 1 week in culture, cells were fixed and double stained for their nuclei with DAPI (blue) and their actin cytoskeleton with rhodamine phallodidin (red). Scale bar corresponds to 1 mm.

Supplementary Figure 4



Supplementary Figure 4 | Correlation between osteogenic differentiation and medium culture conditions.
Combinations of standard MLO, MC3T3-E1, and HUVEC medium compositions were evaluated on 2D MC3T3-E1 monocultures. (a) The enzymatic alkaline phosphatase activity was determined in presence or in absence of rhBMP-2. (b) Bright field images of MC3T3-E1 cells after 6 days in culture in presence of rhBMP-2. Basal HUVEC medium (BECM) significantly decreased osteogenic differentiation as demonstrated by dramatically morphological changes and reduced ALP activity. A combination of 4 parts of MC3T3-E1 medium (MEM-α) and 1 part of HUVEC medium did not apparently affect cell morphology but slightly prevented the rhBMP-2 mediated increase of ALP activity. (c) Bright field images of 2D monocultures of MLO and HUVEC cells. Scale bar corresponds to 100 μm.