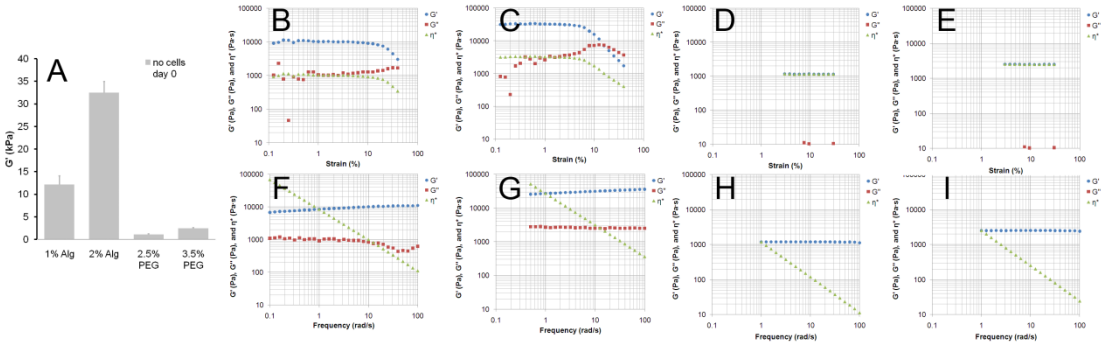
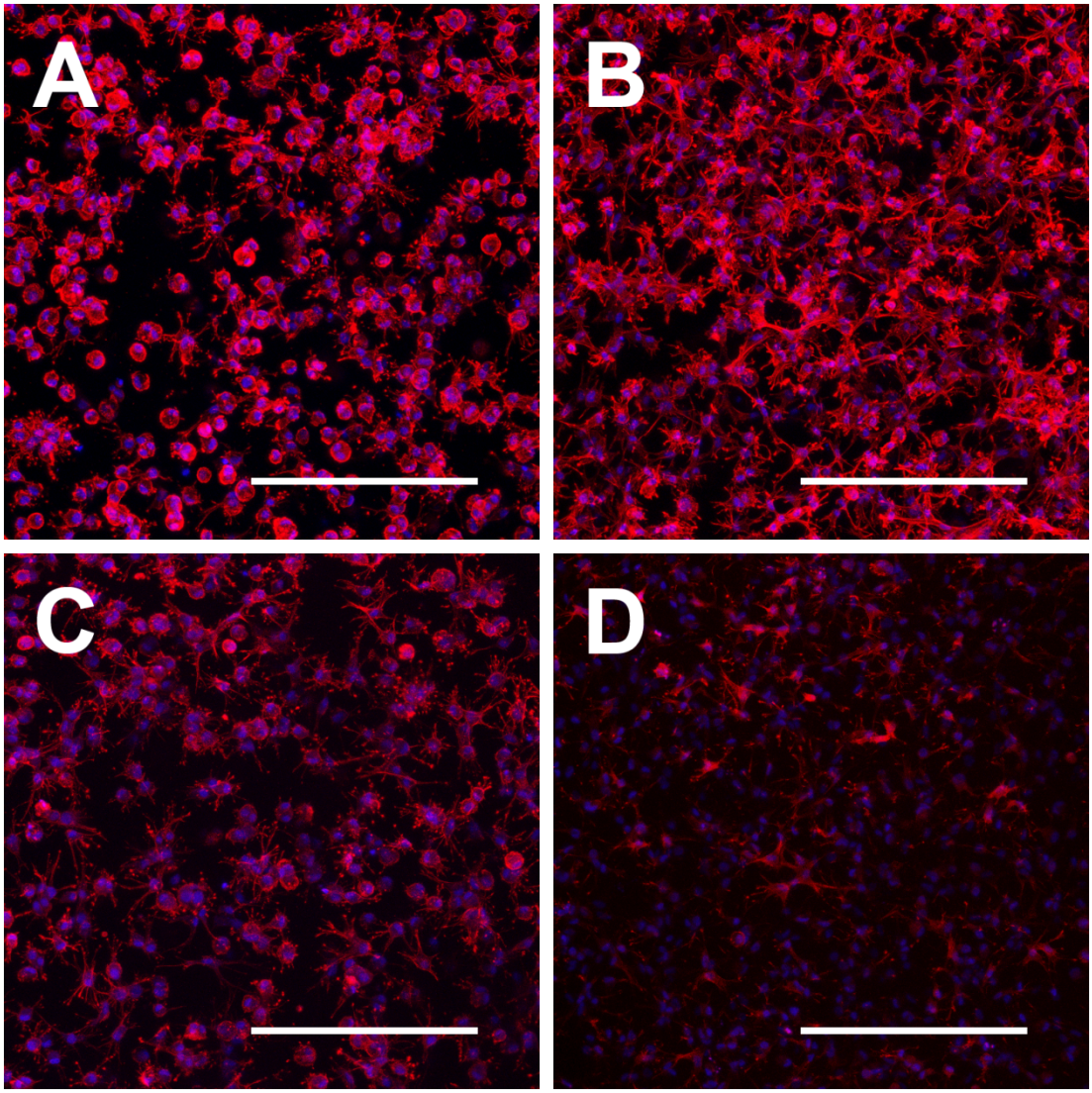
Supplementary Information: Long-term effects of hydrogel properties on human chondrocyte behavior



Supplementary Figure 1. Rheological properties of freshly made (B,F) 1% and (C,G) 2% alginate, and (D,H) 2.5% and (E,I) 3.5% PEG gels. (A) Storage modulus (G') of freshly made hydrogels (n=5-6, mean + SD) varied significantly between all gel types, with PEG gels (G' values recorded from mechanical spectra f=1-10 rad/s; strain=10%) having lower moduli than alginate gels (G' values recorded from mechanical spectra f=10 rad/s; strain=1%). Strain sweeps were performed to determine the strain range in which G' are strain-independent (strain <5% for alginate and <30% for PEG gels for frequencies <10 rad/s). Frequency sweeps show frequency-dependence of G' for alginate gels (F,G, strain = 1%), but not for PEG gels (H,I, strain 10%). In addition, G'' of PEG gels were two or more orders of magnitude lower than G', compared to alginate gels in which they were only approximately one order of magnitude lower than G'. These data indicate that PEG gels behave predominantly as pure elastic materials compared to the more viscous elastic behaviour of alginate gels. The different viscoelastic behavior of the PEG and alginate gels may be due to differences in the molecular arrangements within their gel networks. These are mainly determined by the nature of their cross-links, *i.e.* covalent and physical cross-links for the PEG and alginate gels, respectively.



Supplementary Figure 2. Cells attach to the 2.5% PEG gel matrix (A,B) without incorporation of cell adhesion peptides, but cell spreading is more pronounced at earlier time-points (C,D) with RGD incorporated into the matrix. Confocal images at (A,C) day 5 and (B,D) day 28 show actin filaments (red) and nuclei (blue). Bar = 250 μm.