

Supplementary information

Interplay of matrix stiffness and stress relaxation in directing Mesenchymal Stem Cells osteogenic differentiation

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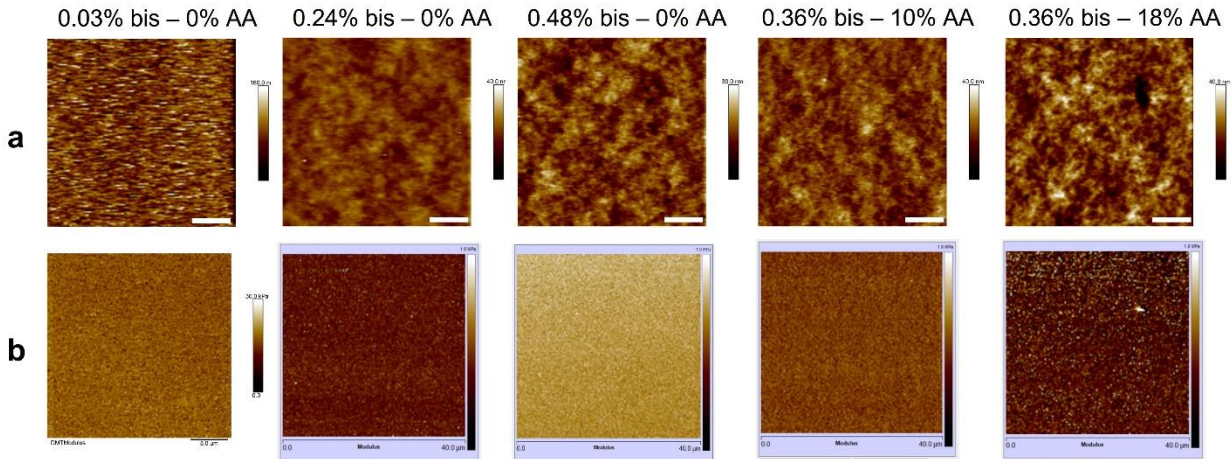


Figure S1. a) AFM images of the surface topography on different hydrogel formulations. Scale bar = 8 μm . b) AFM indentation map on a 40 μm x 40 μm area of five polyacrylamide based hydrogels with varying formulation. The color bar represents the elastic modulus (Young's modulus). The surface topography is similar between the different hydrogel formulations. The surface elastic modulus is highly homogeneous for all the hydrogel formulations. (bis = bis-acrylamide, i.e., the crosslinker, AA = acrylic acid)

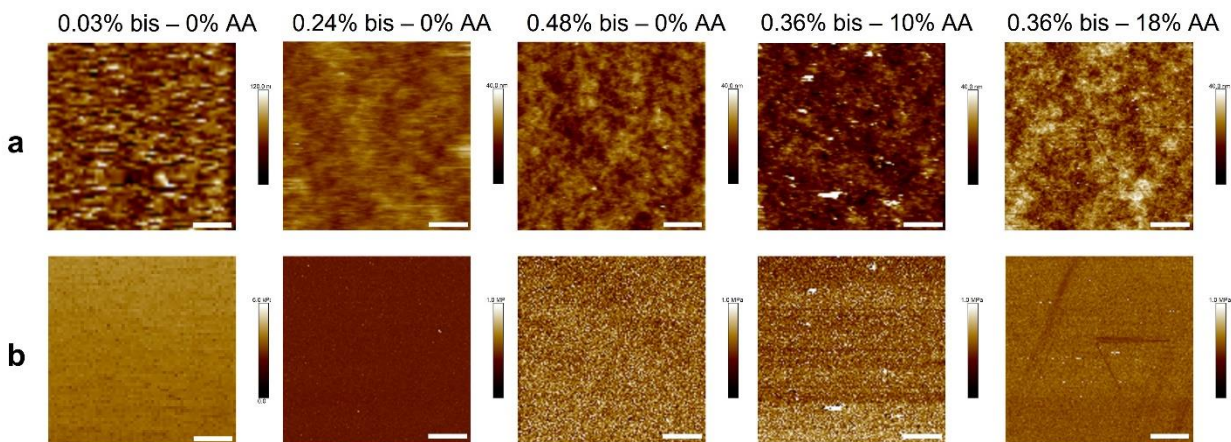


Figure S2. a) AFM images of the surface topography on different hydrogel formulations after surface functionalization with a BMP-2 mimetic peptide. Scale bar = 8 μm . b) AFM indentation map on a 40 μm x 40 μm area of five polyacrylamide based hydrogels with varying formulation after surface functionalization with a BMP-2 mimetic peptide. The color bar represents the elastic modulus (Young's modulus). The surface topography is similar between the different hydrogel formulations. The surface elastic modulus is highly homogeneous for all the hydrogel formulations. In addition, the surface roughness (arithmetic mean roughness R_a) of the hydrogels after the functionalization has been evaluated between 2.1 ± 0.5 nm and 3.4 ± 0.6 nm. Therefore, the roughness of the functionalized hydrogels is similar, which excludes that differences in cell behavior on the different hydrogels could be attributed to differences in surface roughness. (bis = bis-acrylamide, i.e., the crosslinker, AA = acrylic acid)

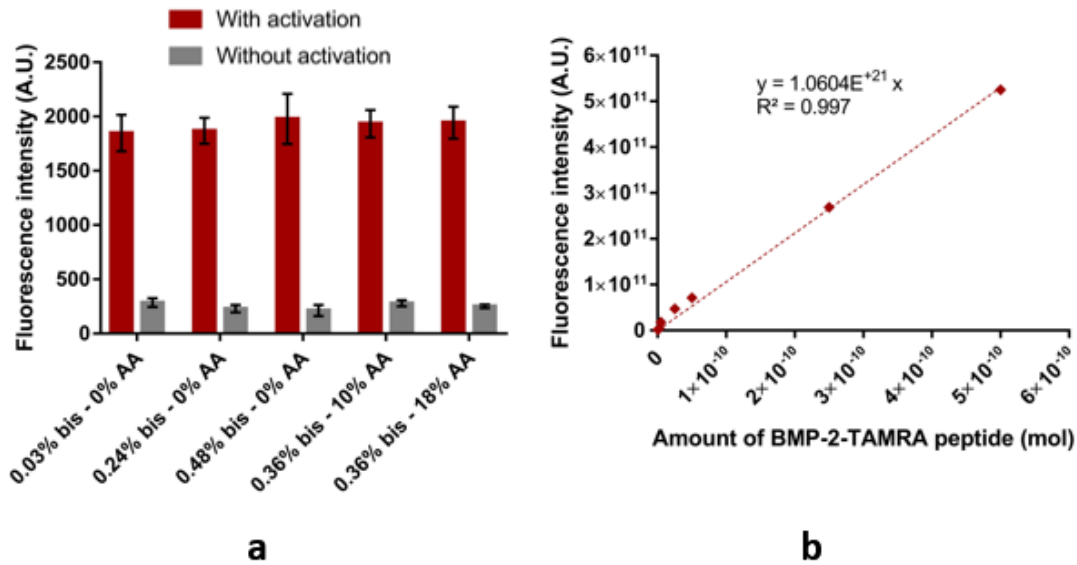


Figure S3. a) Fluorescence intensity of hydrogels functionalized with a fluorescently labelled BMP-2 peptide with Sulfo-SANPAH activation (red) or without activation (grey) after 5 days of rinsing. The fluorescence intensity of the hydrogels activated with Sulfo-SANPAH is approximately ten times higher than the fluorescence intensity of the non-activated hydrogels. b) Standard curve of the total fluorescent intensity as a function of the amount of fluorescently labelled BMP-2 peptide. There is a linear relationship between the fluorescence intensity and the amount of BMP-2-TAMRA peptide. This enables to estimate the density of peptide on the hydrogels. (bis = bis-acrylamide, i.e., the crosslinker, AA = acrylic acid)

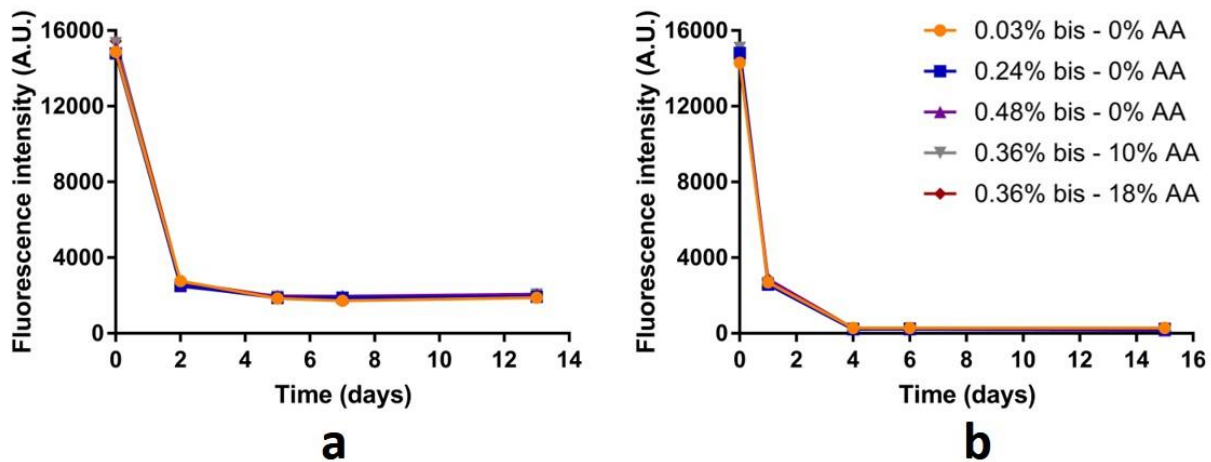


Figure S4. Fluorescence intensity of a) hydrogels functionalized with a fluorescently labelled BMP-2 peptide with Sulfo-SANPAH activation or b) without activation, as a function of the number of days of rinsing. The fluorescence intensity is stable after 5 days of rinsing and remains stable up to at least 15 days of rinsing. The fluorescence intensity of the hydrogels activated with Sulfo-SANPAH is higher than the fluorescence intensity of the non-activated hydrogels. (bis = bis-acrylamide, i.e., the crosslinker, AA = acrylic acid)

Elastic modulus (compression)

a	0.03% bis 0% AA	0.24% bis 0% AA	0.48% bis 0% AA	0.36% bis 10% AA	0.36% bis 18% AA
0.03% bis - 0% AA		*	***	**	*
0.24% bis - 0% AA			***		
0.48% bis - 0% AA				****	***
0.36% bis - 10% AA					
0.36% bis - 18% AA					

Stress relaxation (compression)

b	0.03% bis 0% AA	0.24% bis 0% AA	0.48% bis 0% AA	0.36% bis 10% AA	0.36% bis 18% AA
0.03% bis - 0% AA					*
0.24% bis - 0% AA				*	*
0.48% bis - 0% AA				*	*
0.36% bis - 10% AA					*
0.36% bis - 18% AA					

Elastic modulus (AFM)

c	0.03% bis 0% AA	0.24% bis 0% AA	0.48% bis 0% AA	0.36% bis 10% AA	0.36% bis 18% AA
0.03% bis - 0% AA		*	*	*	*
0.24% bis - 0% AA			*	*	*
0.48% bis - 0% AA				*	*
0.36% bis - 10% AA					*
0.36% bis - 18% AA					

Figure S5. Statistical analysis of the virgin hydrogels mechanical properties a) elastic modulus and b) stress relaxation measured using unconfined compression, c) elastic modulus measured using AFM. The statistical analysis was done by one-way analysis of variance (ANOVA) and Tukey's test for multiple comparisons. Grey boxes represent the absence of statistic difference. P values are represented as following * ≤ 0.05 , ** ≤ 0.01 , *** ± 0.001 , **** ≤ 0.0001 .)

Runx-2 / 24 hours

a	MSCs on glass	Osteoblasts	15 kPa - 15%	60 kPa - 15%	140 kPa - 15%	100 kPa - 30%	140 kPa - 70%
MSCs on glass		****				*	****
Osteoblasts			****	****	****	*	
15 kPa - 15%							****
60 kPa - 15%							****
140 kPa - 15%							****
100 kPa - 30%							*
140 kPa - 70%							

OPN / 24 hours

b	MSCs on glass	Osteoblasts	15 kPa - 15%	60 kPa - 15%	140 kPa - 15%	100 kPa - 30%	140 kPa - 70%
MSCs on glass				****	****	****	**
Osteoblasts				****	****	*	
15 kPa - 15%				****	****		
60 kPa - 15%						****	****
140 kPa - 15%						****	****
100 kPa - 30%							
140 kPa - 70%							

E11 / 24 hours

c	MSCs on glass	Osteoblasts	15 kPa - 15%	60 kPa - 15%	140 kPa - 15%	100 kPa - 30%	140 kPa - 70%
MSCs on glass		****	****	****	****	****	****
Osteoblasts			****	****	****	****	****
15 kPa - 15%				***		****	***
60 kPa - 15%					****	*	
140 kPa - 15%						****	****
100 kPa - 30%							
140 kPa - 70%							

DMP1 / 24 hours

d	MSCs on glass	Osteoblasts	15 kPa - 15%	60 kPa - 15%	140 kPa - 15%	100 kPa - 30%	140 kPa - 70%
MSCs on glass		****	****	****	***	****	****
Osteoblasts				****		****	****
15 kPa - 15%				****		****	****
60 kPa - 15%					****	****	****
140 kPa - 15%						****	****
100 kPa - 30%							
140 kPa - 70%							

SOST / 24 hours

e	MSCs on glass	Osteoblasts	15 kPa - 15%	60 kPa - 15%	140 kPa - 15%	100 kPa - 30%	140 kPa - 70%
MSCs on glass		****	****	****	****	****	****
Osteoblasts			****	****		****	****
15 kPa - 15%					****	****	****
60 kPa - 15%					****	****	****
140 kPa - 15%						****	****
100 kPa - 30%							
140 kPa - 70%							

Figure S6. Statistical analysis of several differentiation markers a) Runx-2, b) Osteopontin, c) E11, d) DMP1, and e) Sclerostin, after 24 hours. The statistical analysis was done by one-way analysis of variance (ANOVA) and Tukey's test for multiple comparisons. Grey boxes represent the absence of statistic difference. P values are represented as following * ≤ 0.05 , ** ≤ 0.01 , *** ≤ 0.001 , **** ≤ 0.0001 .)

Runx-2 / 2 weeks

a	MSCs on glass	Osteoblasts	15 kPa - 15%	60 kPa - 15%	140 kPa - 15%	100 kPa - 30%	140 kPa - 70%
MSCs on glass					***	****	****
Osteoblasts					***	****	****
15 kPa - 15%						****	****
60 kPa - 15%					*	****	****
140 kPa - 15%						****	**
100 kPa - 30%							
140 kPa - 70%							

OPN / 2 weeks

b	MSCs on glass	Osteoblasts	15 kPa - 15%	60 kPa - 15%	140 kPa - 15%	100 kPa - 30%	140 kPa - 70%
MSCs on glass		*	***	****	****	****	****
Osteoblasts				****	****	****	****
15 kPa - 15%				**	****	****	****
60 kPa - 15%						***	**
140 kPa - 15%							
100 kPa - 30%							
140 kPa - 70%							

E11 / 2 weeks

c	MSCs on glass	Osteoblasts	15 kPa - 15%	60 kPa - 15%	140 kPa - 15%	100 kPa - 30%	140 kPa - 70%
MSCs on glass			****	****	****	****	****
Osteoblasts			****	****	****	****	****
15 kPa - 15%					****		****
60 kPa - 15%					***		****
140 kPa - 15%						****	****
100 kPa - 30%							****
140 kPa - 70%							

DMP1 / 2 weeks

d	MSCs on glass	Osteoblasts	15 kPa - 15%	60 kPa - 15%	140 kPa - 15%	100 kPa - 30%	140 kPa - 70%
MSCs on glass		*	****	****	***	****	****
Osteoblasts			****	****	****	****	****
15 kPa - 15%				*	**	***	****
60 kPa - 15%					****		****
140 kPa - 15%						****	****
100 kPa - 30%							****
140 kPa - 70%							

SOST / 2 weeks

e	MSCs on glass	Osteoblasts	15 kPa - 15%	60 kPa - 15%	140 kPa - 15%	100 kPa - 30%	140 kPa - 70%
MSCs on glass			****	****	****	****	****
Osteoblasts			****	****	****	****	****
15 kPa - 15%				****	**	**	****
60 kPa - 15%					****		**
140 kPa - 15%						****	****
100 kPa - 30%							****
140 kPa - 70%							

Figure S7. Statistical analysis of several differentiation markers a) Runx-2, b) Osteopontin, c) E11, d) DMP1, and e) Sclerostin, after 2 weeks. The statistical analysis was done by one-way analysis of variance (ANOVA) and Tukey's test for multiple comparisons. Grey boxes represent the absence of statistic difference. P values are represented as following * ≤ 0.05 , ** ≤ 0.01 , *** ≤ 0.001 , **** ≤ 0.0001 .)

Runx-2 – 24h

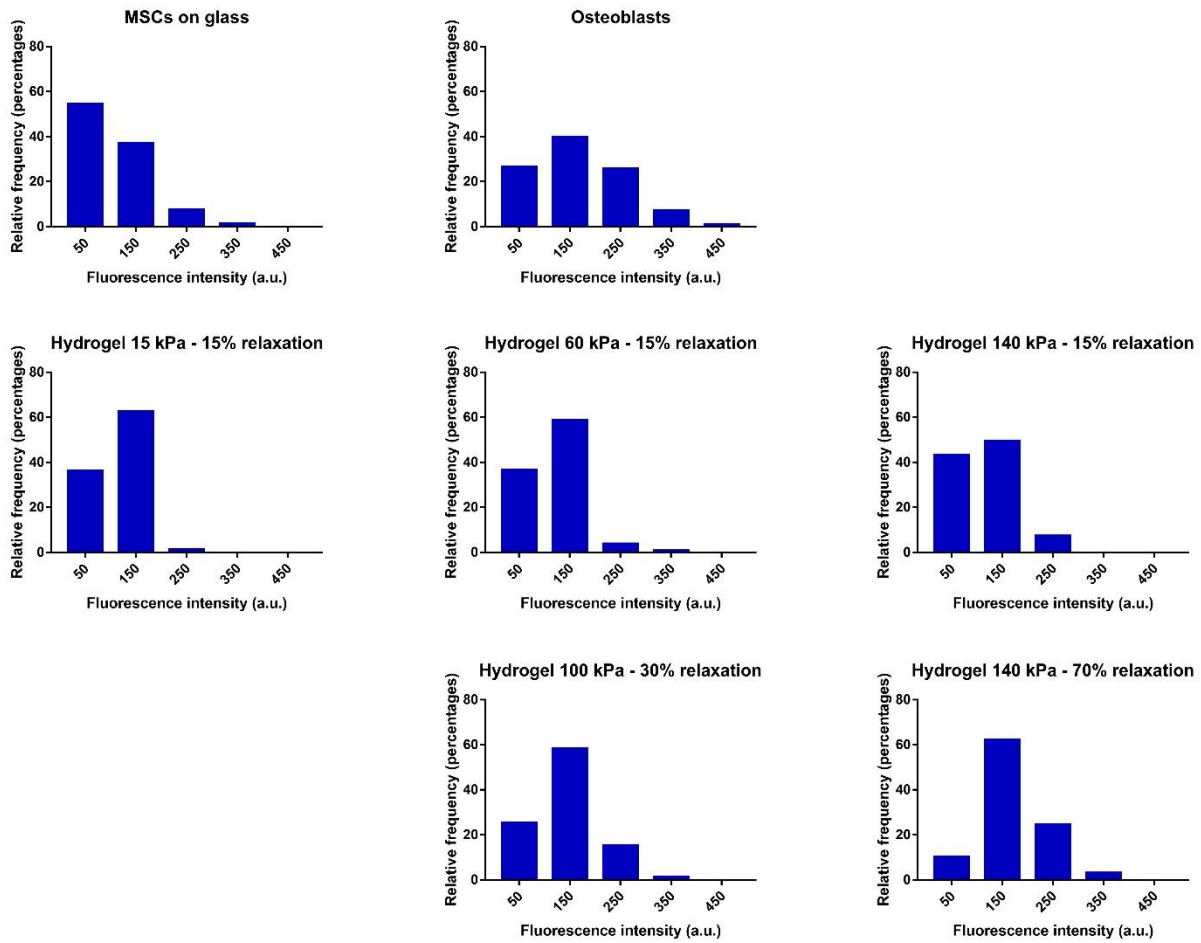


Figure S8. Class distribution of the immunofluorescence expression of Runx-2 after 24 hours for MSCs and osteoblasts cultured on glass (controls), and for MSCs cultured on poly(acrylamide-co-acrylic acid) hydrogels with varying mechanical properties. (n=2) Considering these distributions, the conditions can be ranked from the strongest expression to the lowest : osteoblasts > 140 kPa-70% > 100 kPa-30% > 15 kPa-15% = 60 kPa-15% > 140 kPa-15% > MSCs on glass.

Osteopontin – 24h

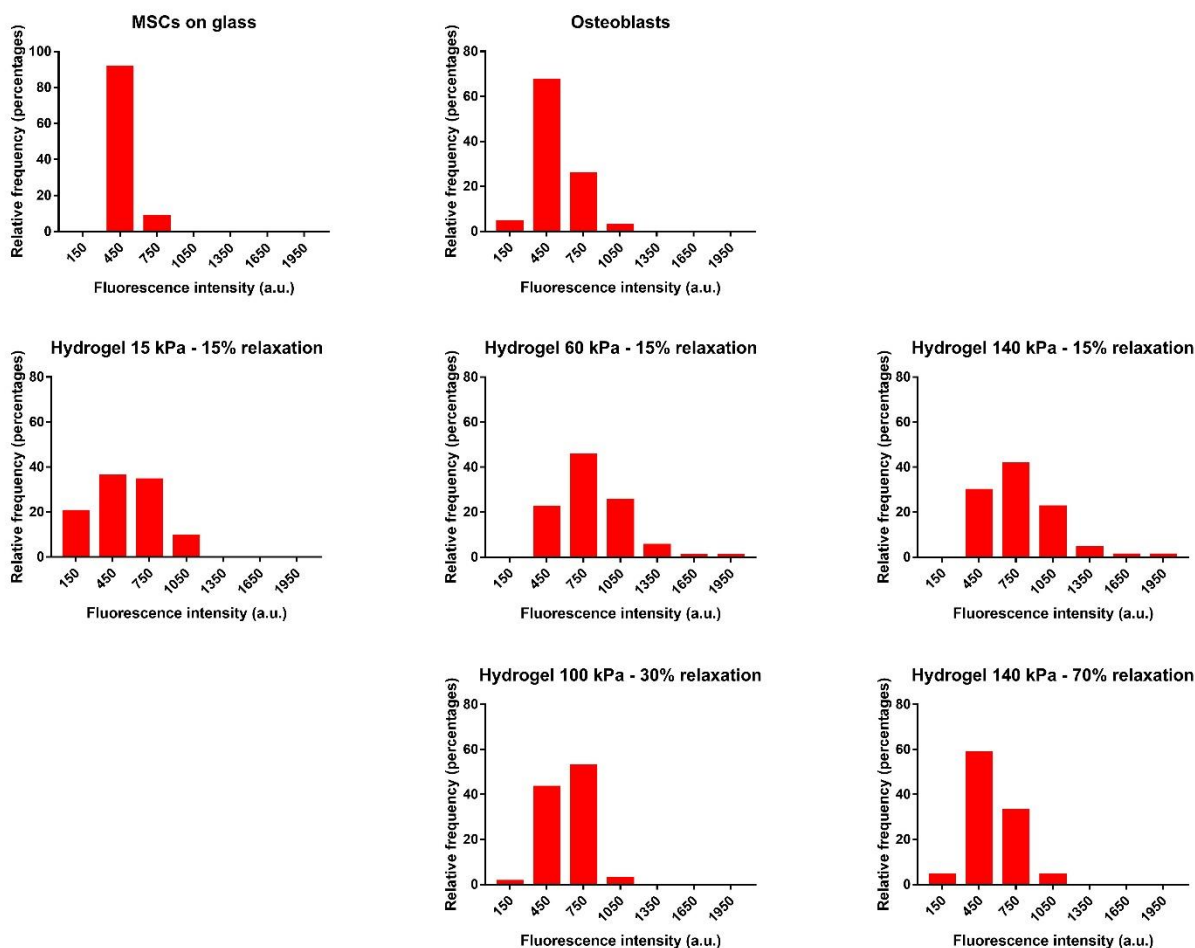


Figure S9. Class distribution of the immunofluorescence expression of osteopontin after 24 hours for MSCs and osteoblasts cultured on glass (controls), and for MSCs cultured on poly(acrylamide-co-acrylic acid) hydrogels with varying mechanical properties. (n=2) Considering these distributions, the conditions can be ranked from the strongest expression to the lowest : 60 kPa-15% = 140 kPa-15% > 100 kPa-30% > 140 kPa-70% > osteoblasts > 15 kPa-15% > MSCs on glass.

E11 – 24h

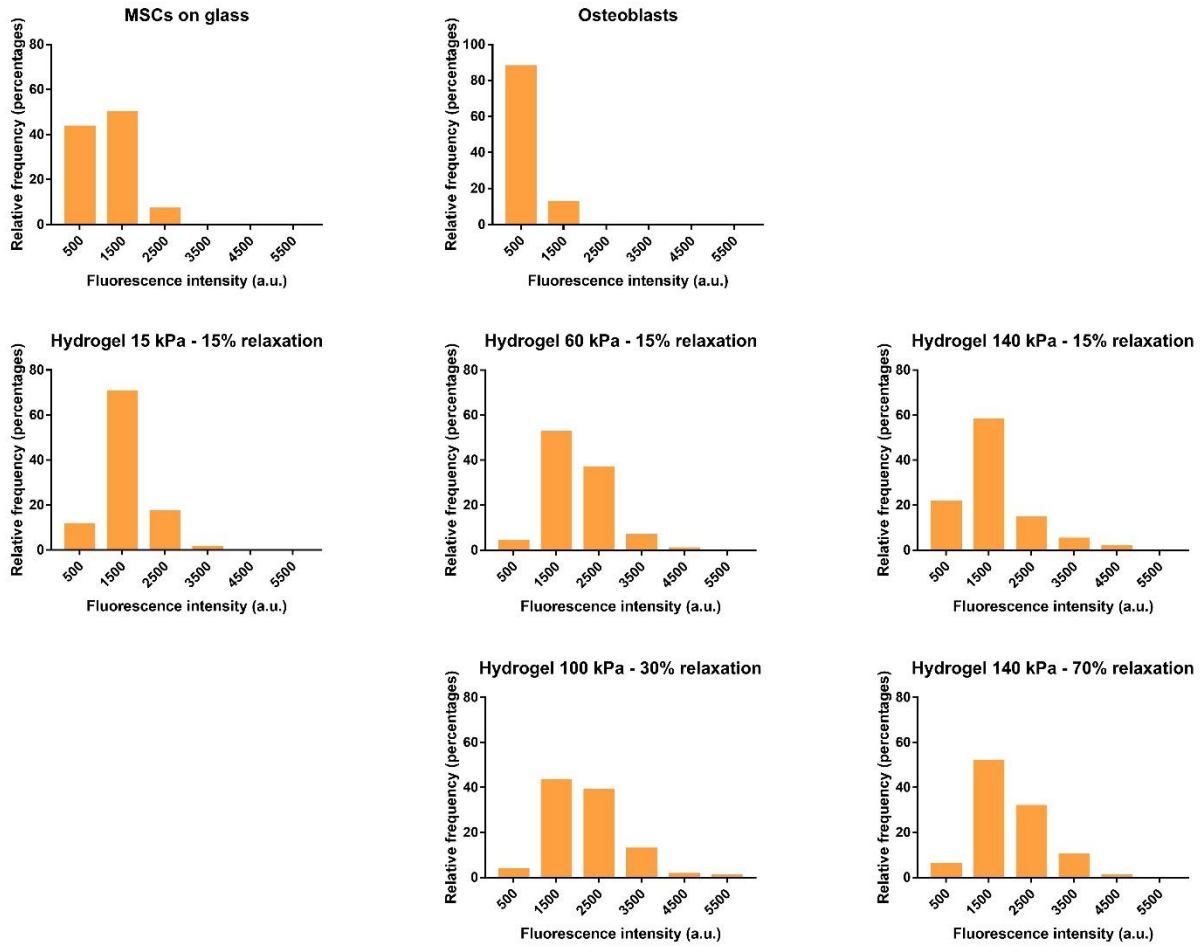


Figure S10. Class distribution of the immunofluorescence expression of E11 after 24 hours for MSCs and osteoblasts cultured on glass (controls), and for MSCs cultured on poly(acrylamide-co-acrylic acid) hydrogels with varying mechanical properties. (n=2) Considering these distributions, the conditions can be ranked from the strongest expression to the lowest : 100 kPa-30% > 60 kPa-15% = 140 kPa-70% > 15 kPa-15% > 140 kPa-15% > MSCs on glass > osteoblasts.

DMP1 – 24h

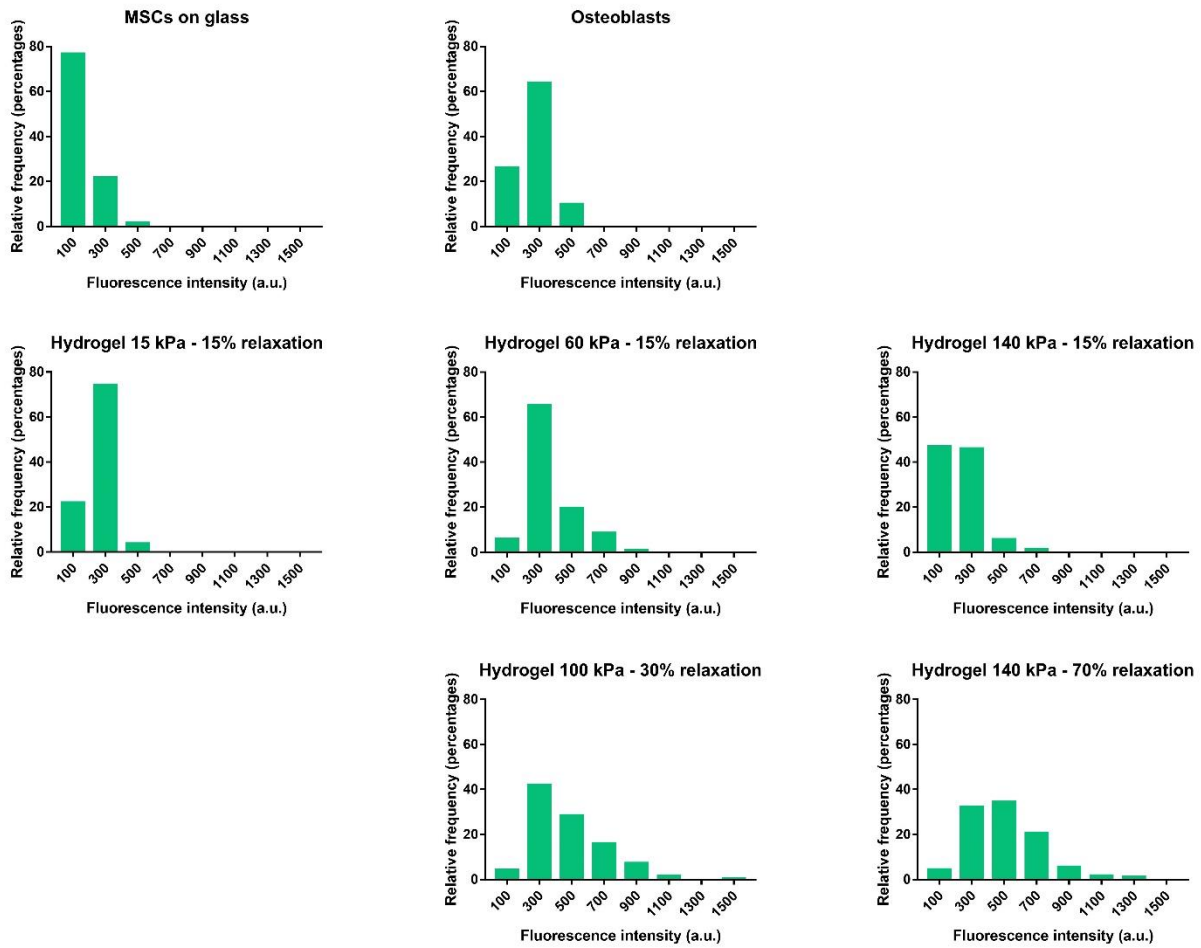


Figure S11. Class distribution of the immunofluorescence expression of DMP1 after 24 hours for MSCs and osteoblasts cultured on glass (controls), and for MSCs cultured on poly(acrylamide-co-acrylic acid) hydrogels with varying mechanical properties. (n=2) Considering these distributions, the conditions can be ranked from the strongest expression to the lowest : 140 kPa-70% > 100 kPa-30% > 60 kPa-15% > 15 kPa-15% = osteoblasts > 140 kPa-15% > MSCs on glass.

Sclerostin – 24h

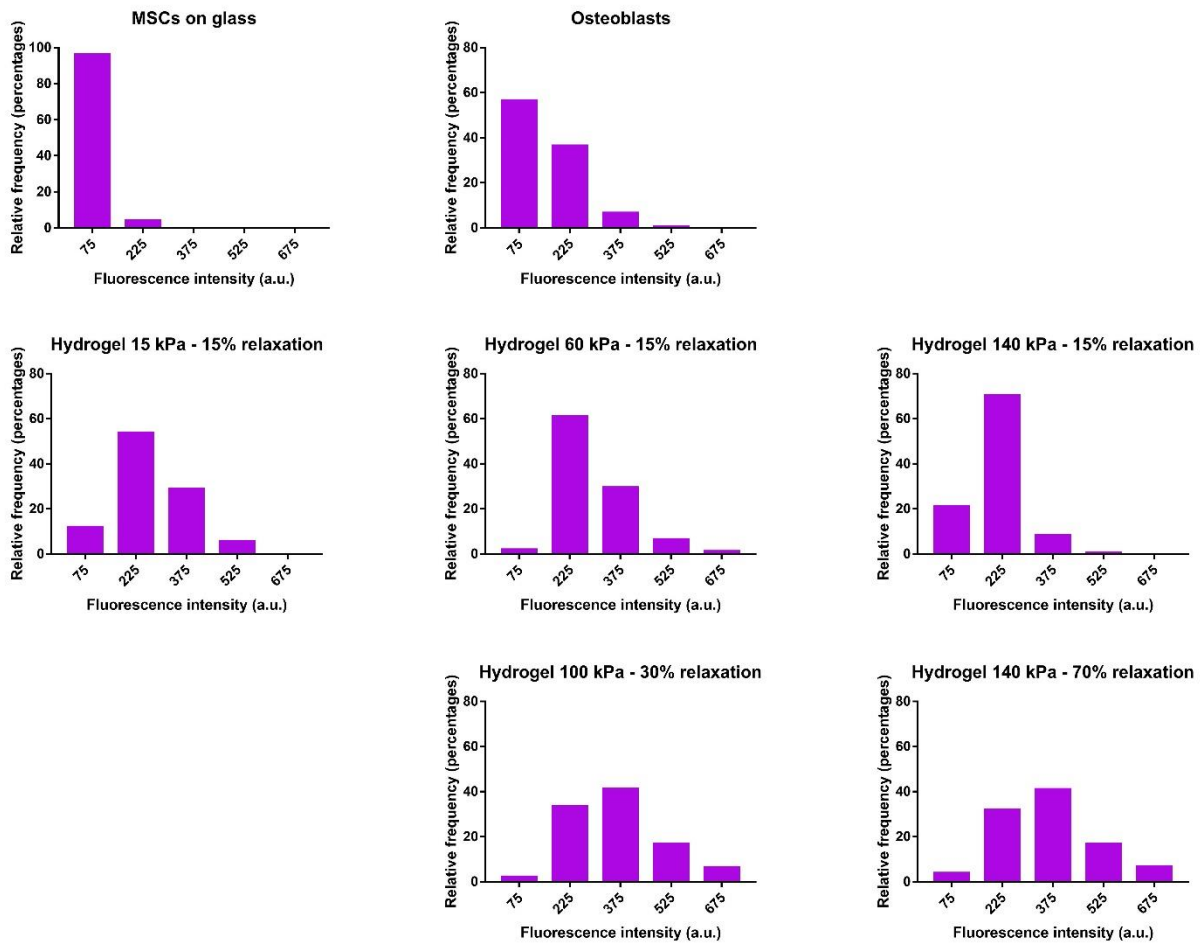


Figure S12. Class distribution of the immunofluorescence expression of sclerostin after 24 hours for MSCs and osteoblasts cultured on glass (controls), and for MSCs cultured on poly(acrylamide-co-acrylic acid) hydrogels with varying mechanical properties. (n=2) Considering these distributions, the conditions can be ranked from the strongest expression to the lowest : 140 kPa-70% = 100 kPa-30% > 60 kPa-15% > 15 kPa-15% > 140 kPa-15% > osteoblasts > MSCs on glass.

Runx-2 – 2 weeks

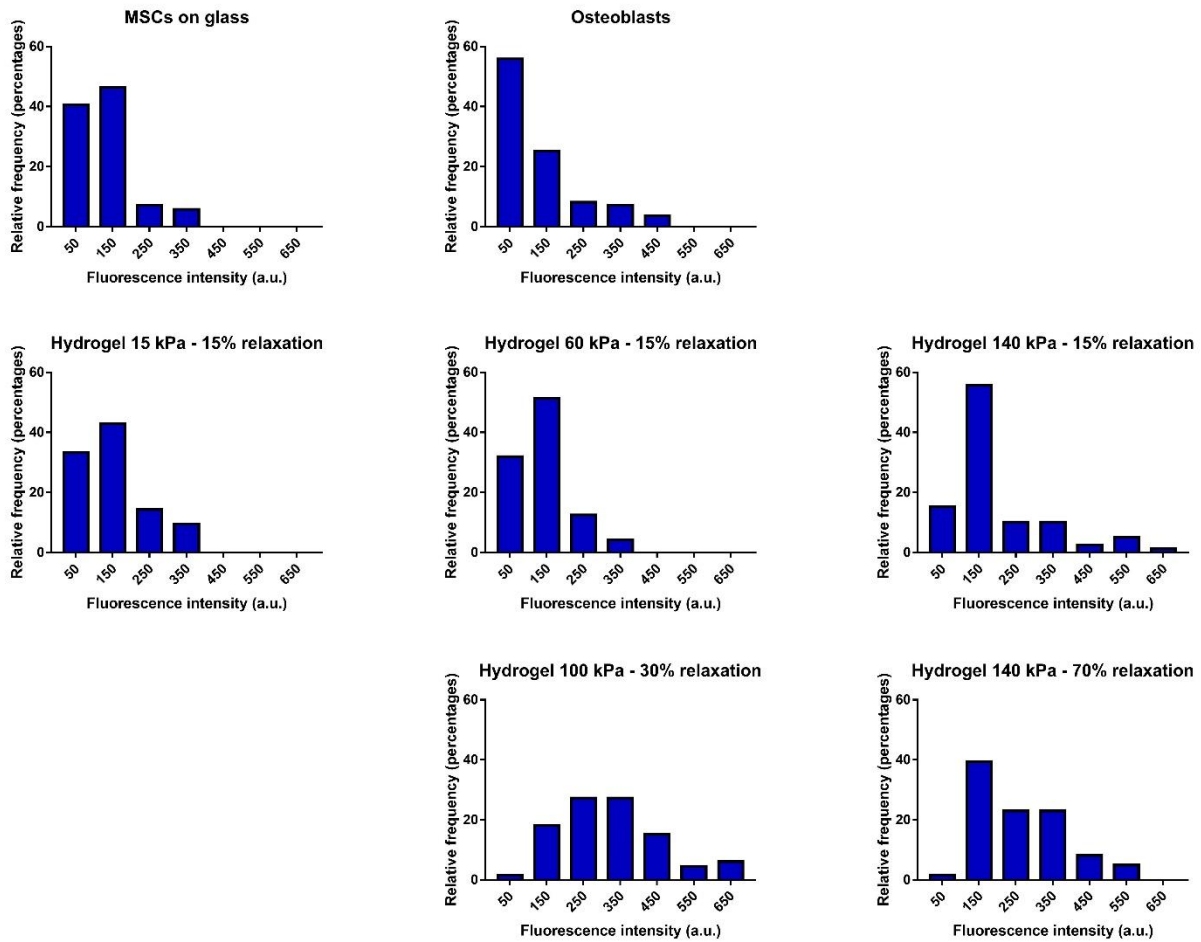


Figure S13. Class distribution of the immunofluorescence expression of Runx-2 after 2 weeks for MSCs and osteoblasts cultured on glass (controls), and for MSCs cultured on poly(acrylamide-co-acrylic acid) hydrogels with varying mechanical properties. (n=2) Considering these distributions, the conditions can be ranked from the strongest expression to the lowest : 100 kPa-30% > 140 kPa-70% > 140 kPa-15% > 15 kPa-15% > 60 kPa-15% > MSCs on glass > osteoblasts.

Osteopontin – 2 weeks

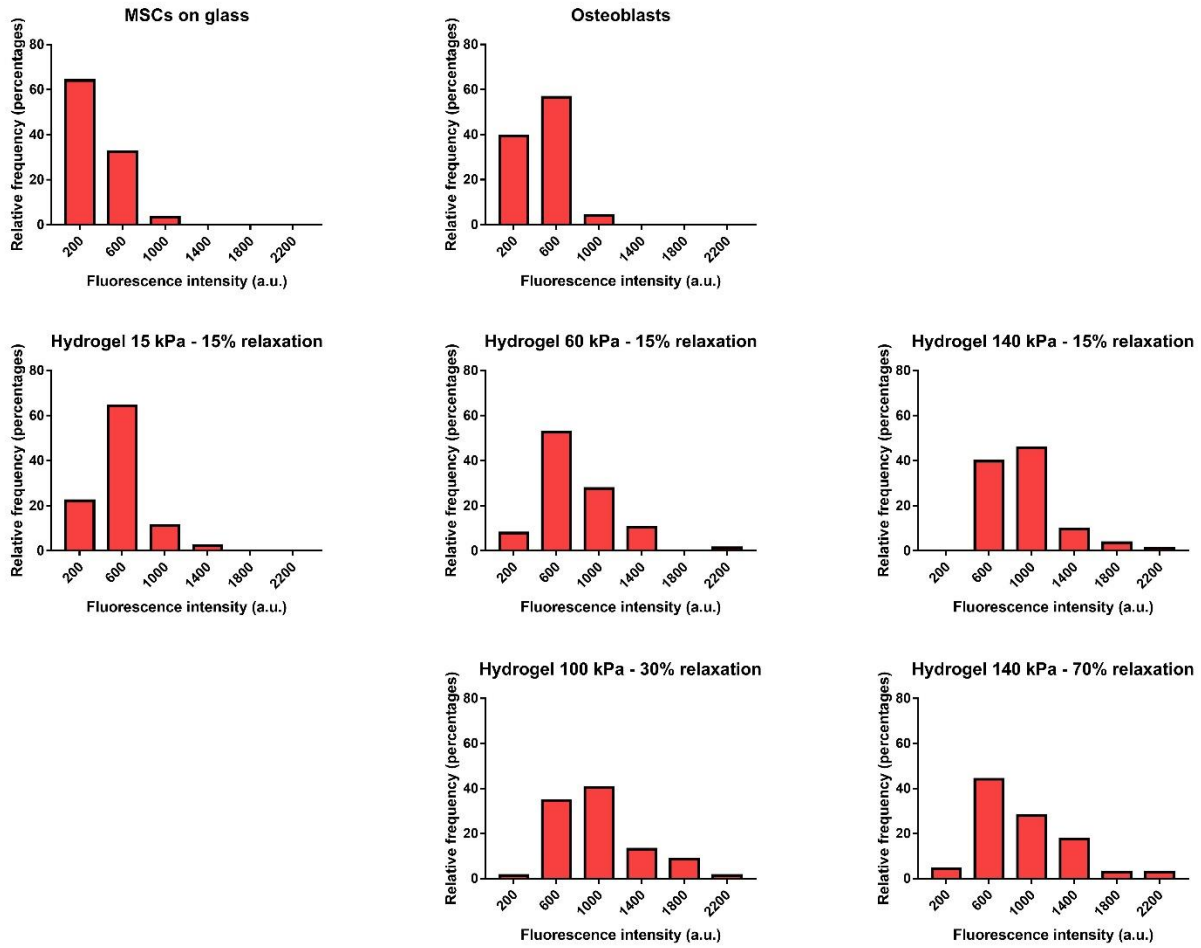


Figure S14. Class distribution of the immunofluorescence expression of OPN after 2 weeks for MSCs and osteoblasts cultured on glass (controls), and for MSCs cultured on poly(acrylamide-co-acrylic acid) hydrogels with varying mechanical properties. (n=2) Considering these distributions, the conditions can be ranked from the strongest expression to the lowest : 100 kPa-30% > 140 kPa-15% > 140 kPa-70% > 60 kPa-15% > 15 kPa-15% > osteoblasts > MSCs on glass.

E11 – 2 weeks

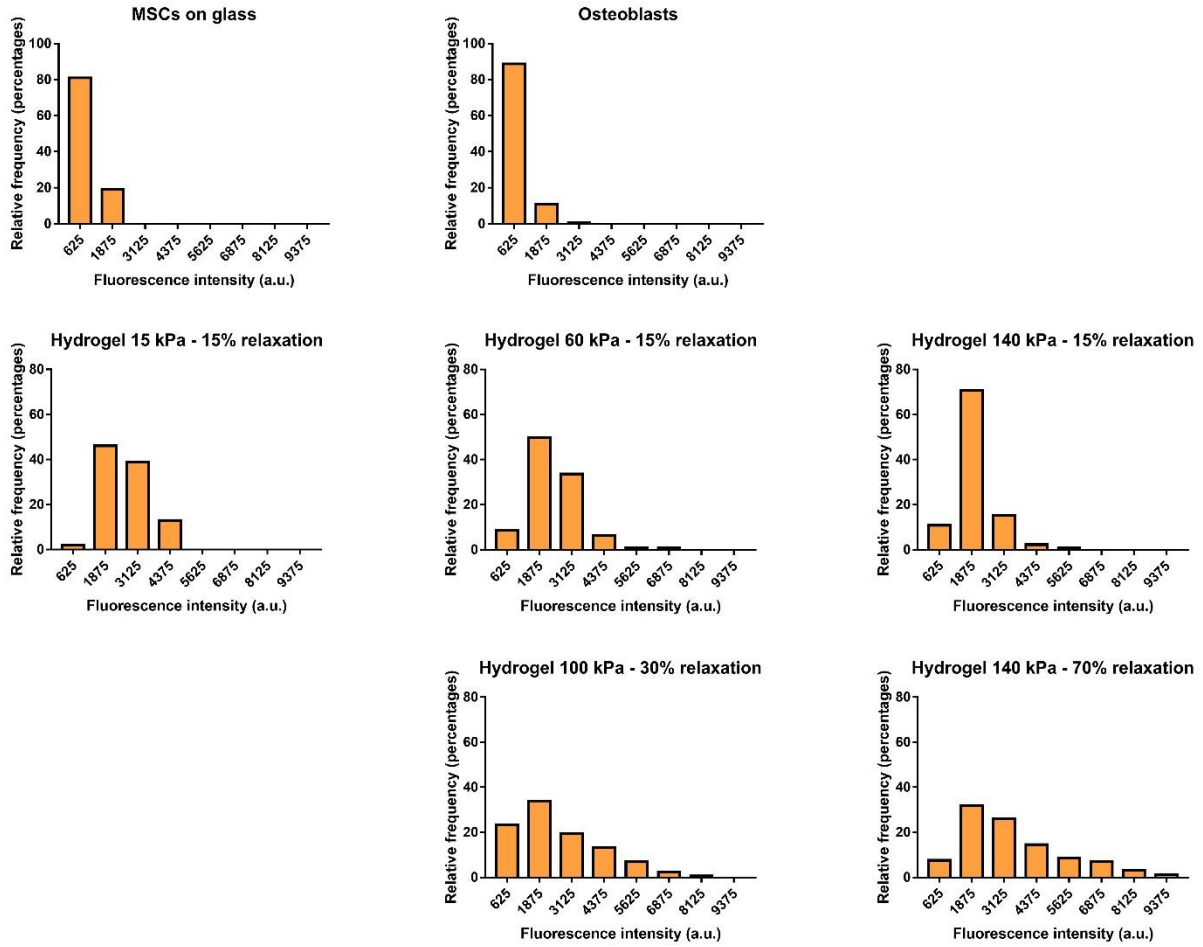


Figure S15. Class distribution of the immunofluorescence expression of E11 after 2 weeks for MSCs and osteoblasts cultured on glass (controls), and for MSCs cultured on poly(acrylamide-co-acrylic acid) hydrogels with varying mechanical properties. (n=2) Considering these distributions, the conditions can be ranked from the strongest expression to the lowest : 140 kPa-70% > 100 kPa-30% > 15 kPa-15% > 60 kPa-15% > 140 kPa-15% > MSCs on glass > osteoblasts.

DMP1 – 2 weeks

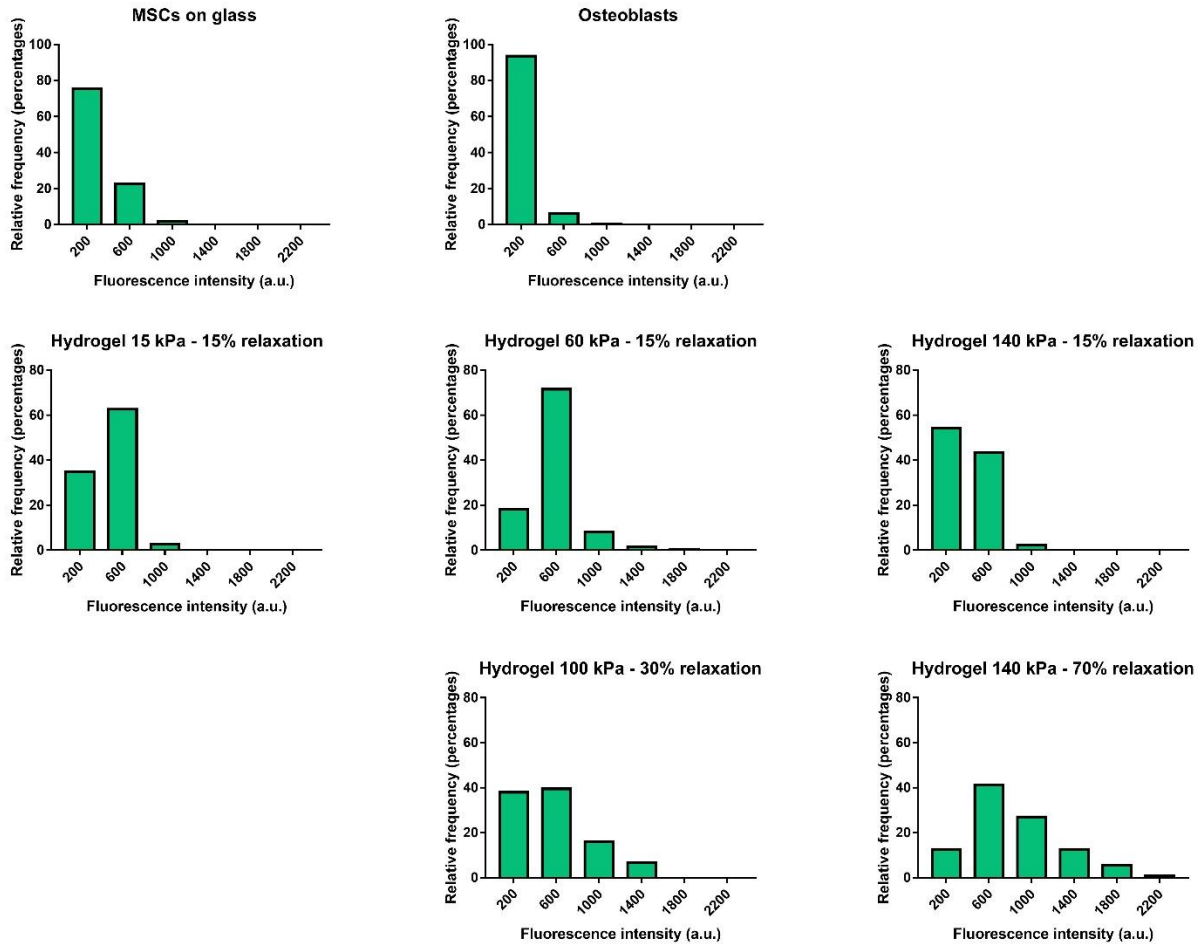


Figure S16. Class distribution of the immunofluorescence expression of DMP1 after 2 weeks for MSCs and osteoblasts cultured on glass (controls), and for MSCs cultured on poly(acrylamide-co-acrylic acid) hydrogels with varying mechanical properties. (n=2) Considering these distributions, the conditions can be ranked from the strongest expression to the lowest : 140 kPa-70% > 60 kPa-15% > 100 kPa-30% > 15 kPa-15% > 140 kPa-15% > MSCs on glass > osteoblasts.

Sclerostin – 2 weeks

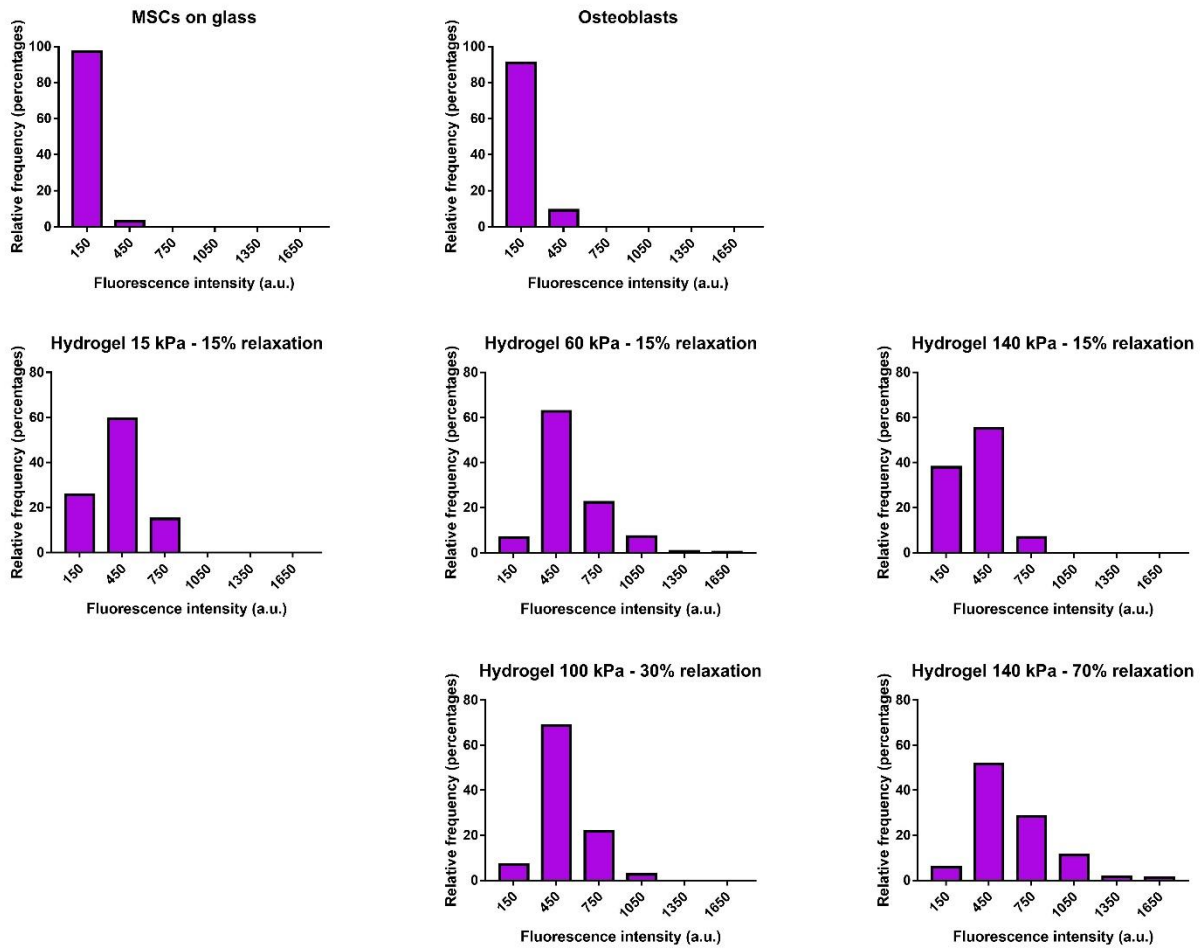


Figure S17. Class distribution of the immunofluorescence expression of sclerostin after 2 weeks for MSCs and osteoblasts cultured on glass (controls), and for MSCs cultured on poly(acrylamide-co-acrylic acid) hydrogels with varying mechanical properties. (n=2) Considering these distributions, the conditions can be ranked from the strongest expression to the lowest : 140 kPa-70% > 60 kPa-15% > 100 kPa-30% > 15 kPa-15% > 140 kPa-15% > osteoblasts > MSCs on glass.