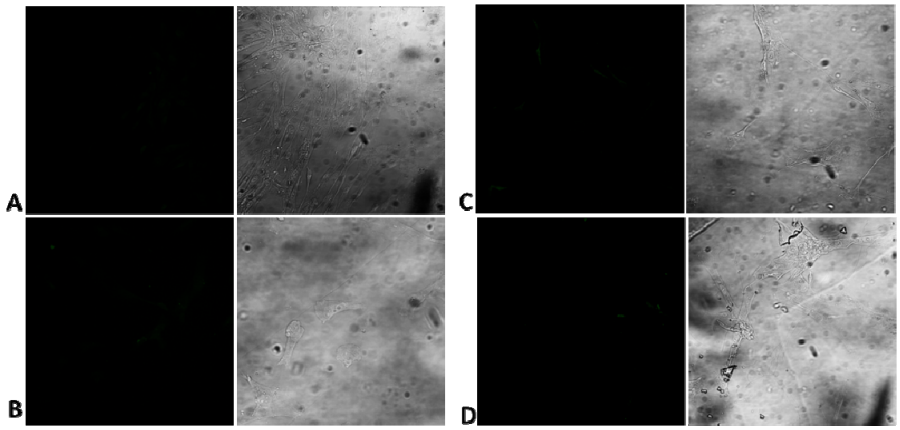

Figure S1. Confocal microscopy images for the negative control for the antibody staining(secondary antibody staining without primary antibodies)

Human MSCs cultured for fourteen days on ppAAm-(A), ppAAc-(B), ppTMP-(C) or collagen- (D) coated polyacrylamide gels.

For each coating, the left image is a fluorescence image (FITC) and the right image is a transmission image.

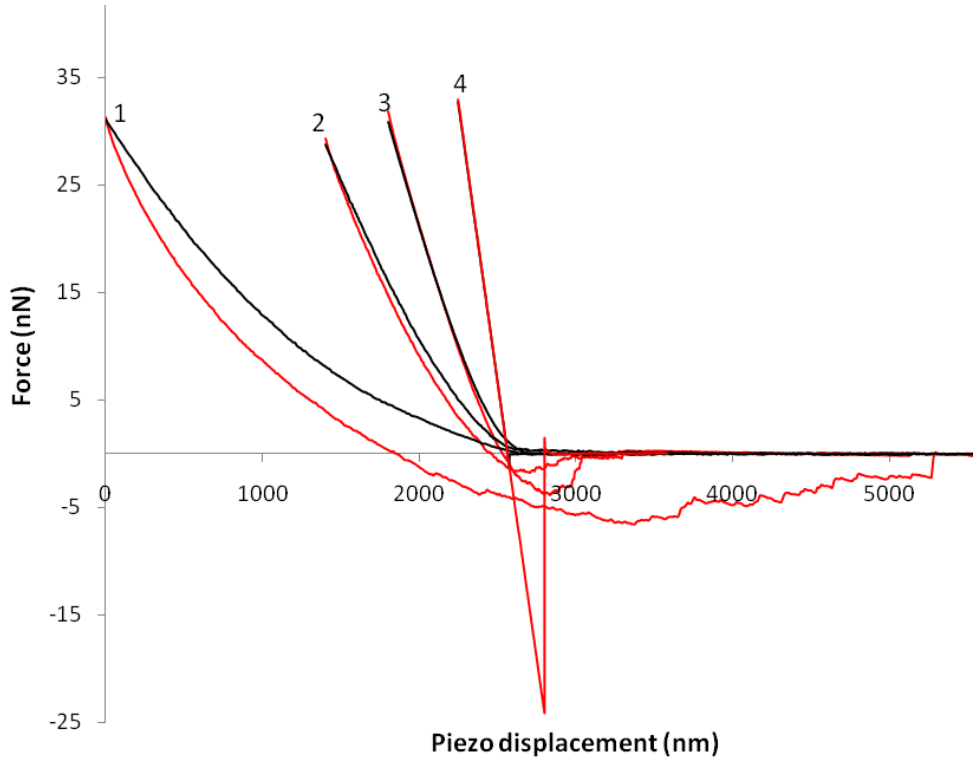

Figure S2. Force versus piezo displacement curves obtained using AFM nanoindentation in water on uncoated polyacrylamide gels with 0.01% (Curve 1), 0.12% (Curve 2) and 0.6% (Curve 3) of bis acrylamide concentrations and glass (Curve 4).

For each force curve, the extending part of the curve is in black, the retracting part is in red.

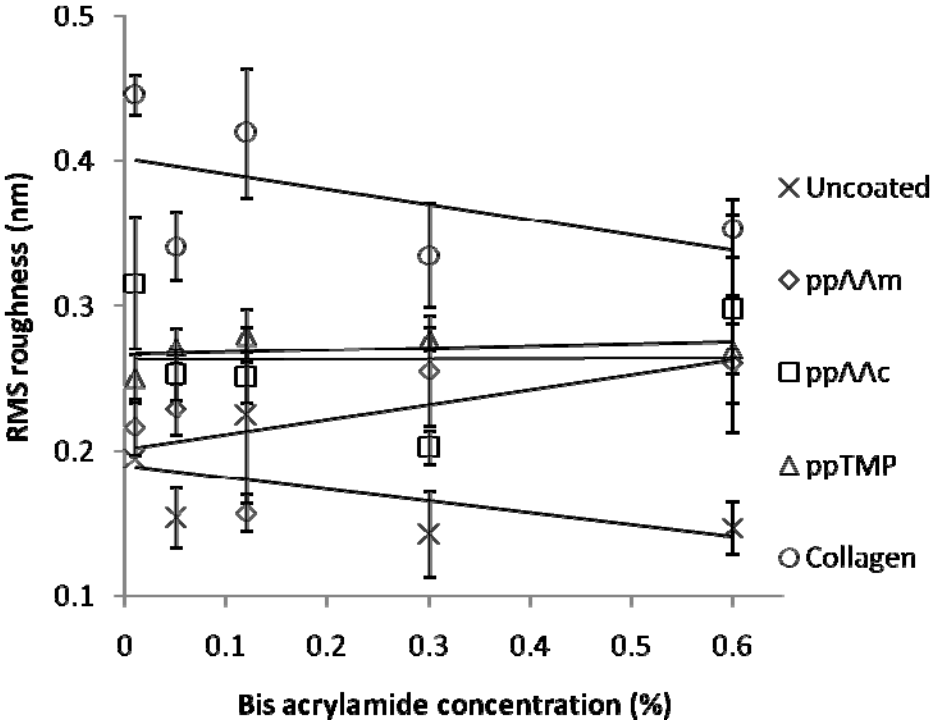

Figure S3. Surface roughness as a function of bis acrylamide concentration in the gel composition for each coating studied. Linear regression fit of the roughness data are shown as trendlines for each coating.

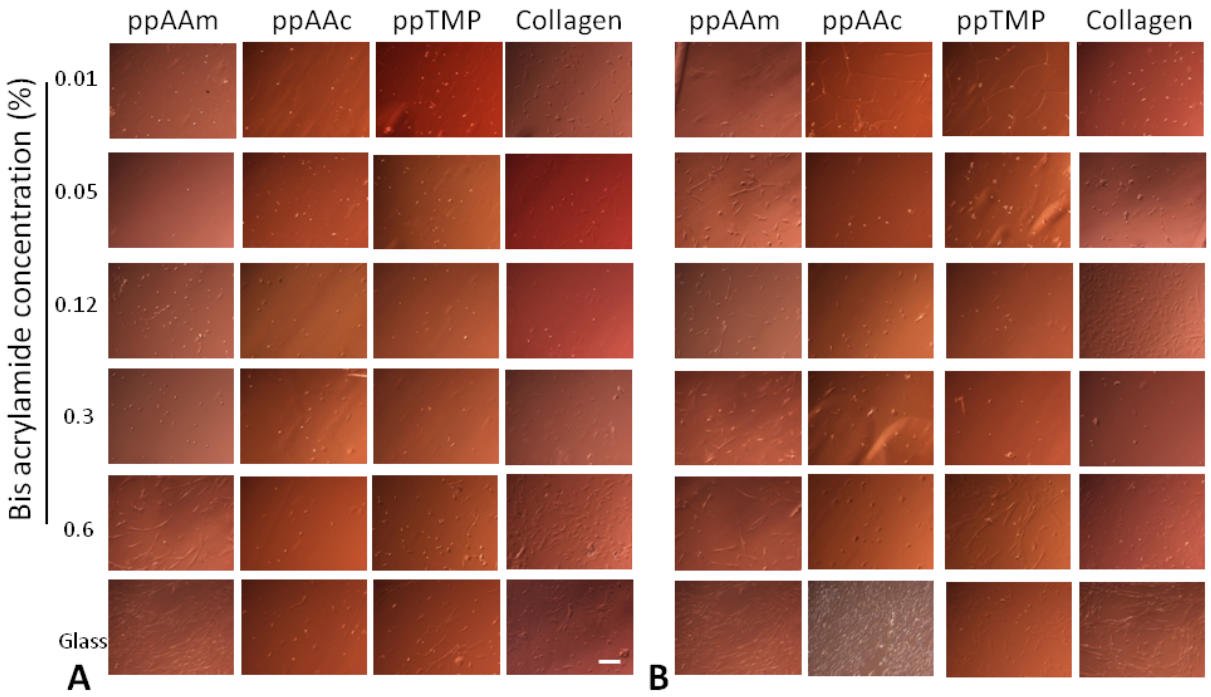

Figure S4. Light microscope images of hMSCs cultured in DMEM on coated polyacrylamide gels of varying bis acrylamide concentrations

A. hMSCs after three days of culture on the polyacrylamide gels. Scale bar is 200 μm.

B. hMSCs after fourteen days of culture on the polyacrylamide gels.

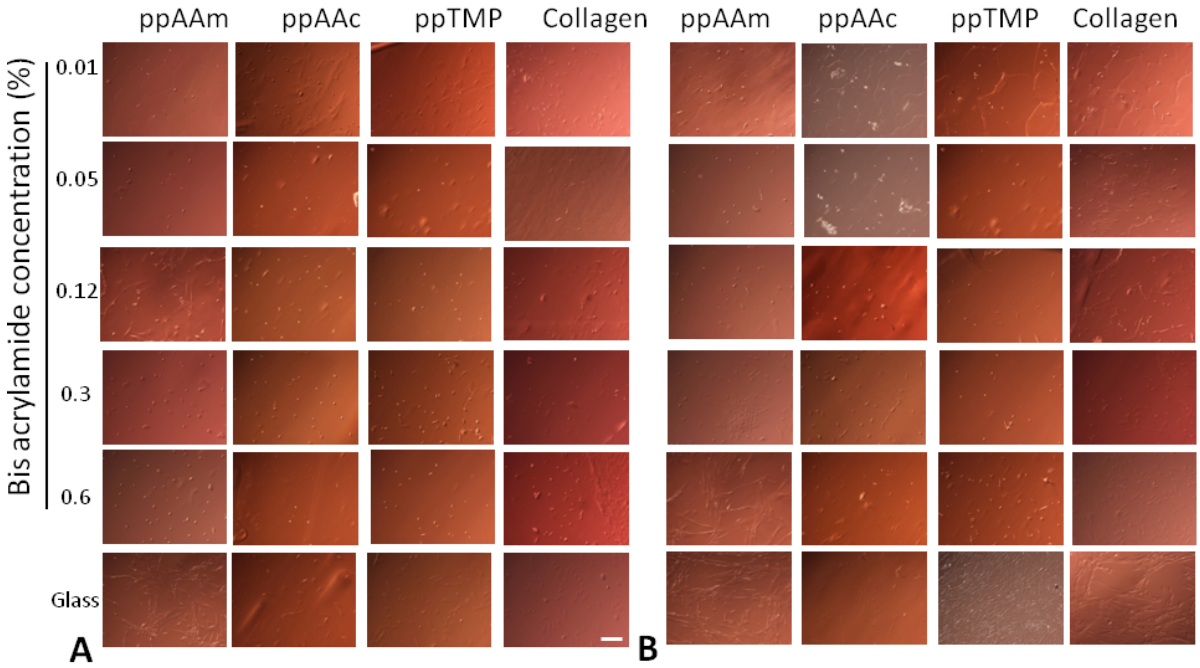

Figure S5. Light microscope images of hMSCs cultured in osteogenic medium on coated polyacrylamide gels of varying bis acrylamide concentrations

A. hMSCs after three days of culture on the polyacrylamide gels. Scale bar is 200 μm.

B. hMSCs after fourteen days of culture on the polyacrylamide gels.

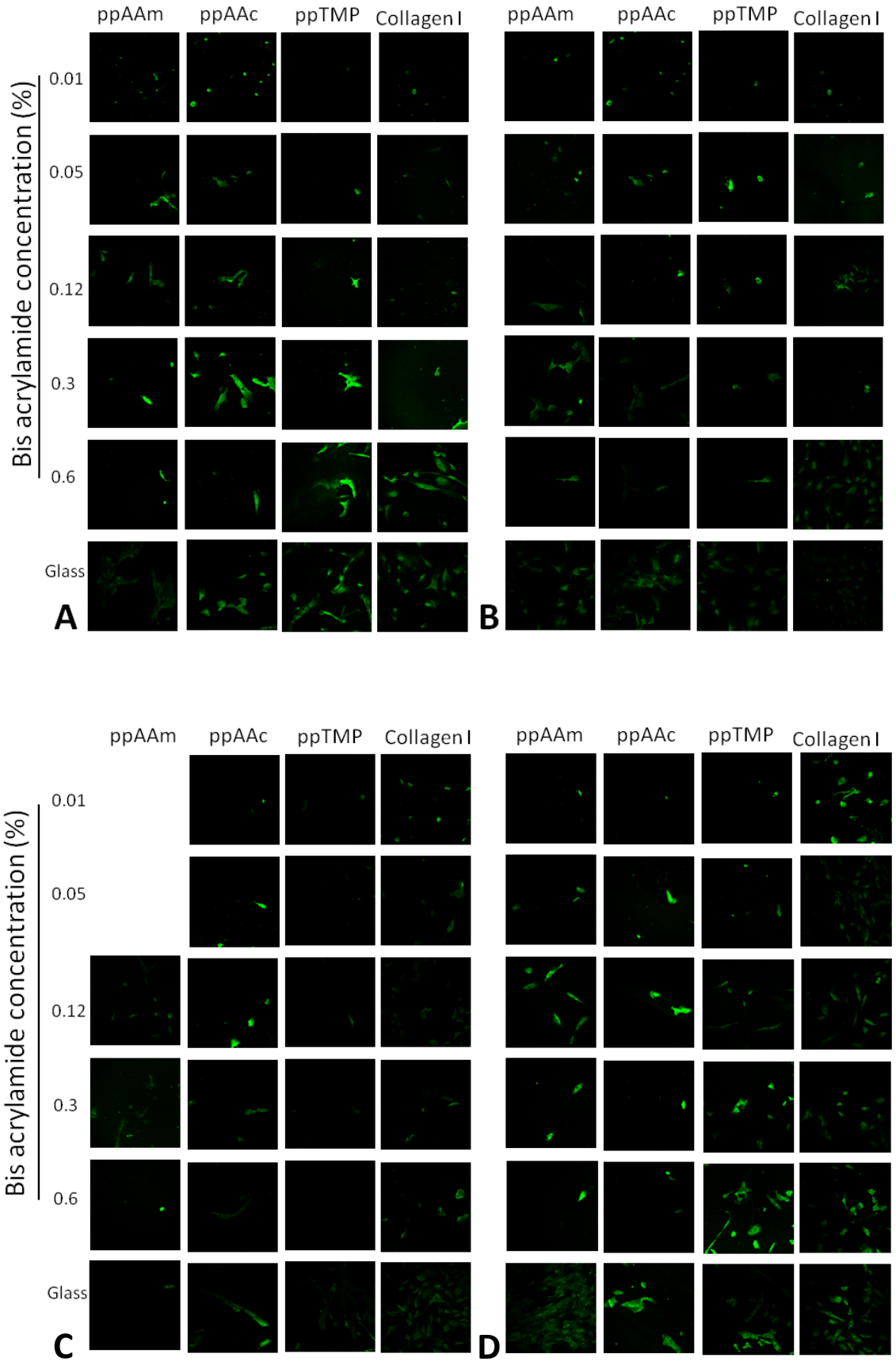

Figure S6. Confocal images of fixed hMSC after fourteen days of culture on the coated gels

A [illegible] stained for β-III tubulin

C [illegible] stained for Runx2 [illegible] enic medium)

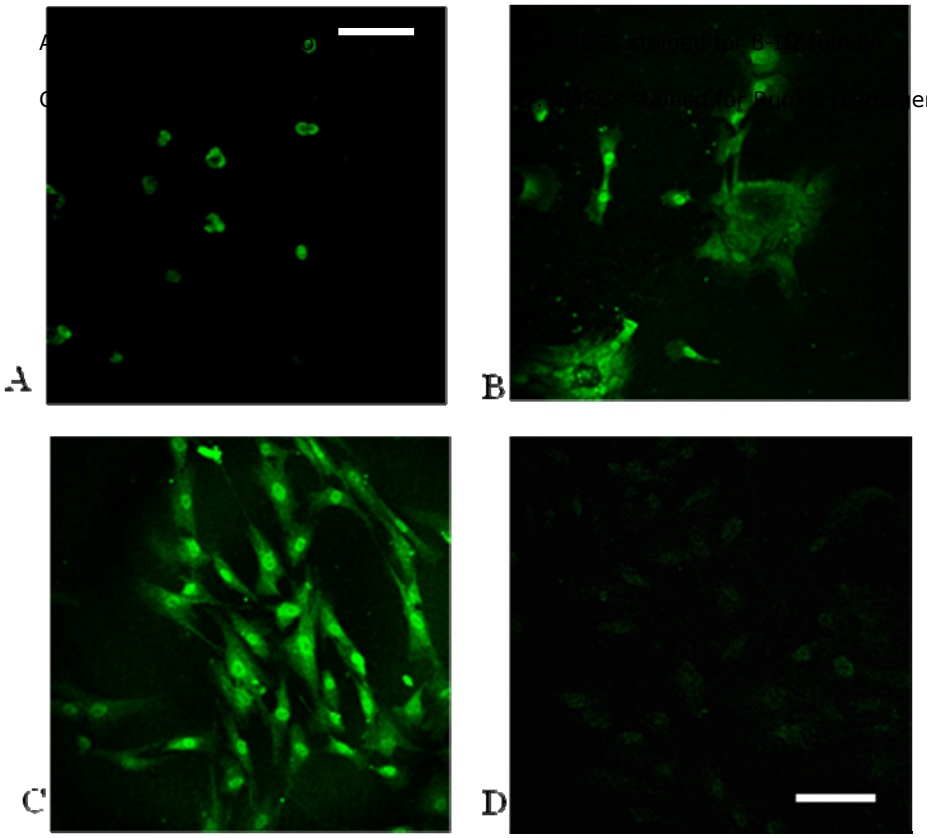

Figure S7. Confocal microscope images for hMSCs cultured on ppTMP coated gels and stained for Runx2

Human MSCs on ppTMP coated gels of :A. 7 kPa Young's modulus, B. 14 kPa Young's modulus, C. 23 kPa Young's modulus. D. Human MSCs on ppTMP coated glass. Scale bar is 200 μm.

The staining is shown to be more intense in the nucleus of the cells.

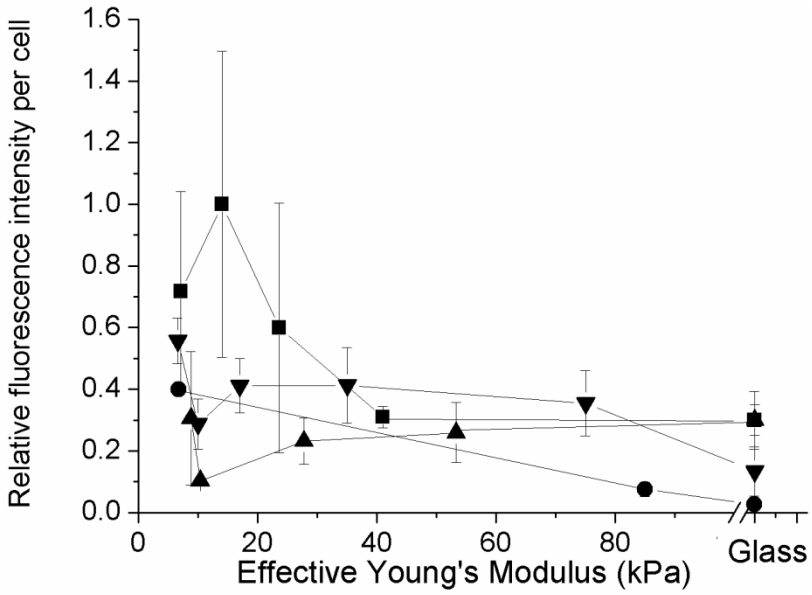

Figure S8. Relative RunX2 fluorescence intensity as a function of surface stiffness in hMSCs cultured in osteogenic medium for fourteen days on polyacrylamide gels or glass coverslips coated with various pulsed plasma polymer coatings (ppAAm (▲), ppTMP (■), ppAAc (▼)) or collagen type I (●) coating).

The fluorescence values are divided by the maximum fluorescence value observed for the marker. Scale bar is 200 μm. Error bars represent standard deviations. An average of four images for each data point was used for quantification.

Supplemental statistical analysis

Effect of coating chemistry and bis acrylamide concentration on the measured Young's modulus values (refers to Figure 1)

Table S1. One way-ANOVA for each tested bis acrylamide concentration (0.01%, 0.05%, 0.12%, 0.3%, 0.6%)

	Coating	Sample Size	Mean Young's modulus values (kPa)	Standard Deviation	Standard error of the mean	F-value	P-value	Conclusions
0.01% bis acrylamide	Uncoated	75	5.54	1	0.12	18.6	5.70E-13	**At least one of the means is significantly different from the others at the 0.05 level**
	ppAAm	59	8.46	1.64	0.21			
	ppAAc	25	6.53	1.99	0.4			
	ppTMP	39	6.91	3.29	0.53			
	Collagen	12	6.89	2.95	0.85			
0.05% bis acrylamide	Uncoated	49	14.08	2.14	0.31	112	0	**At least one of the means is significantly different from the others at the 0.05 level**
	ppAAm	63	10.21	1.89	0.24			
	ppAAc	32	10.1	6.02	1.06			
	ppTMP	40	14.2	1.45	0.23			
	Collagen	12	23.9	2	0.58			
0.12% bis acrylamide	Uncoated	37	33.55	4.75	0.78	180	0	**At least one of the means is significantly different from the others at the 0.05 level**
	ppAAm	70	27.13	3.94	0.47			
	ppAAc	40	17.13	1.32	0.21			
	ppTMP	50	23.05	1.06	0.15			
	Collagen	31	37.84	10.92	1.96			
0.3% bis acrylamide	Uncoated	36	63.16	4.83	0.8	217.9	0	**At least one of the means is significantly different from the others at the 0.05 level**
	ppAAm	70	53.25	3.63	0.43			
	ppAAc	35	35.21	4.61	0.78			
	ppTMP	20	40.85	4.81	1.08			
	Collagen	50	63.15	16.67	2.36			
0.6% bis acrylamide	Uncoated	67	152.16	32.93	4.02	126.1	0	**At least one of the means is significantly different from the others at the 0.05 level**
	ppAAm	27	87.14	9.04	1.74			
	ppAAc	20	75.21	10.36	2.32			
	ppTMP	53	79.22	21.57	2.96			
	Collagen	46	85.05	3.56	0.52			

Effect of coating chemistry and bis acrylamide concentration on the surface roughness values (refers to Figure 2)

Table S2. One-way ANOVA on the mean surface roughness values obtained for each coating

Surface coating	Stiffness values (kPa)	Sample size	Mean roughness value (nm)	Standard deviation	Standard error of the mean	F-value	P-value	Conclusions
Uncoated	5.5	6	0.195	0.013	0.005	5.9	0.002	At least one of the means is significantly different from the others at the 0.05 level
	14	6	0.154	0.021	0.008			
	33.5	6	0.224	0.061	0.025			
	63	6	0.142	0.029	0.012			
	152	6	0.147	0.018	0.007			
ppAAm	8.5	6	0.216	0.019	0.008	11.2	2.3E-05	At least one of the means is significantly different from the others at the 0.05 level
	10	6	0.229	0.017	0.007			
	27	6	0.157	0.013	0.005			
	53	6	0.255	0.038	0.016			
	90	6	0.260	0.047	0.019			
ppAAc	6.5	6	0.316	0.046	0.019	8.4	1.8E-04	At least one of the means is significantly different from the others at the 0.05 level
	10	6	0.252	0.017	0.007			
	17	6	0.250	0.018	0.007			
	35	6	0.202	0.011	0.005			
	75	6	0.298	0.065	0.027			
ppTMP	7	6	0.250	0.017	0.007	3.4	0.023	At least one of the means is significantly different from the others at the 0.05 level
	14	6	0.272	0.012	0.005			
	23	6	0.279	0.018	0.007			
	41	5	0.277	0.008	0.004			
	79	6	0.27	0.018	0.007			
Collagen	7	5	0.446	0.014	0.006	14.4	6.3E-06	At least one of the means is significantly different from the others at the 0.05 level
	24	6	0.341	0.024	0.01			
	38	6	0.420	0.045	0.018			
	63	5	0.335	0.036	0.016			
	85	5	0.354	0.02	0.009			

Table S3. One way ANOVA for the overall roughness value obtained for each coating

Coating	Sample Size	Mean roughness value (nm)	Standard Deviation	Standard error of the mean	F-value	P-value	Conclusions
Uncoated	30	0.170	0.046	0.008	82.4	0	At least one of the means is significantly different from the others at the 0.05 level
ppAAm	30	0.223	0.047	0.009			
ppAAc	30	0.264	0.054	0.010			
ppTMP	30	0.270	0.018	0.003			

Collagen	30	0.379	0.053	0.010			

Cell attachment on the coated gels after 3 days of culture in DMEM (refers to Fig.5 A)

Table S4. One way ANOVA on the mean cell densities after three days of culture in DMEM for all the gel surface coatings studied

Surface coating	Stiffness values (kPa)	Sample size	Mean cell density (cell / mm^2)	Standard deviation	Standard error of the mean	F-value	P-value	Conclusions
ppAAm	8.5	5	5.5	7.78	3.48	17.7	7.60E-04	**At least one of the means is significantly different from the others at the 0.05 level**
	10	5	1	1.41	0.63			
	27	5	43	6.25	2.79			
	53	5	20.5	10.61	4.74			
	90	5	30	7.07	3.16			
	Glass	5	47	2.83	1.26			
ppAAc	6.5	5	7	1.41	0.63	46.3	3.70E-06	**At least one of the means is significantly different from the others at the 0.05 level**
	10	5	4.5	0.71	0.32			
	17	5	15	4.24	1.9			
	35	5	21.67	1.53	0.68			
	75	5	10	2	0.89			
	Glass	5	28	2	0.89			
ppTMP	7	5	28.5	12.02	5.38	3.6	0.039	**At least one of the means is significantly different from the others at the 0.05 level**
	14	5	17.33	9.5	4.25			
	23	5	9.75	3.1	1.38			
	41	5	6	1.41	0.63			
	79	5	35.67	23.44	10.48			
	Glass	5	55.5	27.58	12.33			
Collagen	7	5	23	13.89	6.21	1.2	0.387	The means are not significantly different at the 0.05 level
	24	5	15.5	6.36	2.85			
	38	5	25	7.07	3.16			
	63	5	22.5	4.95	2.21			
	85	5	40.5	16.26	7.27			
	Glass	5	37.5	17.68	7.91			

Table S5. One way ANOVA on the overall cell density mean for each coating chemistry after three days of culture in DMEM

Coating	**Sample Size**	**Mean cell density (cell / mm^2)**	**Standard Deviation**	**Standard error of the mean**	**F-value**	**P-value**	**Conclusions**
ppAAm	30	25.923	18.804	3.433	3.0	0.039	**At least one of the means is significantly different from the others at the 0.05 level**
ppAAc	30	15.467	8.871	1.620			
ppTMP	30	23.625	20.396	3.724			
Collagen	30	27.000	12.987	2.371			

Table S6.*Post hoc* Bonferroni tests for the cell density observations at day 3

Comparisons of cell densities after 3 days	**Probability**	**Significant at the 0.05 level**
ppAAc-ppAAm	0.2451	No
ppTMP- ppAAm	1	No
ppTMP- ppAAc	0.54148	No
Collagen- ppAAm	1	No
Collagen- ppAAc	0.14848	No
Collagen – ppTMP	1	No

Cell attachment on the coated gels after 14 days of culture in DMEM (refers to Fig.5 B)

Table S7. One way ANOVA on the mean cell densities after fourteen days of culture in DMEM for all the gel surface coatings studied

Surface coating	Stiffness values (kPa)	Sample size	Mean cell density (cell / mm^2)	Standard deviation	Standard error of the mean	F-value	P-value	Conclusions
ppAAm	8.5	8	1.63	0.74	0.26	17.3	8.5E-10	**At least one of the means is significantly different from the others at the 0.05 level**
	10	4	3.00	3.56	1.78			
	27	18	8.06	3.19	0.75			
	53	14	13.29	19.96	5.33			
	90	5	6.60	5.32	2.38			
	Glass	5	98.80	62.49	27.95			
ppAAc	6.5	8	1.50	0.53	0.19	5.0	4.8E-04	**At least one of the means is significantly different from the others at the 0.05 level**
	10	13	3.69	2.84	0.79			
	17	13	4.38	2.18	0.60			
	35	23	6.13	4.38	0.91			
	75	11	4.55	3.86	1.16			
	Glass	10	80.70	126.12	39.88			
ppTMP	7	13	1.62	0.77	0.21	5.4	2.4E-04	**At least one of the means is significantly different from the others at the 0.05 level**
	14	7	2.43	1.40	0.53			
	23	16	2.63	2.16	0.54			
	41	17	5.24	4.82	1.17			
	79	14	7.07	4.20	1.12			
	Glass	13	73.54	108.96	30.22			
Collagen	7	13	11.54	4.33	1.20	4.3	1.8E-03	**At least one of the means is significantly different from the others at the 0.05 level**
	24	9	31.00	14.65	4.88			
	38	11	20.09	10.93	3.30			
	63	10	20.50	9.45	2.99			
	85	11	25.09	21.40	6.45			
	Glass	14	106.07	131.89	35.25			

Table S8.*Post hoc* Bonferroni tests for the effect of surface stiffness on cell density after three days of culture in DMEM for each coating

	Comparisons	Probability	Significant at the 0.05 level		Comparisons	Probability	Significant at the 0.05 level
ppAAm	**10 kPa - 8.5 kPa**	1	No	**ppTMP**	**14 kPa - 7 kPa**	1	No
	27 kPa - 8.5 kPa	1	No		**23 kPa - 7 kPa**	1	No
	27 kPa - 10 kPa	1	No		**23 kPa - 14 kPa**	1	No
	53 kPa - 8.5 kPa	1	No		**41 kPa - 7 kPa**	1	No
	53 kPa - 10 kPa	1	No		**41 kPa - 14 kPa**	1	No
	53 kPa - 27 kPa	1	No		**41 kPa - 23 kPa**	1	No
	90 kPa - 8.5 kPa	1	No		**79 kPa - 7 kPa**	1	No
	90 kPa- 10 kPa	1	No		**79 kPa- 14 kPa**	1	No
	90 kPa - 27 kPa	1	No		**79 kPa - 23 kPa**	1	No
	90 kPa - 53 kPa	1	No		**79 kPa - 41 kPa**	1	No
	Glass - 8.5 kPa	2.1E-09	**Yes**		**Glass - 7 kPa**	1.2E-03	**Yes**
	Glass - 10 kPa	2.2E-07	**Yes**		**Glass - 14 kPa**	1.4E-02	**Yes**
	Glass - 27 kPa	4.8E-10	**Yes**		**Glass - 23 kPa**	7.2E-04	**Yes**
	Glass - 53 kPa	6.1E-09	**Yes**		**Glass - 41 kPa**	1.1E-03	**Yes**
	Glass - 90 kPa	1.3E-07	**Yes**		**Glass - 79 kPa**	2.9E-03	**Yes**
	Comparisons	**Probability**	**Significant at the 0.05 level**		**Comparisons**	**Probability**	**Significant at the 0.05 level**
ppAAc	**10 kPa - 6.5 kPa**	1	No	**Collagen**	**24 kPa - 7 kPa**	1	No
	17 kPa - 6.5 kPa	1	No		**38 kPa - 7 kPa**	1	No
	17 kPa - 10 kPa	1	No		**38 kPa - 24 kPa**	1	No
	35 kPa - 6.5 kPa	1	No		**63 kPa - 7 kPa**	1	No
	35 kPa - 10 kPa	1	No		**63 kPa - 24 kPa**	1	No
	35 kPa - 17 kPa	1	No		**63 kPa - 38 kPa**	1	No
	75 kPa - 6.5 kPa	1	No		**85 kPa - 7 kPa**	1	No
	75 kPa- 10 kPa	1	No		**85 kPa- 24 kPa**	1	No
	75 kPa - 17 kPa	1	No		**85 kPa - 38 kPa**	1	No
	75 kPa - 35 kPa	1	No		**85 kPa - 63 kPa**	1	No
	Glass - 6.5 kPa	5.6E-03	**Yes**		**Glass - 7 kPa**	2.7E-03	**Yes**
	Glass - 10 kPa	1.6E-03	**Yes**		**Glass - 24 kPa**	8.7E-02	No
	Glass - 17 kPa	1.9E-03	**Yes**		**Glass - 38 kPa**	1.4E-02	**Yes**
	Glass - 35 kPa	5.4E-04	**Yes**		**Glass - 63 kPa**	2.0E-02	**Yes**
	Glass - 75 kPa	3.2E-03	**Yes**		**Glass - 85 kPa**	2.7E-02	**Yes**

Table S9. One way ANOVA on the overall cell density mean for each coating chemistry after fourteen days of culture in DMEM

Coating	Sample Size	Mean cell density (cell / mm^2)	Standard Deviation	Standard error of the mean	F-value	P-value	Conclusions
ppAAm	54	16.35	33.48	4.56	2.9	0.034	**At least one of the means is significantly different from the others at the 0.05 level**
ppAAc	78	14.29	50.27	5.69			
ppTMP	80	15.30	49.82	5.57			
Collagen	68	38.47	68.83	8.35			

Table S10.*Post hoc* Bonferroni tests on the mean cell densities observed for each coating after three days of culture in DMEM

Comparisons	Probability	Significant at the 0.05 level
ppAAc-ppAAm	1	No
ppTMP- ppAAm	1	No
ppTMP- ppAAc	1	No
Collagen- ppAAm	0.043	**Yes**
Collagen- ppAAc	0.007	**Yes**
Collagen – ppTMP	0.011	**Yes**

Mean cell areas observed after 14 days of culture in DMEM (refers to Fig.6A)

Table S11. One way ANOVA on the mean cell areas after fourteen days of culture in DMEM for all the surface coatings studied

Surface coating	Stiffness values (kPa)	Sample size	Mean cell area (μm^2)	Standard deviation	Standard error of the mean	F-value	P-value	Conclusions
ppAAm	8.5	19	1020.27	491.33	112.72	4.4	6.8E-04	**At least one of the means is significantly different from the others at the 0.05 level**
	10	5	2955.73	1639.65	733.28			
	27	33	4548.88	2656.72	462.48			
	53	62	4404.10	2430.22	308.64			
	Glass	39	4861.98	1389.18	222.45			
ppAAc	6.5	14	870.66	422.39	112.89	10.0	6.5E-09	**At least one of the means is significantly different from the others at the 0.05 level**
	10	55	2636.49	1478.32	199.34			
	17	50	3674.30	1945.19	275.09			
	35	38	4032.02	2036.87	330.42			
	75	16	3385.85	2584.76	646.19			
	Glass	86	3657.75	1652.36	178.18			
ppTMP	7	17	1400.98	881.04	213.68	4.9	2.7E-04	**At least one of the means is significantly different from the others at the 0.05 level**
	14	11	2952.90	2039.68	614.99			
	23	28	3385.16	1769.02	334.31			
	41	47	2858.65	1744.07	254.40			
	79	57	3794.97	2459.31	325.74			
	Glass	94	3596.53	1793.18	184.95			
Collagen	7	102	2304.65	3309.57	327.70	8.6	5.9E-08	**At least one of the means is significantly different from the others at the 0.05 level**
	24	79	3074.79	2040.98	229.63			
	38	99	4257.96	2470.11	248.26			
	63	84	3798.25	2465.97	269.06			
	85	134	3439.94	2071.21	178.93			
	Glass	129	3916.50	1833.41	161.42			

Table S12. *Post hoc* Bonferroni tests on the mean cell areas after fourteen days of culture in DMEM for all studied coatings

	Comparisons	Probability	Significant at the 0.05 level
ppAAm	**10 kPa - 8.5 kPa**	1	No
	27 kPa - 8.5 kPa	8.4E-04	**Yes**
	27 kPa - 10 kPa	1	No
	53 kPa - 8.5 kPa	0.0016	**Yes**
	53 kPa - 10 kPa	1	No
	53 kPa - 27 kPa	1	No
	Glass - 8.5 kPa	0.014	**Yes**
	Glass - 10 kPa	1	No
	Glass - 27 kPa	1	No
	Glass - 53 kPa	1	No
ppAAc	**10 kPa - 6.5 kPa**	0.019	**Yes**
	17 kPa - 6.5 kPa	0	**Yes**
	17 kPa - 10 kPa	0.056	No
	35 kPa - 6.5 kPa	0	**Yes**
	35 kPa - 10 kPa	0	**Yes**
	35 kPa - 17 kPa	1	No
	75 kPa - 6.5 kPa	0.003	**Yes**
	75 kPa- 10 kPa	1	No
	75 kPa - 17 kPa	1	No
	75 kPa - 35 kPa	1	No
	Glass - 6.5 kPa	0	**Yes**
	Glass - 10 kPa	0.019	**Yes**
	Glass - 17 kPa	1	No
	Glass - 35 kPa	1	No
	Glass - 75 kPa	1	No

	Comparisons	Probability	Significant at the 0.05 level
ppTMP	**14 kPa - 7 kPa**	0.68	No
	23 kPa - 7 kPa	0.018	**Yes**
	23 kPa - 14 kPa	1	No
	41 kPa - 7 kPa	0.16	No
	41 kPa - 14 kPa	1	No
	41 kPa - 23 kPa	1	No
	79 kPa - 7 kPa	2.4E-04	**Yes**
	79 kPa- 14 kPa	1	No
	79 kPa - 23 kPa	1	No
	79 kPa - 41 kPa	0.211	No
	Glass - 7 kPa	5.1E-04	**Yes**
	Glass - 14 kPa	1	No
	Glass - 23 kPa	1	No
	Glass - 41 kPa	0.49	No
	Glass - 79 kPa	1	No
Collagen	**24 kPa - 7 kPa**	0.47	No
	38 kPa - 7 kPa	1.5E-07	**Yes**
	38 kPa - 24 kPa	0.016	**Yes**
	63 kPa - 7 kPa	3.7E-04	**Yes**
	63 kPa - 24 kPa	0.80	No
	63 kPa - 38 kPa	1	No
	85 kPa - 7 kPa	0.004	**Yes**
	85 kPa- 24 kPa	1	No
	85 kPa - 38 kPa	0.16	No
	85 kPa - 63 kPa	1	No
	Glass - 7 kPa	6.8E-06	**Yes**
	Glass - 24 kPa	0.207	No
	Glass - 38 kPa	1	No
	Glass - 63 kPa	1	No
	Glass - 85 kPa	1	No

Mean cell areas observed after 14 days of culture in osteogenic medium (refers to Fig.6B)

Table S13. One way ANOVA on the mean cell areas after fourteen days of culture in osteogenic medium for all the surface coatings studied

Surface coating	Stiffness values (kPa)	Sample size	Mean cell area (μm^2)	Standard deviation	Standard error of the mean	F-value	P-value	Conclusions
ppAAm	8.5	6	2162.91	2787.87	1138.14	0.9	0.474	The means are not significantly different at the 0.05 level
	10	5	1148.23	70.16	31.38			
	27	14	4796.96	4784.35	1278.67			
	53	15	3709.85	2652.25	684.81			
	Glass	40	4003.45	1833.41	289.89			
ppAAc	6.5	14	849.33	680.87	181.97	18.6	1.0E-11	**At least one of the means is significantly different from the others at the 0.05 level**
	10	55	1370.14	369.80	49.86			
	17	50	2165.75	1421.90	201.09			
	35	38	4466.01	2228.77	361.55			
	75	16	3220.28	1704.93	426.23			
	Glass	86	3962.65	1241.25	133.85			
ppTMP	7	17	965.19	659.88	160.04	4.1	6.8E-03	**At least one of the means is significantly different from the others at the 0.05 level**
	14	11	1381.21	426.59	128.62			
	23	28	4167.25	1537.96	290.65			
	41	47	1690.95	259.67	37.88			
	Glass	94	4672.25	2386.94	246.19			
Collagen	7	102	2201.42	836.31	82.81	0.9	0.321	The means are not significantly different at the 0.05 level
	38	99	4908.34	757.16	76.10			
	85	134	3766.91	1671.77	144.42			
	Glass	129	3344.02	822.78	72.44			

Table S14. *Post hoc* Bonferroni tests on the mean cell areas after fourteen days of culture in osteogenic medium for the ppAAc and ppTMP coated samples

	Comparisons	Probability	Significant at the 0.05 level
ppAAc	**10 kPa - 6.5 kPa**	1	No
	17 kPa - 6.5 kPa	0.85	No
	17 kPa - 10 kPa	1	No
	35 kPa - 6.5 kPa	2.2E-11	**Yes**
	35 kPa - 10 kPa	7.2E-05	**Yes**
	35 kPa - 17 kPa	2.7E-02	**Yes**
	75 kPa - 6.5 kPa	2.2E-03	**Yes**
	75 kPa- 10 kPa	0.24	No
	75 kPa - 17 kPa	1	No
	75 kPa - 35 kPa	0.75	No
	Glass - 6.5 kPa	9.4E-07	**Yes**
	Glass - 10 kPa	5.4E-03	**Yes**
	Glass - 17 kPa	0.33	No
	Glass - 35 kPa	1	No
	Glass - 75 kPa	1	No
ppTMP	**14 kPa - 7 kPa**	1	No
	23 kPa - 7 kPa	0.57	No
	23 kPa - 14 kPa	1	No
	41 kPa - 7 kPa	1	No
	41 kPa - 14 kPa	1	No
	41 kPa - 23 kPa	1	No
	Glass - 7 kPa	6.8E-02	No
	Glass - 14 kPa	0.42	No
	Glass - 23 kPa	1	No
	Glass - 41 kPa	0.13	No

Expression of ß-III tubulin for hMSCs cultured in DMEM for fourteen days (refers to Fig.8 A)

Table S15. One way ANOVA on the mean relative fluorescence intensities (ß-III tubulin) after fourteen days of culture in DMEM for all the surface coatings studied

Surface coating	Stiffness values (kPa)	Sample size	Mean relative fluorescence intensity	Standard deviation	Standard error of the mean	F-value	P-value	Conclusions
ppAAm	8.5	17	0.69	0.31	0.07	4.4	1.5E-03	**At least one of the means is significantly different from the others at the 0.05 level**
	10	10	0.57	0.11	0.04			
	27	40	0.54	0.13	0.02			
	53	10	0.50	0.17	0.06			
	Glass	40	0.23	0.11	0.02			
ppAAc	6.5	13	1.00	0.05	0.01	7.9	8.6E-04	**At least one of the means is significantly different from the others at the 0.05 level**
	10	13	0.81	0.23	0.06			
	17	14	0.90	0.09	0.02			
	35	14	0.91	0.11	0.03			
	75	14	0.70	0.16	0.04			
	Glass	30	0.71	0.11	0.02			
ppTMP	7	16	0.41	0.03	0.01	15.4	1.2E-11	**At least one of the means is significantly different from the others at the 0.05 level**
	14	17	0.23	0.11	0.03			
	23	12	0.16	0.06	0.02			
	41	20	0.26	0.11	0.02			
	79	33	0.36	0.07	0.01			
	Glass	47	0.29	0.07	0.01			
Collagen	7	50	0.51	0.25	0.04	25.3	5.6E-12	**At least one of the means is significantly different from the others at the 0.05 level**
	24	33	0.22	0.11	0.02			
	38	64	0.28	0.14	0.02			
	63	61	0.31	0.11	0.01			
	Glass	70	0.19	0.07	0.01			

Table S16. *Post hoc* Bonferroni tests on the mean relative fluorescence intensities (ß-III tubulin) after fourteen days of culture in DMEM for hMSCs on ppAAc coated samples

	Comparisons	Probability	Significant at the 0.05 level
ppAAc coated samples	**10 kPa - 6.5 kPa**	1	No
	17 kPa - 6.5 kPa	1	No
	17 kPa - 10 kPa	1	No
	35 kPa - 6.5 kPa	1	No
	35 kPa - 10 kPa	1	No
	35 kPa - 17 kPa	1	No
	75 kPa - 6.5 kPa	1.08E-04	**Yes**
	75 kPa- 10 kPa	1	No
	75 kPa - 17 kPa	0	No
	75 kPa - 35 kPa	0	No
	Glass - 6.5 kPa	0.002	**Yes**
	Glass - 10 kPa	1	No
	Glass - 17 kPa	1	No
	Glass - 35 kPa	0.203	No
	Glass - 75 kPa	1	No

Expression of MyoD1 for hMSCs cultured in DMEM for fourteen days (refers to Fig.8B)

Table S17. One way ANOVA on the mean relative fluorescence intensities (MyoD1) after fourteen days of culture in DMEM for all the surface coatings studied

Surface coating	Stiffness values (kPa)	Sample size	Mean relative fluorescence intensity	Standard deviation	Standard error of the mean	F-value	P-value	Conclusions
ppAAm	27	25	0.26	0.11	0.02	3.78	2.0E-02	**At least one of the means is significantly different from the others at the 0.05 level**
	53	48	0.28	0.08	0.01			
	90	15	0.47	0.06	0.01			
	Glass	44	0.34	0.12	0.02			
ppAAc	6.5	15	0.90	0.07	0.02	39.38	8.6E-07	**At least one of the means is significantly different from the others at the 0.05 level**
	10	38	1.00	0.13	0.02			
	17	19	0.99	0.10	0.02			
	35	35	0.71	0.26	0.04			
	75	16	0.14	0.07	0.02			
	Glass	47	0.67	0.18	0.03			
ppTMP	7	15	0.25	0.36	0.09	8.03	3.6E-06	**At least one of the means is significantly different from the others at the 0.05 level**
	14	15	0.38	0.45	0.12			
	23	12	0.43	0.25	0.07			
	41	20	0.23	0.16	0.04			
	79	13	0.06	0.05	0.01			
	Glass	31	0.26	0.11	0.02			
Collagen	7	50	0.53	0.15	0.02	49.07	9.5E-09	**At least one of the means is significantly different from the others at the 0.05 level**
	24	33	0.22	0.10	0.02			
	38	64	0.14	0.06	0.01			
	63	61	0.47	0.25	0.03			
	85	79	0.36	0.28	0.03			
	Glass	70	0.14	0.04	0.01			

Table S18. *Post hoc* Bonferroni tests on the mean relative fluorescence intensities (MyoD1) after fourteen days of culture in DMEM for hMSCs on ppAAc coated samples

Comparisons	Probability	Significant at the 0.05 level
10 kPa - 6.5 kPa	1	No
17 kPa - 6.5 kPa	1	No
17 kPa - 10 kPa	1	No
35 kPa - 6.5 kPa	0.382	No
35 kPa - 10 kPa	3.1E-10	**Yes**
35 kPa - 17 kPa	0	**Yes**
75 kPa - 6.5 kPa	8.0E-10	**Yes**
75 kPa- 10 kPa	0	**Yes**
75 kPa - 17 kPa	0	**Yes**
75 kPa - 35 kPa	0	**Yes**
Glass - 6.5 kPa	0.104	No
Glass - 10 kPa	6.9E-14	**Yes**
Glass - 17 kPa	0	**Yes**
Glass - 35 kPa	1	No
Glass - 75 kPa	0	**Yes**

Expression of RunX2 for hMSCs cultured in DMEM for fourteen days (refers to Fig.8C)

Table S19. One way ANOVA on the mean relative fluorescence intensities (Runx2) after fourteen days of culture in DMEM for all the surface coatings studied

Surface coating	Stiffness values (kPa)	Sample size	Mean relative fluorescence intensity	Standard deviation	Standard error of the mean	F-value	P-value	Conclusions
ppAAm	8.5	16	0.41	0.11	0.03	11.23	1.2E-06	**At least one of the means is significantly different from the others at the 0.05 level**
	27	29	0.26	0.10	0.02			
	53	15	0.57	0.07	0.02			
	90	10	0.12	0.04	0.01			
	Glass	29	0.19	0.03	0.01			
ppAAc	6.5	16	0.59	0.07	0.02	9.97	1.7E-06	**At least one of the means is significantly different from the others at the 0.05 level**
	10	12	0.65	0.03	0.01			
	17	13	0.41	0.22	0.06			
	35	25	0.42	0.10	0.02			
	75	16	0.48	0.12	0.03			
	Glass	20	0.27	0.09	0.02			
ppTMP	7	12	0.49	0.16	0.05	8.85	1.7E-05	**At least one of the means is significantly different from the others at the 0.05 level**
	23	14	0.89	0.52	0.14			
	41	17	1.00	0.41	0.10			
	79	11	0.81	0.37	0.11			
	Glass	26	0.24	0.05	0.01			
Collagen	7	19	0.41	0.07	0.02	1.25	0.304	**At least one of the means is significantly different from the others at the 0.05 level**
	85	34	0.15	0.10	0.02			
	Glass	38	0.15	0.05	0.01			

Table S20. *Post hoc* Bonferroni tests on the mean relative fluorescence intensities (Runx2) after fourteen days of culture in DMEM for hMSCs on ppTMP coated samples

Comparisons	Probability	Significant at the 0.05 level
14 kPa - 7 kPa	1	No
23 kPa - 7 kPa	1	No
23 kPa - 14 kPa	1	No
41 kPa - 7 kPa	0.68	No
41 kPa - 14 kPa	0.98	No
41 kPa - 23 kPa	1	No
79 kPa - 7 kPa	1	No
79 kPa- 14 kPa	1	No
79 kPa - 23 kPa	1	No
79 kPa - 41 kPa	1	No
Glass - 7 kPa	1	No
Glass - 14 kPa	1	No
Glass - 23 kPa	8.3E-03	**Yes**
Glass - 41 kPa	5.0E-05	**Yes**
Glass - 79 kPa	4.2E-04	**Yes**

RunX2 expression for hMSCs cultured in osteogenic medium for fourteen days (refers to Fig 9 and Fig. S8)

Table S21. One way ANOVA on the mean relative fluorescence intensities (Runx2) after fourteen days of culture in osteogenic medium for all the surface coatings studied

Surface coating	Stiffness values (kPa)	Sample size	Mean relative fluorescence intensity	Standard deviation	Standard error of the mean	F-value	P-value	Conclusions
ppAAm	8.5	14	0.31	0.22	0.06	1.09	0.385	The means are not significantly different at the 0.05 level
	10	8	0.10	0.01	0.00			
	27	14	0.23	0.07	0.02			
	53	15	0.26	0.10	0.03			
	Glass	31	0.26	0.07	0.01			
ppAAc	6.5	29	0.56	0.07	0.01	35.07	1.9E-11	**At least one of the means is significantly different from the others at the 0.05 level**
	10	17	0.29	0.08	0.02			
	17	15	0.41	0.09	0.02			
	35	18	0.41	0.12	0.03			
	75	17	0.35	0.11	0.03			
	Glass	30	0.13	0.08	0.01			
ppTMP	7	13	0.72	0.32	0.09	9.62	5.4E-06	**At least one of the means is significantly different from the others at the 0.05 level**
	14	12	1.00	0.50	0.14			
	23	14	0.60	0.40	0.11			
	41	14	0.31	0.03	0.01			
	Glass	30	0.30	0.09	0.02			
Collagen	7	11	0.10	0.07	0.02	16.53	1.5E-10	**At least one of the means is significantly different from the others at the 0.05 level**
	85	51	0.07	0.02	0.00			
	Glass	47	0.03	0.01	0.00			

Table S22. *Post hoc* Bonferroni tests on the mean relative fluorescence intensities (Runx2) after fourteen days of culture in osteogenic medium for hMSCs on ppTMP coated samples

Comparisons	Probability	Significant at the 0.05 level
14 kPa - 7 kPa	1	No
23 kPa - 7 kPa	1	No
23 kPa - 14 kPa	0.20	No
41 kPa - 7 kPa	7.3E-02	No
41 kPa - 14 kPa	1.0E-03	**Yes**
41 kPa - 23 kPa	0.41	No
Glass - 7 kPa	6.3E-03	**Yes**
Glass - 14 kPa	6.1E-05	**Yes**
Glass - 23 kPa	4.7E-02	**Yes**
Glass - 41 kPa	1	No

Nodule density for hMSCs cultured for 14 days in osteogenic medium (refers to Fig.10 A)

Table S23. One way ANOVA on the mean nodule densities depending on surface stiffness value for each coating chemistry after cell culture for fourteen days in osteogenic medium.

Surface coating	Stiffness values (kPa)	Sample size	Mean nodule density (nodule / mm^2)	Standard deviation	Standard error of the mean	F-value	P-value	Conclusions
ppAAm	8.5	6	0.00	0.00	0.00	11.0	1.6E-05	**At least one of the means is significantly different from the others at the 0.05 level**
	10	6	2.67	1.03	0.42			
	27	6	0.67	0.52	0.21			
	53	6	1.00	0.89	0.37			
	90	6	2.33	0.52	0.21			
	Glass	6	0.00	0.00	0.00			
ppAAc	6.5	6	0.00	0.00	0.00	4.6	5.8E-03	**At least one of the means is significantly different from the others at the 0.05 level**
	10	6	0.00	0.00	0.00			
	17	6	0.00	0.00	0.00			
	35	6	1.00	0.89	0.37			
	75	6	0.00	0.00	0.00			
	Glass	6	0.00	0.00	0.00			
ppTMP	7	6	0.00	0.00	0.00	1.1	0.37	The means are not significantly different at the 0.05 level
	14	6	0.00	0.00	0.00			
	23	6	2.00	3.10	1.26			
	41	6	0.00	0.00	0.00			
	79	6	0.00	0.00	0.00			
	Glass	6	0.00	0.00	0.00			
Collagen	7	6	0.00	0.00	0.00	1.5	0.22	The means are not significantly different at the 0.05 level
	24	6	0.00	0.00	0.00			
	38	6	0.00	0.00	0.00			
	63	6	0.00	0.00	0.00			
	85	6	0.00	0.00	0.00			
	Glass	6	0.33	0.52	0.21			

Table S24. One way ANOVA on the overall mean nodule densities for each surface coating

Surface coating	Sample size	Mean nodule density (nodule / mm^2)	Standard deviation	Standard error of the mean	F-value	P-value	Conclusions
ppAAm	36	1.37931	1.20753	0.20	6.8	2.96E-04	**At least one of the means is significantly different from the others at the 0.05 level**
ppAAc	36	0.23077	0.58704	0.10			
ppTMP	36	0.46154	1.63048	0.27			
Collagen	36	0.07692	0.27175	0.05			

Table S25.*Post hoc* Bonferroni tests for the overall nodule densities for each surface coating

Comparisons	Probability	Significant at the 0.05 level
ppAA c- ppAAm	7.61E-04	**Yes**
ppTMP - ppAAm	0.01148	**Yes**
ppTMP - ppAAc	1	No
Collagen - ppAAm	1.01E-04	**Yes**
Coolagen - ppAAc	1	No
Collagen - ppTMP	1	No