

SUPPORTING INFORMATION

Unravelling the Synergies: Effects of Hydrogel Mechanics and Biofunctionalization on the Osteogenic Differentiation of Mesenchymal Stem Cells

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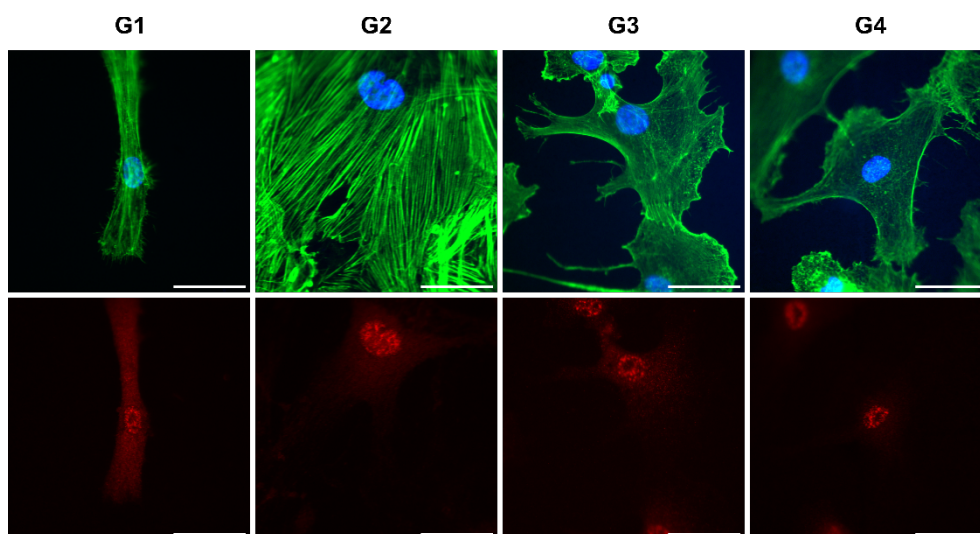


Figure S1: Representative fluorescence images of OPN staining of cells culture on different hydrogels (G1 to G4) functionalized with Mix1. Cytoskeleton (green) and nucleus (blue) on the top row and OPN (red) on the bottom row. Scale bar = 50 μ m.

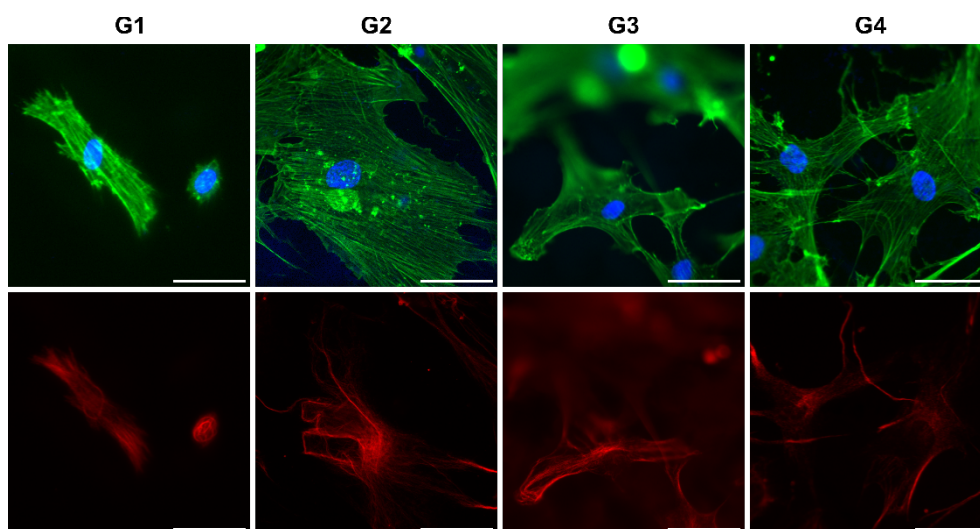


Figure S2: Representative fluorescence images of PDPN staining of cells culture on different hydrogels (G1 to G4) functionalized with Mix1. Cytoskeleton (green) and nucleus (blue) on the top row and E11 (red) on the bottom row. Scale bar = 50 μ m.

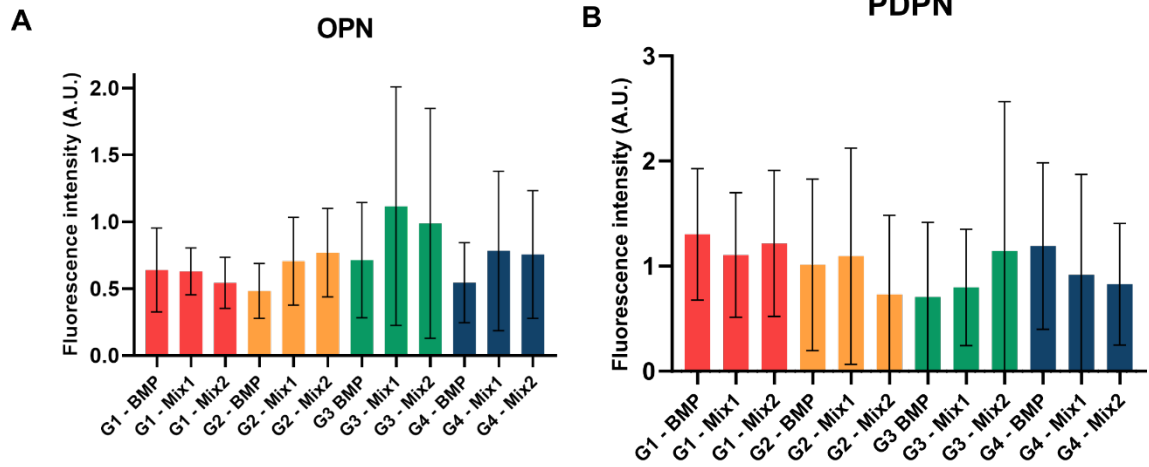


Figure S3: Evaluation of OPN and PDPN fluorescence intensity from immunocytochemistry assay.

	G1 - BMP	G1 - Mix1	G1 - Mix2	G2 - BMP	G2 - Mix1	G2 - Mix2	G3 BMP	G3 - Mix1	G3 - Mix2	G4 - BMP	G4 - Mix1	G4 - Mix2
G1 - BMP		ns	ns	ns	****	****	ns	****	***	ns	****	*
G1 - Mix1			ns	ns	****	****	ns	****	****	ns	****	**
G1 - Mix2				ns	****	****	ns	****	****	ns	****	**
G2 - BMP					****	****	ns	*	ns	ns	ns	ns
G2 - Mix1						ns	****	***	***	****	****	****
G2 - Mix2							****	ns	ns	****	ns	***
G3 BMP								*	ns	ns	ns	ns
G3 - Mix1									ns	****	ns	ns
G3 - Mix2										**	ns	ns
G4 - BMP											***	ns
G4 - Mix1												ns
G4 - Mix2												

Table S1: Statistical analyses of total cell area. Statistical analyses were done by one-way analysis of variance (ANOVA) and Tukey's test for multiple comparisons. P values are represented as follows * < 0.05, ** < 0.01, *** < 0.001, **** < 0.0001. ns indicates the absence of statistical significance at alpha equal to 0.05.

	G1 - BMP	G1 - Mix1	G1 - Mix2	G2 - BMP	G2 - Mix1	G2 - Mix2	G3 BMP	G3 - Mix1	G3 - Mix2	G4 - BMP	G4 - Mix1	G4 - Mix2
G1 - BMP		ns	ns	ns	****	****	ns	**	ns	ns	ns	ns
G1 - Mix1			ns	**	****	****	ns	***	*	*	*	*
G1 - Mix2				***	****	****	ns	****	***	**	***	***
G2 - BMP					**	*	ns	ns	ns	ns	ns	ns
G2 - Mix1						ns	****	ns	*	***	**	**
G2 - Mix2							****	ns	ns	**	**	**
G3 BMP								ns	ns	ns	ns	ns
G3 - Mix1									ns	ns	ns	ns
G3 - Mix2										ns	ns	ns
G4 - BMP											ns	ns
G4 - Mix1												ns
G4 - Mix2												

Table S2: Statistical analyses of nuclei area. Statistical analyses were done by one-way analysis of variance (ANOVA) and Tukey's test for multiple comparisons. P values are represented as follows * < 0.05, ** < 0.01, *** < 0.001, **** < 0.0001. ns indicates the absence of statistical significance at alpha equal to 0.05.

	G1 - BMP	G1 - Mix1	G1 - Mix2	G2 - BMP	G2 - Mix1	G2 - Mix2	G3 BMP	G3 - Mix1	G3 - Mix2	G4 - BMP	G4 - Mix1	G4 - Mix2
G1 - BMP		ns	ns	ns	ns	ns	ns	**	ns	ns	ns	ns
G1 - Mix1			ns	ns	ns	ns	ns	**	ns	ns	ns	ns
G1 - Mix2				ns	ns	ns	ns	****	*	ns	ns	ns
G2 - BMP					ns	ns	ns	****	**	ns	ns	ns
G2 - Mix1						ns	ns	*	ns	ns	ns	ns
G2 - Mix2							ns	ns	ns	ns	ns	ns
G3 BMP								*	ns	ns	ns	ns
G3 - Mix1									ns	****	ns	ns
G3 - Mix2										*	ns	ns
G4 - BMP											ns	ns
G4 - Mix1												ns
G4 - Mix2												

Table S3: Statistical analyses of OPN. Statistical analyses were done by one-way analysis of variance (ANOVA) and Tukey's test for multiple comparisons. P values are represented as follows * < 0.05, ** < 0.01, *** < 0.001, **** < 0.0001. ns indicates the absence of statistical significance at alpha equal to 0.05.

	G1 - BMP	G1 - Mix1	G1 - Mix2	G2 - BMP	G2 - Mix1	G2 - Mix2	G3 BMP	G3 - Mix1	G3 - Mix2	G4 - BMP	G4 - Mix1	G4 - Mix2
G1 - BMP		ns	ns	ns	ns	*	*	ns	ns	ns	ns	ns
G1 - Mix1			ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
G1 - Mix2				ns	ns	ns	ns	ns	ns	ns	ns	ns
G2 - BMP					ns	ns	ns	ns	ns	ns	ns	ns
G2 - Mix1						ns	ns	ns	ns	ns	ns	ns
G2 - Mix2							ns	ns	ns	ns	ns	ns
G3 BMP								ns	ns	ns	ns	ns
G3 - Mix1									ns	ns	ns	ns
G3 - Mix2										ns	ns	ns
G4 - BMP											ns	ns
G4 - Mix1												ns
G4 - Mix2												

Table S4: Statistical analyses of E11 intensity. Statistical analyses were done by one-way analysis of variance (ANOVA) and Tukey's test for multiple comparisons. P values are represented as follows * < 0.05, ** < 0.01, *** < 0.001, **** < 0.0001. ns indicates the absence of statistical significance at alpha equal to 0.05.