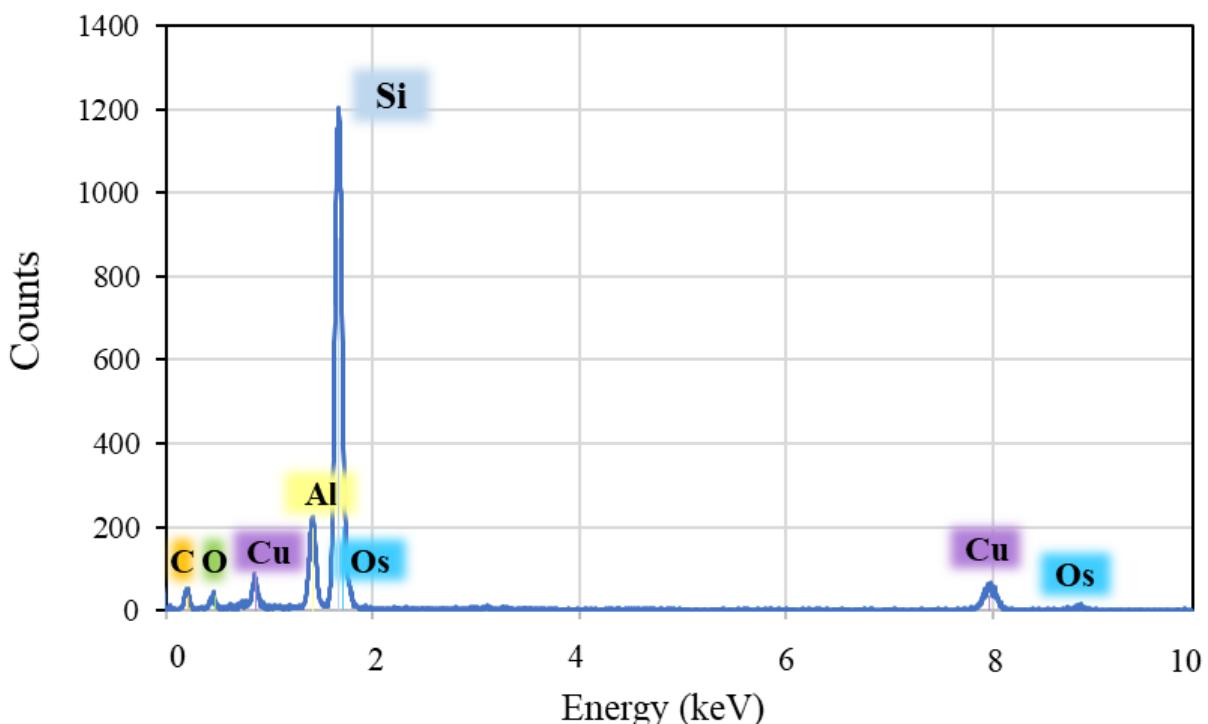


Assessment of laser-synthesized Si nanoparticles effects on myoblasts motility, proliferation and differentiation: towards potential tissue engineering applications

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Supplementary Material



Supplementary Figure 1. Energy-dispersive X-ray spectroscopy of Si-NPs. EDS was performed in the section of the sample where the nanoparticles were detected. The obtained spectrum confirmed the internalization of Si-NPs while the other elements displayed derive by the composition of the resin, the products applied for fixing the cells and the grid used to observe the sample.

	Significativity	P values
ctrl vs 14	**	0.0070
ctrl vs 28	****	<0.0001
ctrl vs 56	*	0.0133
14 vs 28	***	0.0003
14 vs 56	ns	0.9574
28 vs 56	***	0.0002

Supplementary Table 1. Statistical validation of Fusion Index analysis. For each Si-NPs concentration tested p values and statistical significance are reported. The p values are calculated using a one-way ANOVA with Tukey's multiple comparisons test.

	day 1		day 3		day 6		day 8	
	signif.	P value						
ctrl vs 14	ns	0.6909	ns	0.9805	ns	0.4143	ns	0.1818
ctrl vs 28	ns	0.0506	**	0.0025	***	0.0006	****	<0.0001
ctrl vs 56	ns	0.1192	*	0.0165	*	0.0203	ns	0.8206
14 vs 28	ns	0.2382	**	0.0038	**	0.0034	***	0.0002
14 vs 56	*	0.0250	*	0.0100	**	0.0026	ns	0.5256
28 vs 56	**	0.0019	****	<0.0001	****	<0.0001	****	<0.0001

Supplementary Table 2. Statistical validation of the growth curves analysis. For each Si-NPs concentration tested at each experimental point, p values and statistical significance are reported. The concentration of 28 µg/mL is the only one that is significant compared to the other conditions starting on day 3 (light blue lines).

	hours post-scratch																				
	7.5	8	8.5	9	9.5	10	10.5	11	11.5	12	12.5	13	13.5	14	14.5	15	15.5	16	16.5	17.5	18
ctrl vs 14											*										
ctrl vs 28					*	**															
ctrl vs 56																					
14 vs 28																					
14 vs 56	*	*	*	**	**	**	**	*	***	**	**	**	**	**	*	*	***	**	**	*	
28 vs 56	*	*	*	*	****	***	**	**	***	**	*	**	*				**	*	**		

Supplementary Table 3. Statistical validation of the Scratch-wound healing assay. For each Si-NPs concentration tested at each experimental point, p values and statistical significance are reported. Only experimental time points showing a statistically significant result are presented.

Gene	Time 2				Time 3					
	28 µg/ml		56 µg/ml		14 µg/ml		28 µg/ml			
		p value		p value		p value		p value		
MyoD	*	0.0259	*	0.0124	*	0.0259	*	0.0439	*	0.0324
Desmin	*	0.0126								
MHC									*	0.0127
Myomaker					*	0.0115			**	0.0054
Ki-67	**	0.0058	**	0.0060					**	0.0068
C-met	***	0.0006	***	0.0001						

Supplementary Table 4. Statistical validation of qRT-PCR analysis. For each gene studied at each Si-NPs concentration tested and each experimental time point, p values and statistical significance are reported. Values were calculated using the ΔCt of the target gene *versus* the ΔCt of the control gene. Statistical significance was determined using the Ordinary one-way ANOVA with Dunnett's multiple comparisons test.

Gene	Si-NPs - 14 µg/ml			Si-NPs - 28 µg/ml			Si-NPs - 56 µg/ml		
	T1 vs T2	T1 vs T3	T2 vs T3	T1 vs T2	T1 vs T3	T2 vs T3	T1 vs T2	T1 vs T3	T2 vs T3
MyoD	\$\$\$	\$\$\$		\$	\$		\$\$	\$\$	
p value	0.0005	0.0001		0.0037	0.0448		0.0024		
Desmin	\$\$\$	\$\$\$							
p value	0.001	0.0007							
MHC	\$		\$	\$			\$\$	\$\$	
p value	0.0159		0.0272	0.0019			0.0009	0.0058	
Myomaker	\$\$\$	\$\$	\$\$	\$\$\$		\$	\$\$	\$\$	
p value	0.0005	0.0014	0.0015	0.0002		0.0464	0.0034		
Ki-67	\$\$	\$		\$\$\$		\$\$			
p value	0.0079	0.013		0.0002		0.0023			
C-met				\$\$\$		\$\$\$	\$\$\$	\$\$\$	
p value				0.0010		0.0016	0.0001		0.0007

Supplementary Table 5. Significance and p values based on the $2^{-\Delta\Delta Ct}$ values. For each gene studied at each Si-NPs concentration tested and each experimental time point, p values and statistical significance are reported. In particular, for each gene the trend during the 3 experimental time points for each concentration of Si-NPs tested was investigated, using the Ordinary one-way ANOVA with Tukey's multiple comparisons test.