Electronic Supplementary Material (ESI) for Biomaterials Science. This journal is © The Royal Society of Chemistry 2021

## **Supplementary Information**

**Table S1.** Calculated salt concentrations and corresponding ion concentrations of test and controls to assess osteogenic effects of Laponite degradation products.

Laponite Conc. (µg/mL)	Salt & corresponding ion conc. (μg/mL)						
	Test (Na₂SiO₃) – to provide Si⁴+			Control group (NaCl) – to exclude Na <sup>+</sup>			
	Salt	Si	Na	Salt	Si	Na	
0	0	0	0	0	0	0	
25	31.835	7.325	10.3	26.15	0	10.3	
50	63.67	14.65	20.6	52.3	0	20.6	
100	127.34	29.3	41.2	104.6	0	41.2	

Laponite Conc. (µg/mL)	Salt & corresponding ion conc. (µg/mL)						
	Group A (MgSO <sub>4</sub> ) – to provide Mg <sup>2+</sup>			Group B (Na <sub>2</sub> SO <sub>4</sub> ) – to exclude SO <sub>4</sub> <sup>2</sup> -			
	Salt	Mg	SO <sub>4</sub>	Salt	Mg	SO <sub>4</sub>	
0	0	0	0	0	0	0	
25	21.675	4.375	17.25	25.5	0	17.25	
50	43.35	8.75	34.5	51	0	34.5	
100	86.7	17.5	69	102	0	69	

Laponite Conc. (µg/mL)	Salt & corresponding ion conc. (µg/mL)						
	Group A (LiCl) – to provide Li⁺			Group B (NaCl) – to exclude Cl-			
	Salt	Li	CI	Salt	Li	CI	
0	0	0	0	0	0	0	
25	0.4125	0.0675	0.345	0.56875	0	0.345	
50	0.825	0.135	0.69	1.1375	0	0.69	
100	1.65	0.27	1.38	2.275	0	1.38	

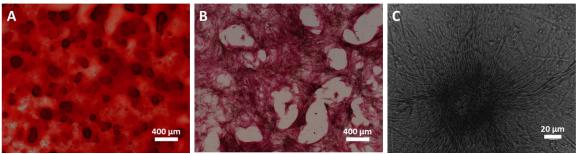


Figure S1. Mineralised nodule formation by HBMSCs following exposure to Laponite. Laponite treatment over days 1-7 resulted in the formation of mineralized high cell density nodules at day 14. Alizarin Red staining for calcium (A) co-localised with regions of high cell density staining positive for Alkaline Phosphatase (B). High magnification phase microscopy images (C) reveal nodules are formed of dense cellular clusters.

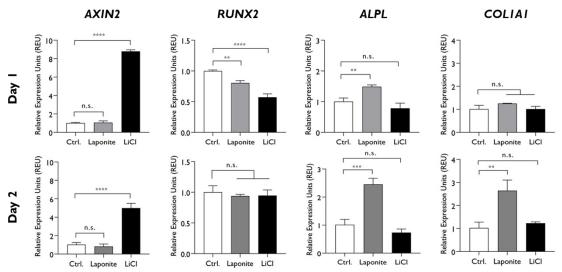


Figure S2. Early Wnt and osteogenic gene expression by HBMSCs following exposure to Laponite and LiCl. AXIN2 expression in HBMSCs was upregulated at days 1 and 2 by LiCl (50mM) but not Laponite (10  $\mu$ g/mL). RUNX2 was downregulated by both treatments at day 1. ALPL and COL1A1 expression increased over 48 hours with addition of Laponite but not LiCl. Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparisons test. Data represent mean  $\pm$  SD, N = 3. \*\*P < 0.01; \*\*\*P < 0.001; \*\*\*\*P<0.0001; n.s. = non-significant.

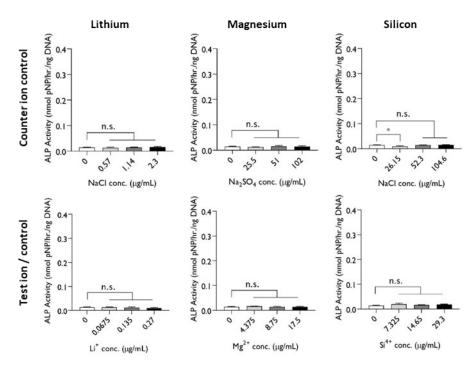


Figure S3. Effect of Laponite degradation products on alkaline phosphatase activity against counter-ion controls. Additional control salts were applied to exclude any influence of counter ions (Na<sup>+</sup>, Cl<sup>-</sup> & SO<sub>4</sub><sup>2-</sup>) on the effect of test salts. Upper panel reports background effects of control salts and lower panel reports effects of test salts normalized to effect of control salts. Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparisons test. Data represent mean  $\pm$  SD, N = 3. \*\*\*P < 0.001; \*\*\*\*P < 0.0001; n.s. = not significant.