

Supplementary Material

Mn²⁺ vs Co²⁺ substitution into β -TCP: structural details and bone cells response

Elisa Boanini, Stefania Pagani, Massimo Gazzano, Katia Rubini, Lavinia Raimondi, Angela De Luca,

Alessia Romanelli, Gianluca Giavaresi, Adriana Bigi

Table S1 – Details of primers used for gene expression analysis.

Gene	Primer forward	Primer reverse	Amplicon Length	Annealing Temperature
<i>Gapdh</i>	5'-AATGGTGAAGGTCGGTGTG-3'	5'-GTGGAGTCATACTGGAACATGTAG-3'	150 bp	60°C
<i>Col1a1</i>	5'-CGCAAAGAGTCTACATGTCTAGG-3'	5'-CATTGTGTATGCAGCTGACTTC-3'	134 bp	60°C
<i>Alpl</i>	5'-CAAGGACATCGCATATCAGCTA-3'	5'-GCCTTCTCATCCAGTTCGTAT-3'	123 bp	60°C
<i>Runx2</i>	5'-GATGATGACACTGCCACCTC-3'	5'-AGGGATGAAATGCTTGGGAA-3'	125 bp	60°C
<i>Bglap</i>	5'-AGCAGAGTGAGCAGAAAGATG-3'	5'-GAACAGACAAGTCCCACACAG-3'	77 bp	60°C
<i>Vegfa</i>	5'-AGAAAGACAGAAACAAAGCCAGA-3'	5'-TGGTGACATGGTTAATCGGT-3'	127 bp	60°C

Table S2 – Cell parameters (e.s.d. in parentheses).

Sample	a (Å)	c (Å)	V (Å ³)*
β -TCP	10.439(2)	37.406(3)	3530.3
Co1	10.439(3)	37.405(3)	3530.2
Co2	10.436(3)	37.399(3)	3527.4
Co5	10.407(3)	37.327(3)	3501.0
Co10	10.354(3)	37.178(3)	3451.5
Co15	10.326(2)	37.251(3)	3440.0
Mn1	10.433(3)	37.378(3)	3523.6
Mn2	10.430(3)	37.376(3)	3521.2
Mn5	10.413(2)	37.311(3)	3503.4
Mn10	10.384(3)	37.212(4)	3475.2
Mn20	10.356(3)	37.202(4)	3455.6

* e.s.d. lower than 0.1

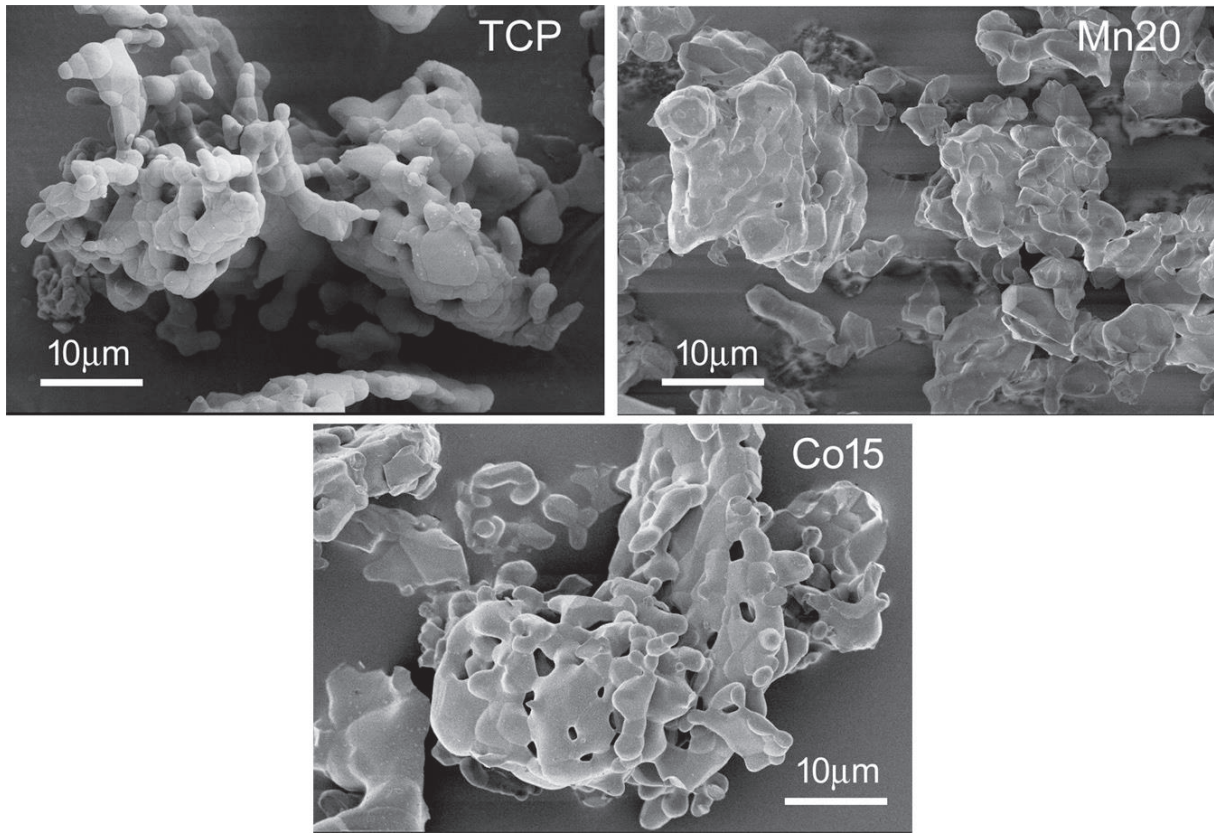


Figure S1 – SEM images of TCP, Mn20 and Co15 show the characteristic morphology of solid-state reaction products although the presence of substituting metal provokes some variations.

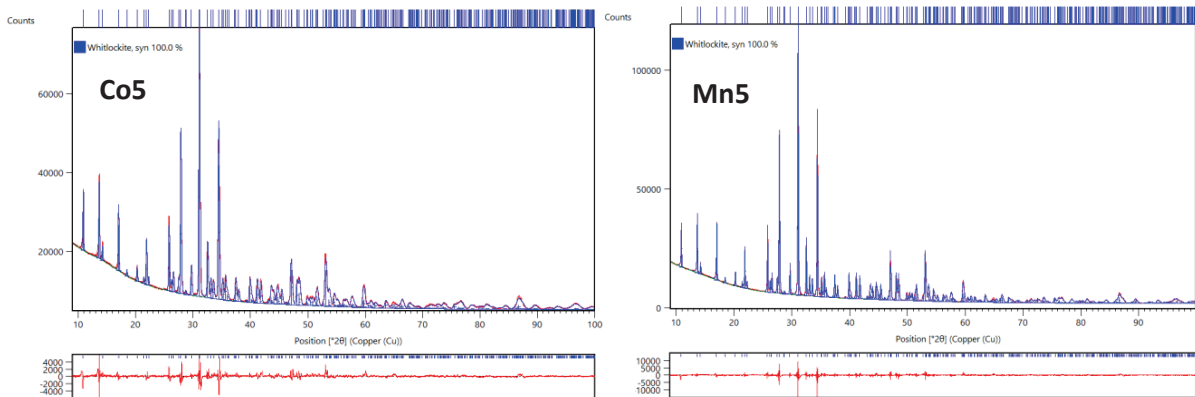


Figure S2 - Comparison of the observed (blue) and calculated (red) patterns of Co5 and Mn5. At the bottom reflection markers and difference curve are displayed. Final Rietveld plots confirm an overall good agreement and the absence of any secondary crystal phase.

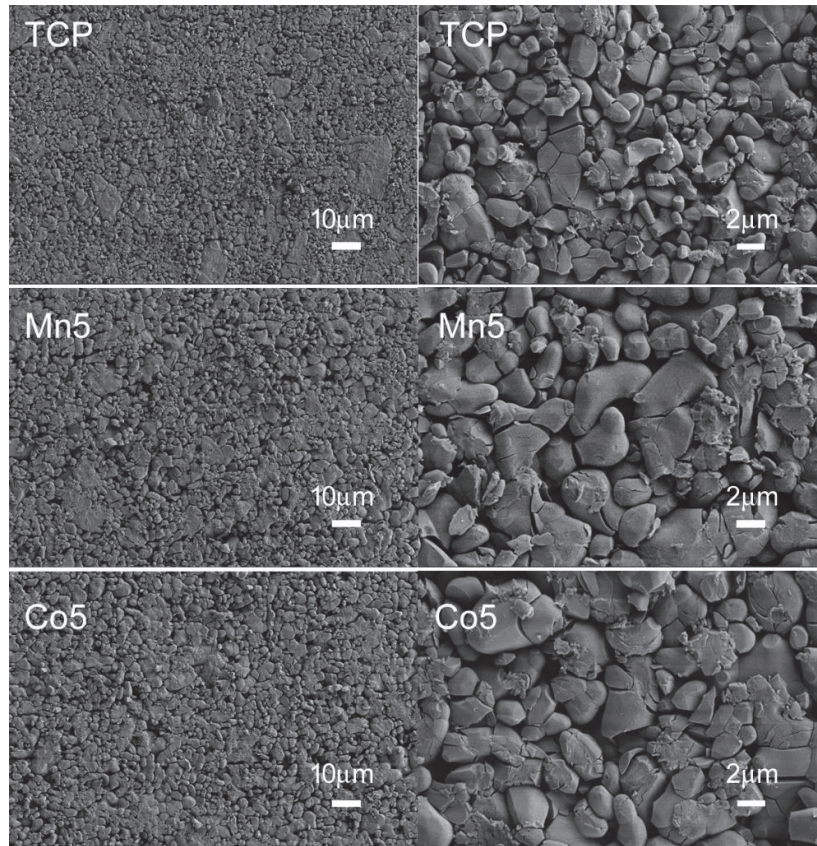


Figure S3 - SEM investigation shows that the surfaces of the disk-shaped samples do not exhibit relevant morphological differences.

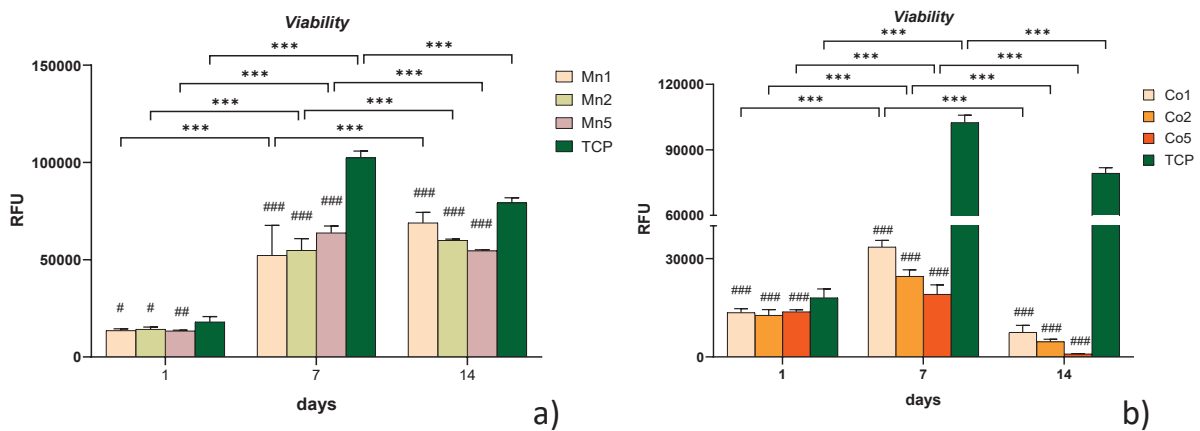


Figure S4 - Cell viability of pre-osteoblasts on β -TCP-Mn substituted (a) and Co-substituted (b), evaluated by Alamar blue assay after 1, 7 and 14 days (Mean \pm SD, n = 4 replicates). The results are expressed as relative fluorescent units (RFU) and statistical analysis is reported in the graph. Dunnet's test (#, p < 0.05, ##, p < 0.005, ###, p < 0.001): each material versus control (β -TCP) at each timepoint. Holm-Sidak's test (*, p < 0.05, **, p < 0.005, ***, p < 0.001): comparison among experimental time for the same material.

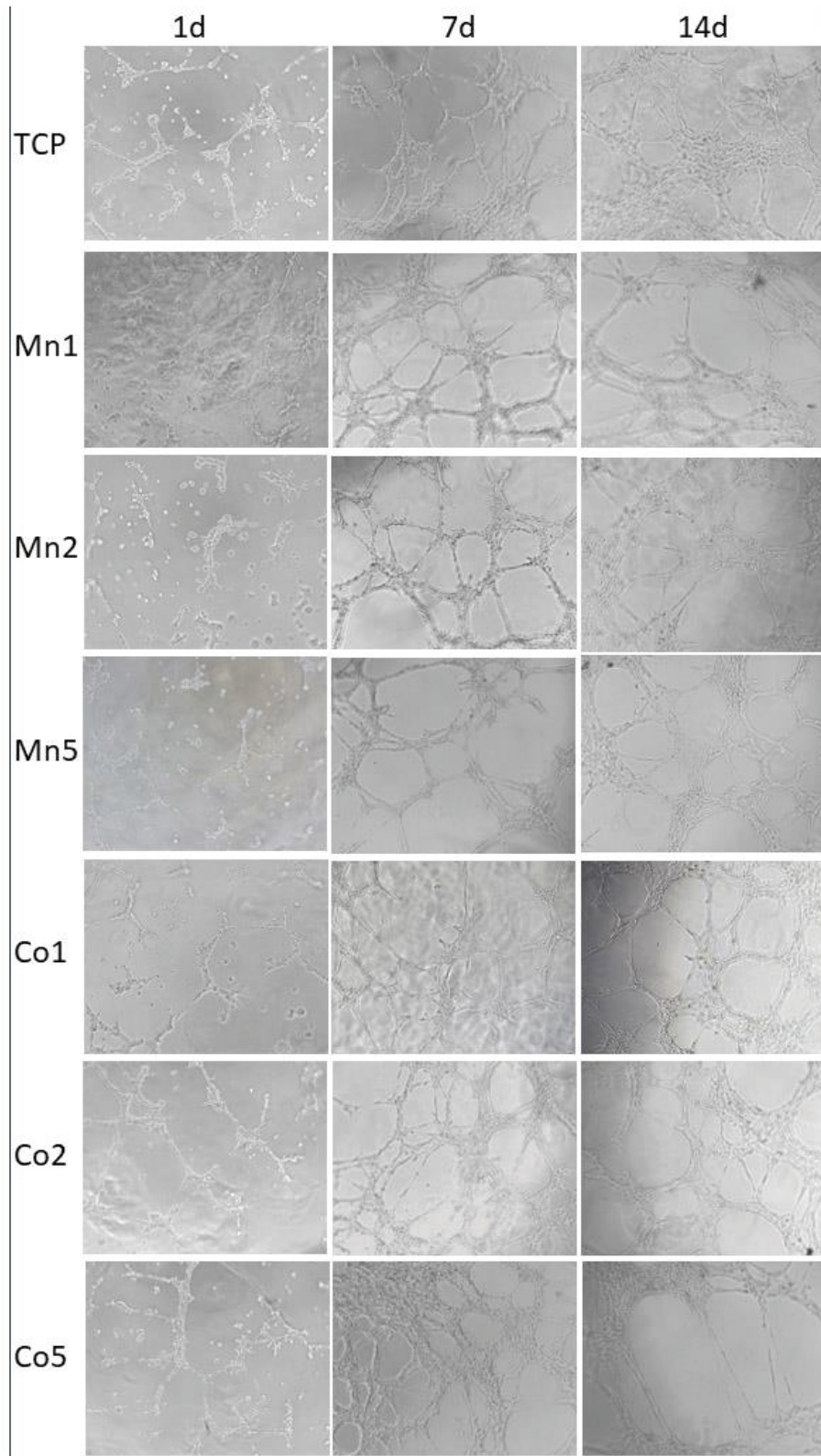


Figure S5 - Representative images (magnification 10×) of the tube formation assay performed using HUVEC cells exposed for six hours to conditioned medium obtained from MC3T3-E1 cells treated with Mn- or Co-substituted β -TCP (50% of conditioned medium and 50% of HUVEC medium).

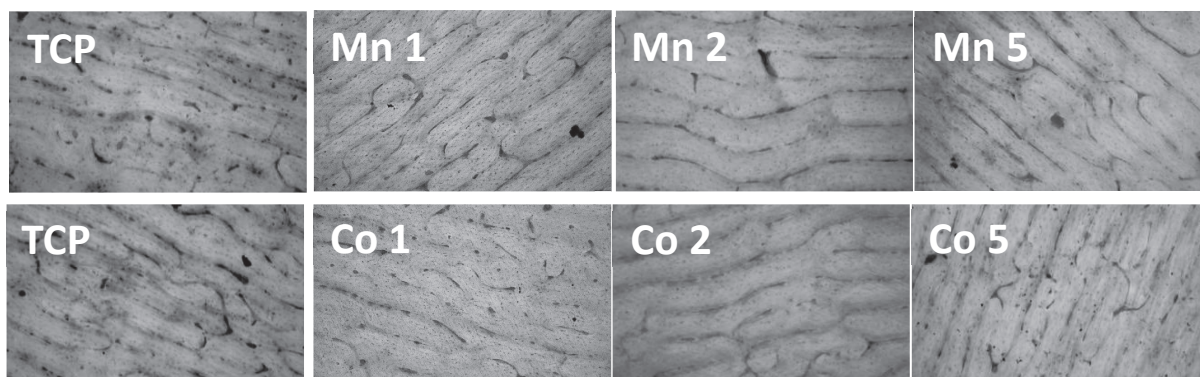


Figure S6 - Effect of Mn (1,2,5) substituted-, Co (1,2,5) substituted- and β -TCP on osteoclast activity as indicated by resorption pit area. Raw264.7 cells were seeded on dentine discs and cultured with RANKL (25 ng/ml) for 6 days in the presence of the conditioned medium obtained from MC3T3-E1 cells treated with Mn or Co-substituted β -TCP for 14 days (50% of conditioned medium and 50% of osteoclastic medium). Representative pictures of lacunae (dark areas) formed by cells (original magnification 10x) are reported.

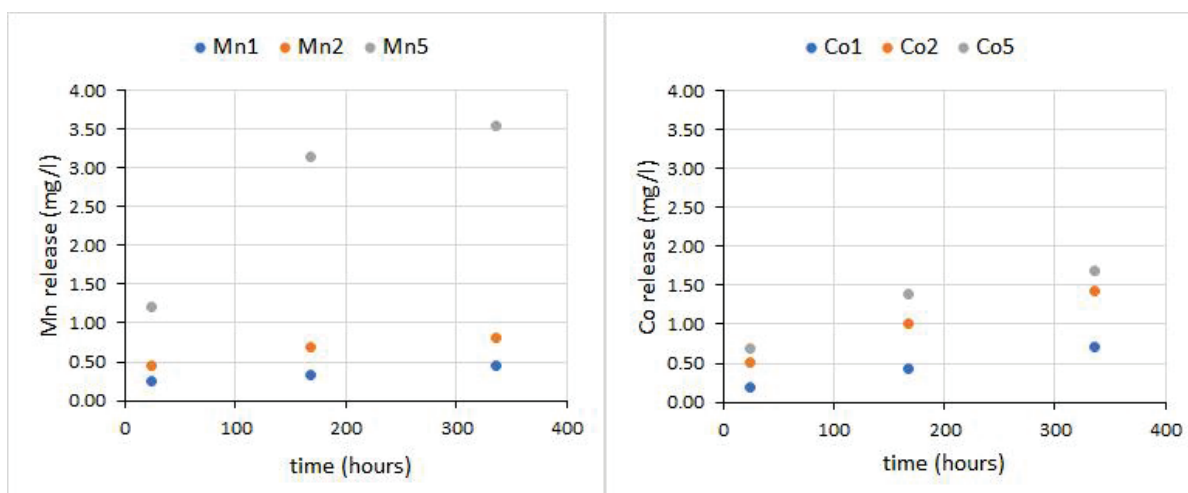


Figure S7 – Mn and Co release from disk-shaped samples in cell growth medium at 1, 7 and 14 days.