

## Supplementary Information for

### **Chemical fingerprint of Zn-hydroxyapatite in the early stages of osteogenic differentiation**

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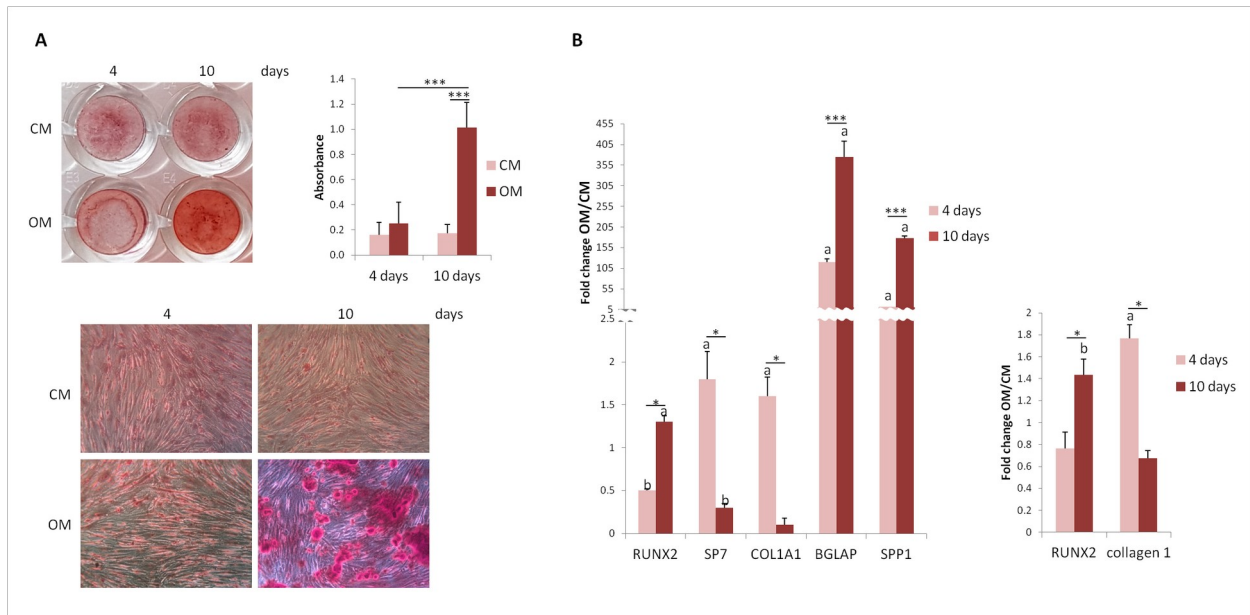
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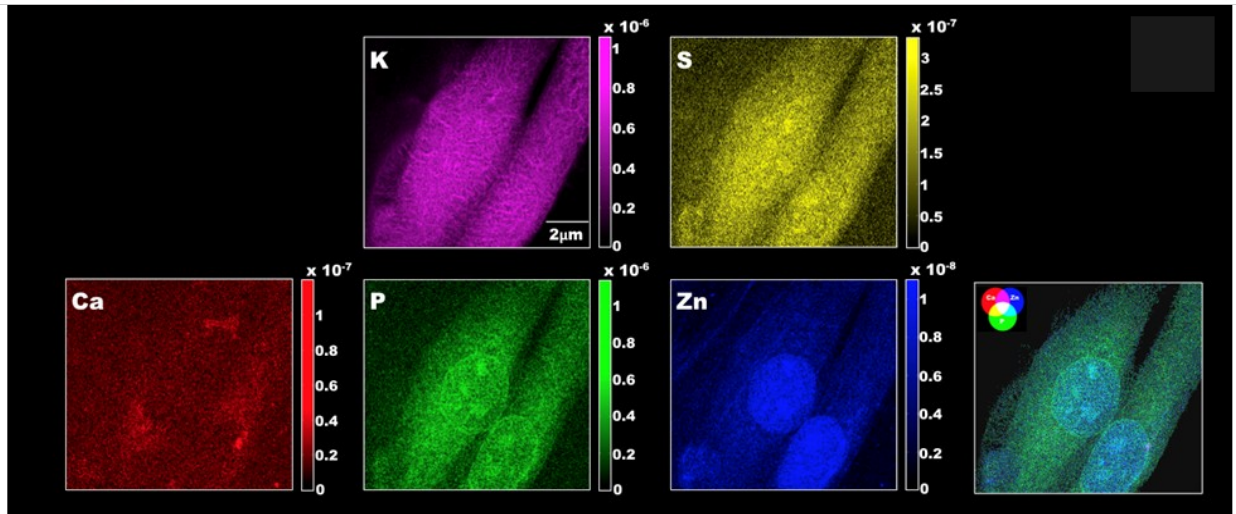
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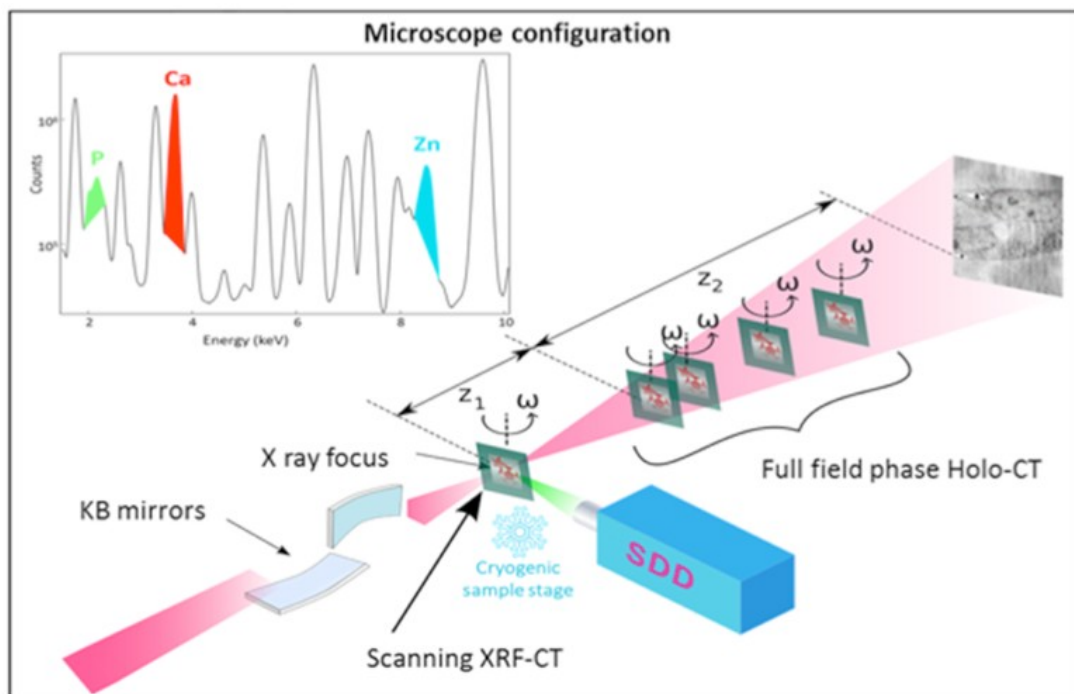
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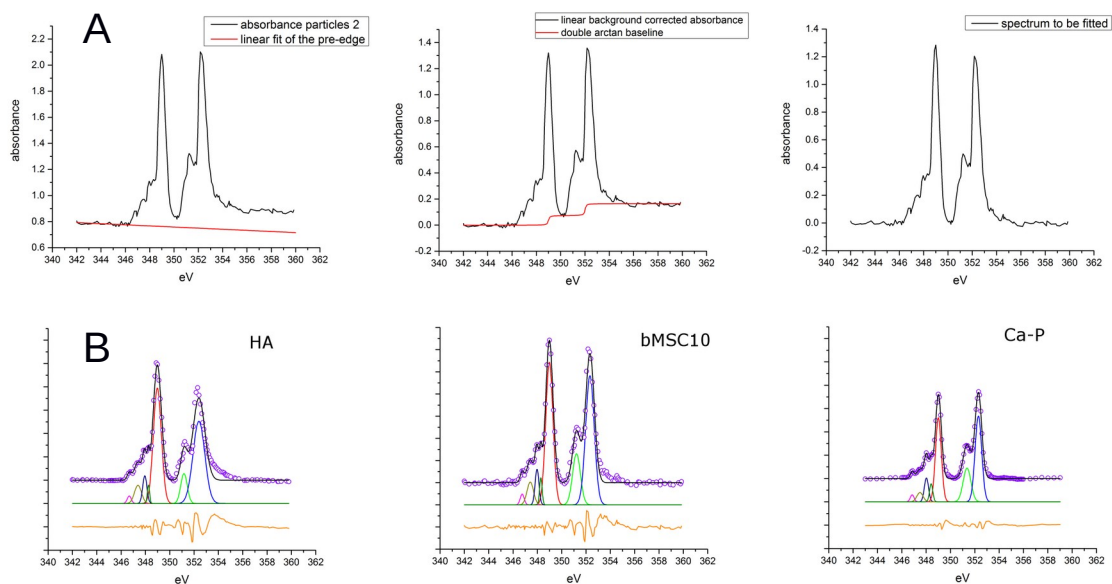
**Figure S1.** (A) Alizarin Red staining was performed after exposure to culture medium (CM) or osteogenic medium (OM) for 4 and 10 days. Photographs of a representative plate (upper left panel) and photographs taken at 10X magnification (lower panel) are shown. After acid extraction the absorbance was measured at 562 nm (upper right panel). The results are shown as the mean  $\pm$  standard deviation of four experiments in triplicate. Statistical significance was determined using Student's t test. \*\*\* $p < 0.001$ . (B) Real-Time PCR (left panel) was performed three times in triplicate on RNA extracted from bMSC exposed to CM or OM for 4 and 10 days using primers designed on *RUNX2*, *SP7*, *COL1A1*, *BGLAP* and *SPP1* sequence. ELISA for RUNX2 and collagen type 1 (right panel) was conducted on extracts from bMSC cultured in CM or OM for 4 and 10 days. All the values were normalized with respect to their controls cultured in CM. Statistical significance was determined using Student's t test. \* $p < 0.05$ , \*\*\* $p < 0.001$ . Different letters indicate the statistically significant effect of OM vs CM (a:  $p < 0.05$ , b:  $p < 0.01$ ).



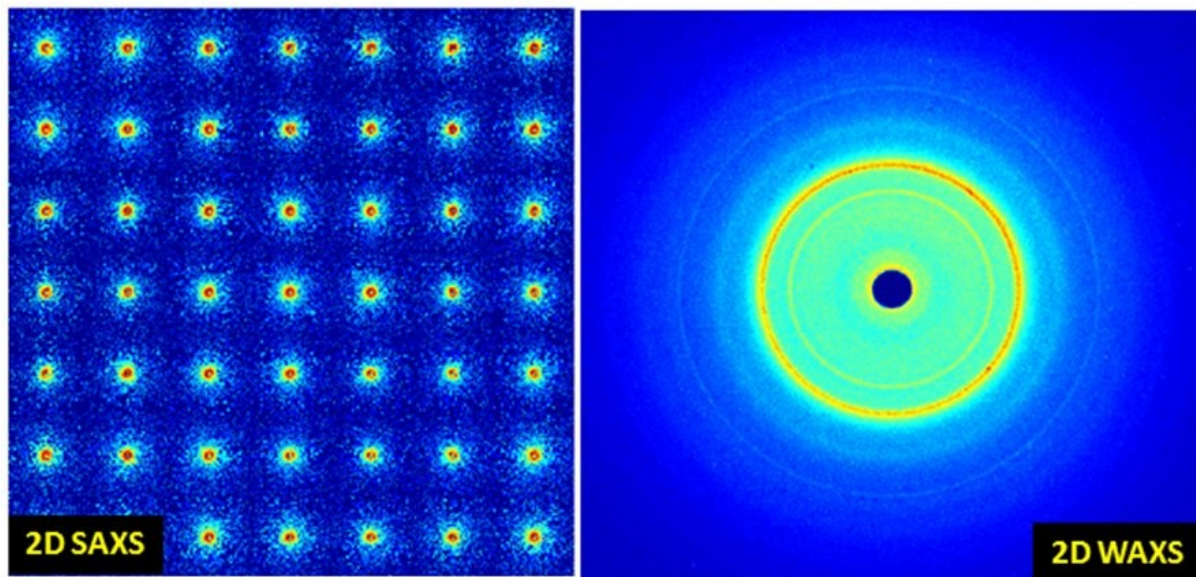
**Figure S2.** 2D x-ray fluorescence maps (pixel size 70 nm, expressed in areal mass ( $\text{g}/\text{cm}^2$ )) of non-induced bMSC. Magenta: elemental map of K. Yellow: elemental map of S. Red: elemental map of Ca. Green: elemental map of P. Blue: elemental map of Zn. Composite elemental distribution of Ca, P and Zn to better understand the correspondence of elements accumulation.



**Figure S3.** The experimental set-up at the ID16A-NI ‘Nano-Imaging’ beamline of the ESRF Synchrotron.



**Figure S4.** Panel A: Linear background (left) and double arctan subtraction (middle) showed for the bMSC at 10 days sample. The last plot on the right (“subtracted data”) is the experimental spectrum on which the Gaussians fit was performed. Panel B: Ca L edge Gaussian fits for some subtracted data reported in the main text (HA left, bMSC10 middle and Ca-P right). The experimental points (empty circles) are over imposed with the cumulative fit (black line) which is simply the sum of the used Gaussian curves also reported (coloured lines). The corresponding residual, calculated as the difference between the subtracted data and the cumulative fit is reported in orange. In the plots, both the Gaussians curves and the residual are vertically shifted for sake of clarity.



**Figure S5.** Scanning SAXS 2D data recorded across a  $0.9 \times 0.9 \text{ mm}^2$  area (left); 2D WAXS (right), averaged in the same area

**HA REFERENCE FIT (peaks numbers are not following the energy position)**

PEAK	PARAMETER	VALUE	STANDARD ERROR
Peak1(Gaussian)	xc	348.98026	0.0098
Peak1(Gaussian)	A	0.81583	0.02125
Peak1(Gaussian)	w	0.77461	0.02471
Peak2(Gaussian)	xc	351.16799	0.04205
Peak2(Gaussian)	A	0.17238	0.02689
Peak2(Gaussian)	w	0.63182	0.09627
Peak3(Gaussian)	xc	352.39493	0.01798
Peak3(Gaussian)	A	0.85465	0.02912
Peak3(Gaussian)	w	1.13889	0.0489
Peak4(Gaussian)	xc	346.67251	0.18247
Peak4(Gaussian)	A	0.02713	0.03385
Peak4(Gaussian)	w	0.39838	0.37859
Peak5(Gaussian)	xc	347.38889	0.2512
Peak5(Gaussian)	A	0.11324	0.12111
Peak5(Gaussian)	w	0.6829	0.72708
Peak6(Gaussian)	xc	347.94857	0.09769
Peak6(Gaussian)	A	0.10287	0.14565
Peak6(Gaussian)	w	0.4067	0.37902
Peak7(Gaussian)	xc	348.25275	0.07655
Peak7(Gaussian)	A	0.03621	0.06602
Peak7(Gaussian)	w	0.21488	0.19018

**PARTICLE bMSC at 10 days FIT**

Peak1(Gaussian)	xc	348.9898	0.00702
Peak1(Gaussian)	A	0.95868	0.01968
Peak1(Gaussian)	w	0.70077	0.01743
Peak2(Gaussian)	xc	351.21612	0.0261
Peak2(Gaussian)	A	0.37274	0.02898
Peak2(Gaussian)	w	0.75995	0.06302
Peak3(Gaussian)	xc	352.32152	0.00993
Peak3(Gaussian)	A	1.0346	0.02583
Peak3(Gaussian)	w	0.83484	0.02441
Peak4(Gaussian)	xc	346.75437	0.10632
Peak4(Gaussian)	A	0.03895	0.02971
Peak4(Gaussian)	w	0.38001	0.2394
Peak5(Gaussian)	xc	347.41877	0.11025
Peak5(Gaussian)	A	0.13713	0.07904
Peak5(Gaussian)	w	0.62696	0.40037
Peak6(Gaussian)	xc	347.97447	0.06242
Peak6(Gaussian)	A	0.12703	0.09315
Peak6(Gaussian)	w	0.37246	0.19573
Peak7(Gaussian)	xc	348.28872	0.05715
Peak7(Gaussian)	A	0.06396	0.05212
Peak7(Gaussian)	w	0.24608	0.09938

### PARTICLE bMSC at 4 days FIT

Peak1(Gaussian)	xc	348.9931	0.00559
Peak1(Gaussian)	A	0.27314	0.00652
Peak1(Gaussian)	w	0.40554	0.01597
Peak2(Gaussian)	xc	351.3148	0.04513
Peak2(Gaussian)	A	0.12128	0.00744
Peak2(Gaussian)	w	0.82879	0.12052
Peak3(Gaussian)	xc	352.30000	0.00761
Peak3(Gaussian)	A	0.43744	0.00631
Peak3(Gaussian)	w	0.58772	0.01786
Peak4(Gaussian)	xc	346.8634	0.27232
Peak4(Gaussian)	A	0.00632	0.00603
Peak4(Gaussian)	w	0.43784	0.73408
Peak5(Gaussian)	xc	348.5958	0.12042
Peak5(Gaussian)	A	0.23678	0.01162
Peak5(Gaussian)	w	2.29355	0.32177

### Ca-P FIT

Peak1(Gaussian)	xc	349.01023	0.00427
Peak1(Gaussian)	A	0.43131	0.0071
Peak1(Gaussian)	w	0.56216	0.01111
Peak2(Gaussian)	xc	351.35918	0.0126
Peak2(Gaussian)	A	0.23067	0.00814
Peak2(Gaussian)	w	0.74947	0.03063
Peak3(Gaussian)	xc	352.29333	0.00466
Peak3(Gaussian)	A	0.50254	0.00816
Peak3(Gaussian)	w	0.64214	0.01109
Peak4(Gaussian)	xc	346.8544	0.05433
Peak4(Gaussian)	A	0.02318	0.00914
Peak4(Gaussian)	w	0.36179	0.10812
Peak5(Gaussian)	xc	347.49449	0.12196
Peak5(Gaussian)	A	0.05086	0.03203
Peak5(Gaussian)	w	0.6	0.36372
Peak6(Gaussian)	xc	348.0256	0.03131
Peak6(Gaussian)	A	0.08818	0.03884
Peak6(Gaussian)	w	0.40528	0.12095
Peak7(Gaussian)	xc	348.41407	0.03783
Peak7(Gaussian)	A	0.05208	0.01959
Peak7(Gaussian)	w	0.31695	0.06955

**Table S1. Fitting results.** The used formula for the Gaussian function is:

$$y = \left( \frac{A}{w \cdot \sqrt{\frac{PI}{4 \cdot \ln(2)}}} \right) e^{\left( -4 \ln(2) \times \left( \frac{(x-xc)^2}{w^2} \right) \right)}$$

Fits were shifted to have the L<sub>2</sub> peak centered at 352.3eV.



sample	crystallographic unit cell parameters			cell volume	volume contraction factor
	a (Å)	b (Å)	c (Å)		
10days	9.480±0.005	9.480±0.005	6.906±0.005	538±2	0.97±0.01
	alpha (°)	beta (°)	gamma (°)		
	90	90	120		
10days_A	a (Å)	b (Å)	c (Å)		
	9.521±0.005	9.521±0.005	6.910±0.005	543±2	0.98±0.01
	alpha (°)	beta (°)	gamma (°)		
	90	90	120		
4days	a (Å)	b (Å)	c (Å)		
	9.579±0.005	9.579±0.005	6.949±0.005	552±2	1
	alpha (°)	beta (°)	gamma (°)		
	90	90	120		

**Table S2.** Crystallographic unit cell parameters (a, b, c, alpha, beta, gamma) and cell volume (V), after Rietveld analysis, and relative volume contraction factor X, given by the ratio V(10 days)/V(4 days).

**Movie S1.**

Virtual stack of x-ray phase-contrast tomography of bMSC after 4 days of the osteogenic induction (pixel size 50 nm).

**Movie S2.**

Virtual stack of x-ray phase-contrast tomography of bMSC after 10 days of the osteogenic induction (pixel size 15 nm).

**Movie S3.**

3D nano-rendering of x-ray phase-contrast tomography of bMSC after 4 days of differentiation.

**Movie S4.**

3D nano-rendering of phase-contrast tomography of bMSC after 10 days of differentiation.

**Movie S5.**

Virtual stack of X-ray fluorescence projections of Ca, P and Zn of bMSC after 10 days of the osteogenic induction (pixel size 125 nm). Red: virtual stack projections of Ca. Green: virtual stack projections of P. Blue: virtual stack projections of Zn. Composite elemental distribution of Ca, P and Zn.

**Movie S6.**

Zoomed 3D nano-rendering of Ca (red spots), P (green spots) and Zn (blue spots) of bMSC after 10 days of the osteogenic induction. In cyan was represented the nucleus.