Supplementary Information for

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

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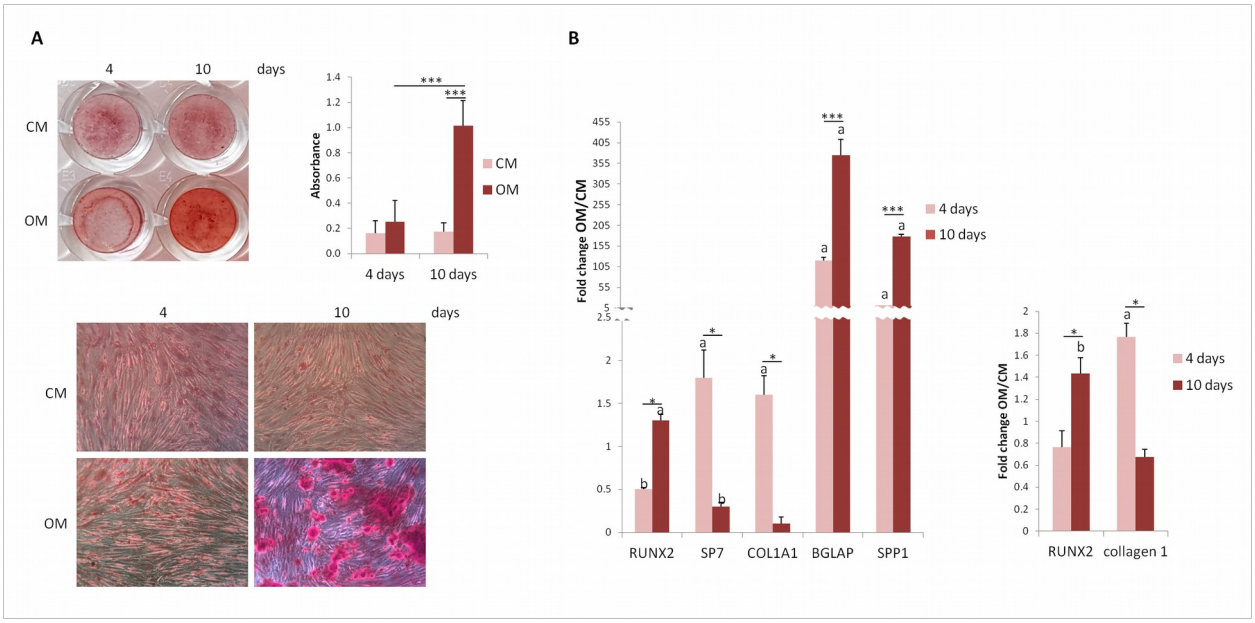

Figure S1. (A) Alizarin Red staining was performed after exposure to culture medium (CM) or osteogenic medium (OM) for 4 and 10 days. Photographs of a representative plate (upper left panel) and photographs taken at 10X magnification (lower panel) are shown. After acid extraction the absorbance was measured at 562 nm (upper right panel). The results are shown as the mean ± 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).

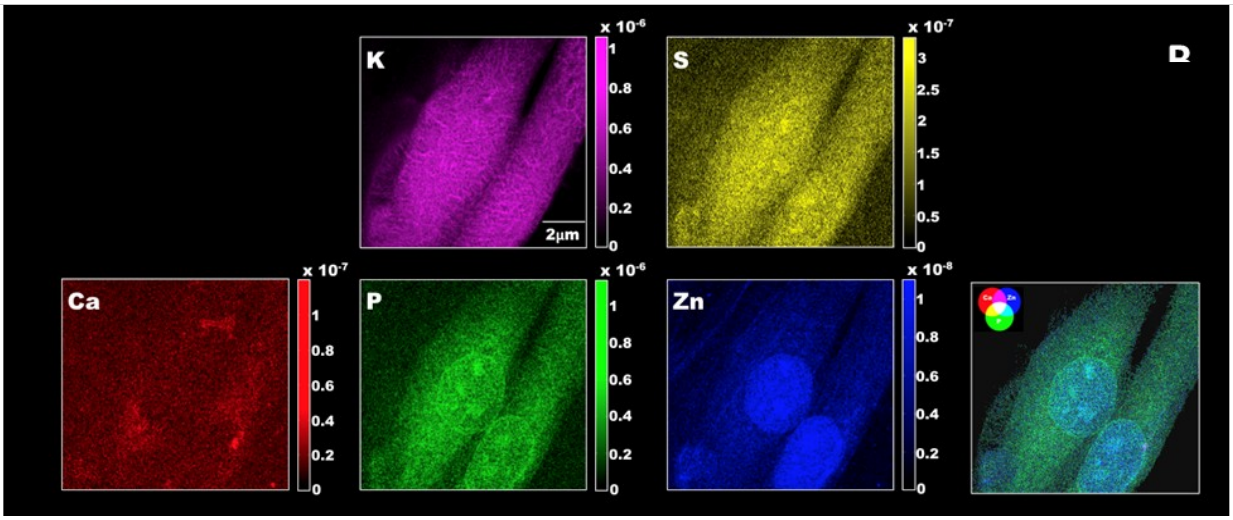

Figure S2. 2D x-ray fluorescence maps (pixel size 70 nm, expressed in areal mass (g/cm3)) of non-induced bMSC. Magenta: elemental map of K. Yellow: elemental map of S. Red: elemental map of Ca. Green: elemental map of P. Blue: elemental map of Zn. Composite elemental distribution of Ca, P and Zn to better understand the correspondence of elements accumulation.

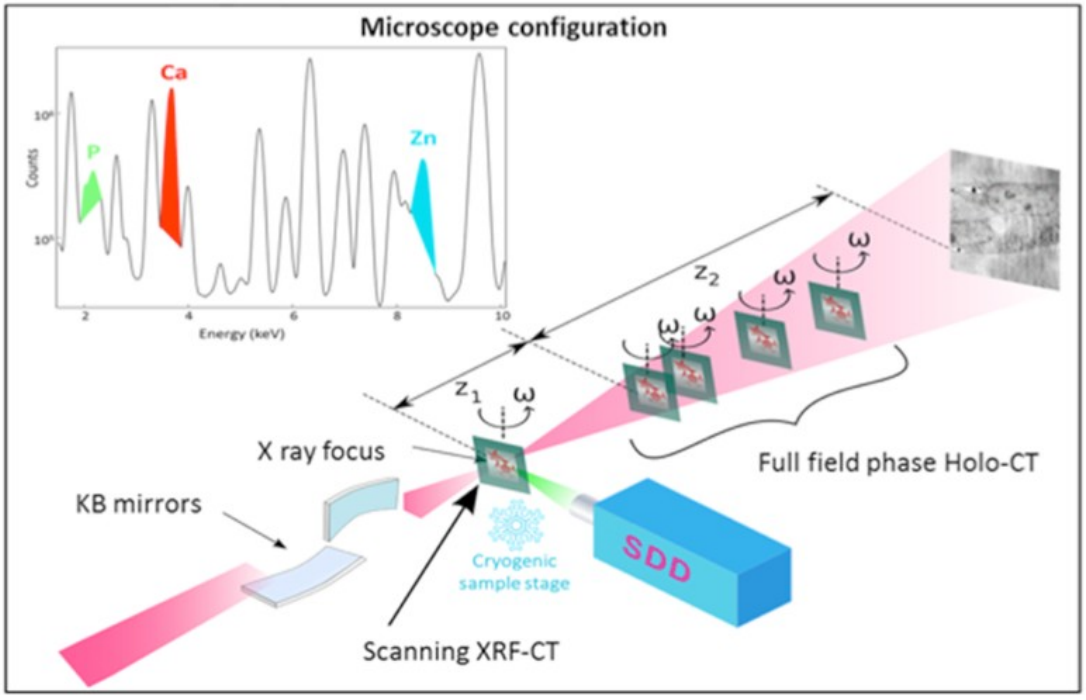

Figure S3. The experimental set-up at the ID16A-NI 'Nano-Imaging' beamline of the ESRF Synchrotron.

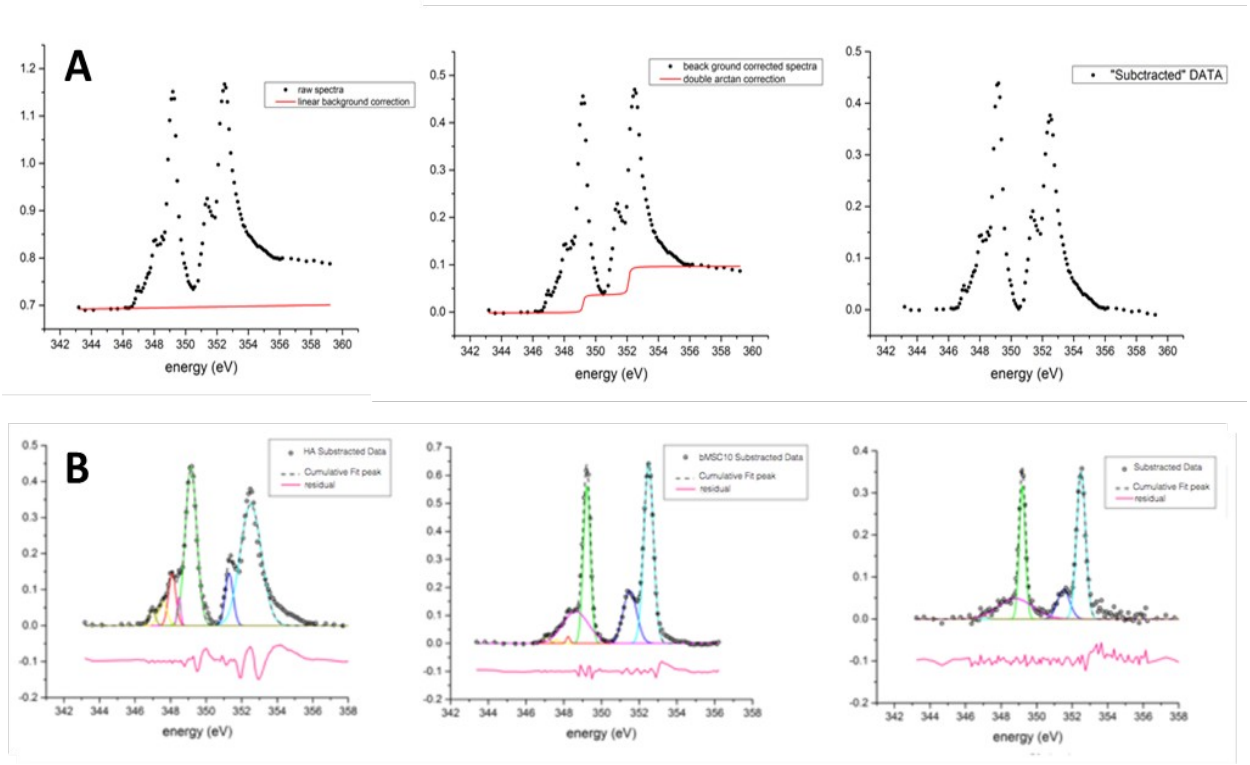

Figure S4. Panel A: Linear background and double arctan subtraction showed for the HA reference sample. The "subtracted DATA" are the experimental points on which the Gaussians fit was performed. Panel B: Ca L edge Gaussian fits for the subtracted data reported in the main text (HA, bMSC10 and BMSC4). The subtracted data (empty circles) are over imposed with the Gaussians used for the fits and the cumulative fit which is simply the sum of the Gaussian curves (dashed lines). The corresponding residual, calculated as the difference between the subtracted data and the cumulative fit is also reported, vertically shifted for sake of clarity.

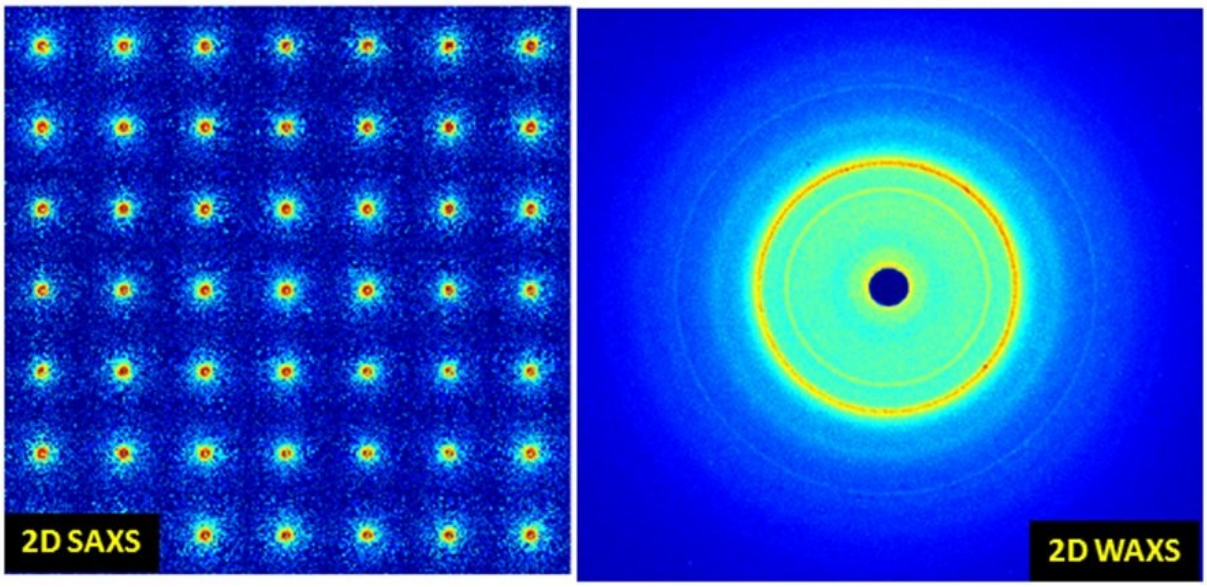

Figure S5. Scanning SAXS 2D data recorded across a 0.9x0.9 mm^2 area (left); 2D WAXS (right), averaged in the same area

BMSC4		Value	Standard Error
Peak1(Gaussian)	xc	347.0634	0.27232
Peak1(Gaussian)	A	0.00316	0.00603
Peak1(Gaussian)	w	0.43784	0.73408
Peak2(Gaussian)	xc	348.7958	0.12042
Peak2(Gaussian)	A	0.11839	0.01162
Peak2(Gaussian)	w	2.29355	0.32177
Peak3(Gaussian)	xc	349.1931	0.00559
Peak3(Gaussian)	A	0.13657	0.00652
Peak3(Gaussian)	w	0.40554	0.01597
Peak4(Gaussian)	xc	351.5148	0.04513
Peak4(Gaussian)	A	0.06064	0.00744
Peak4(Gaussian)	w	0.82879	0.12052
Peak5(Gaussian)	xc	352.50000	0.00761
Peak5(Gaussian)	A	0.21872	0.00631
Peak5(Gaussian)	w	0.58772	0.01786
BMSC10		**Value**	**Standard Error**
Peak1(Gaussian)	xc	348.98978	0.00775
Peak1(Gaussian)	A	0.51081	0.01331
Peak1(Gaussian)	w	0.60646	0.01854
Peak2(Gaussian)	xc	351.20877	0.03643
Peak2(Gaussian)	A	0.16659	0.01822
Peak2(Gaussian)	w	0.72996	0.0912
Peak3(Gaussian)	xc	352.30259	0.00907
Peak3(Gaussian)	A	0.56612	0.01528
Peak3(Gaussian)	w	0.69512	0.02175
Peak4(Gaussian)	xc	346.72671	0.22735
Peak4(Gaussian)	A	0.01104	0.01863
Peak4(Gaussian)	w	0.35674	0.53551
Peak5(Gaussian)	xc	347.40298	0.22689
Peak5(Gaussian)	A	0.05346	0.05586
Peak5(Gaussian)	w	0.62507	0.70939
Peak6(Gaussian)	xc	347.97583	0.08754
Peak6(Gaussian)	A	0.0629	0.06329
Peak6(Gaussian)	w	0.40201	0.30606
Peak7(Gaussian)	xc	348.30887	0.06793
Peak7(Gaussian)	A	0.02674	0.02976
Peak1(Gaussian)	xc	348.98978	0.00775
HA		**Value**	**Standard Error**
Peak1(Gaussian)	xc	346.96418	0.23915
Peak1(Gaussian)	A	0.01871	0.02901
Peak1(Gaussian)	w	0.41351	0.37816
Peak2(Gaussian)	xc	347.55357	0.47415
Peak2(Gaussian)	A	0.03911	0.11437
Peak2(Gaussian)	w	0.56412	1.28968
Peak3(Gaussian)	xc	348.08607	0.17356
Peak3(Gaussian)	A	0.07534	0.1132
Peak3(Gaussian)	w	0.48911	0.48683
Peak4(Gaussian)	xc	348.47364	0.05218
Peak4(Gaussian)	A	0.01881	0.02459
Peak4(Gaussian)	w	0.22477	0.13979
Peak5(Gaussian)	xc	349.15099	0.01168
Peak5(Gaussian)	A	0.35646	0.01179
Peak5(Gaussian)	w	0.76594	0.03117
Peak6(Gaussian)	xc	351.29468	0.02867

Peak6(Gaussian)	A	0.0826	0.01256
Peak6(Gaussian)	w	0.533	0.07166
Peak7(Gaussian)	xc	352.50000	0.02346
Peak7(Gaussian)	A	0.49785	0.01759
Peak7(Gaussian)	w	1.38511	0.059
$Ca(H_2PO_4)_2$		**Value**	**Standard Error**
Peak1(Gaussian)	xc	348.0256	0.03131
Peak1(Gaussian)	A	0.08818	0.0384
Peak1(Gaussian)	w	0.40528	0.12095
Peak2(Gaussian)	xc	349.01023	0.00427
Peak2(Gaussian)	A	0.43131	0.0071
Peak2(Gaussian)	w	0.56216	0.01111
Peak3(Gaussian)	xc	351.35918	0.0126
Peak3(Gaussian)	A	0.23067	0.00814
Peak3(Gaussian)	w	0.74947	0.03063
Peak4(Gaussian)	xc	352.29333	0.00466
Peak4(Gaussian)	A	0.50254	0.00816
Peak4(Gaussian)	w	0.64214	0.01109
Peak5(Gaussian)	xc	346.8544	0.05433
Peak5(Gaussian)	A	0.02318	0.00914
Peak5(Gaussian)	w	0.36179	0.10812
Peak6(Gaussian)	xc	347.49449	0.12196
Peak6(Gaussian)	A	0.05086	0.03203
Peak6(Gaussian)	w	0.6	0.36372
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_2 peak centered at 352.5 eV.

sample	crystallographic unit cell parameters			cell volume	volume contraction factor
10days	**a (*Å*)**	**b (*Å*)**	**c (*Å*)**	**V($Å^3$)**	**X**
	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 (blu spots) of bMSC after 10 days of the osteogenic induction. In cyan was represented the nucleus.