Low doses of uranium and osteoclastic bone resorption: key reciprocal effects evidenced using new *in vitro* biomimetic models of bone matrix.

Tatiana Gritsaenko¹, Valérie Pierrefite-Carle¹, Gaëlle Creff², Bastien Simoneau¹, Agnès Hagège³, Delphine Farlay⁴, Sophie Pagnotta⁵, François Orange⁵, Xavier Jaurand⁶, Christophe Den Auwer², Georges F. Carle¹, Sabine Santucci-Darmanin^{1*}

Electronic Supplementary Material

Supplementary Methods

Mineralization and collagen deposition assays

The Saos-2s were seeded in 24 well plates at a density of 20.000 cells/well. When subconfluence was reached, the growth medium was replaced with differentiation medium containing either 0. 0.5. 1 or 2 μ M U(VI) and the culture was maintained for 10 days with a change of medium every 2-3 days.

For mineralization evaluation. cultures were fixed with 4% formaldehyde solution (Sigma-Aldrich). rinsed with deionized water and stained with 1% Alizarin Red S solution (Alfa Aesar. ThermoFisher Scientific) for 5 min. Intensive washes with deionised water were then carried out before the plates were left to dry and photographed. Images of each well were analyzed with ImageJ software to evaluate the percentage of mineralized area.

Supplementary Data

samples	path and distance (Å)	σ² (Ų)	$\Delta e_0 (eV); SO_2^2$
U(VI)-COAS matrix	2 O _{ax} at 1.83(1)	0.0015	3.36; 1.1
	5.5(6) Oeq at 2.40(3)	0.0099	
(1.8%. 2) ^a	4.2(10) P at 3.65(4)	0.0021	
U(VI)- Saos-2 matrix	2 O _{ax} at 1.81(2)	0.0036	-0.75; 1.1
	5.5(13) Oeq at 2.34(1)	0.0135	
(3.3%; 40) ^a	6.2(34) P at 3.59(2)	0.0149	
Autunite	2 O _{ax} at 1.81(2)	0.0042	0.35; 1.1
	4.1(10) Oeq at 2.33(5)	0.0164	
(2.3%; 30) ^a	5.7(12) P at 3.59(5)	0.0039	

Supplementary Table 1 Best fit metrical parameters for the EXAFS spectra. σ^2 is the Debye Waller factor. Δe_0 and $S0_2^2$ are the energy threshold and global amplitude factor of the fit. Numbers in italics have been fixed. ^aRefers to the *agreement factor* (r %) and *reduced quality factor* ($\chi^2_{/n}$) of the fit. Uncertainties on the last quoted digit are given in bracket.

		Day 3		Day 4	
[U(VI)] in exposure medium (µM)	Average amount of U(VI) in initial matrix (µg)	without RANKL	with RANKL	without RANKL	with RANKL
5	0.16	0.32 ± 0.07	0.34 ± 0.09	0.04 ± 0.01	0.05
10	0.24	0.59 ± 0.13	0.57 ± 0.14	0.07 ± 0.01	0.09
25	0.45	1.13 ± 0.19	1.13 ± 0.22	0.12 ± 0.04	0.23 ± 0.05
50	0.75	1.80 ± 0.26	1.92 ± 0.34	0.20 ± 0.01	0.33 ± 0.05
100	1	2.95 ± 0.28	2.97 ± 0.36	0.27 ± 0.03	0.41 ± 0.1

[U(VI)] in culture supernatant (µM)

Supplementary Table 2 Uranium concentrations in culture supernatants of synthetic matrices at days 3 and 4 were calculated based on ICP-MS quantification. Data are given as mean \pm SD when n=3 or as mean when n=2.

 $[U(VI)] = 0 \ \mu M \ (0 \ \mu g/well)$





200 µm



200 µm

Osteoclast on synthetic matrices

Supplementary Fig.1 Osteoclasts behavior on synthetic matrices containing U(VI). RAW 264.7 cells were cultured with RANKL onto synthetic apatite matrix. prepared in the presence of the indicated concentration (μ M) of U(VI) and containing. on average. the indicated amount (μ g / well) of U(VI). Representative micrographs of TRAP-positive osteoclasts (> 3 nuclei) obtained after 4 days of culture on synthetic matrix in the absence or presence of U(VI)

Alzarin Red staining of Saos-2 matrices



	0	0.5	I	2
[U(VI)] µg/well	0	≈0.28	≈0.54	≈1

b



Supplementary Fig. 2 Calcium deposition by Saos-2 cells cultivated in the presence of U(VI). Saos-2 cells were cultured in the presence of the indicated concentrations of U(VI) and under mineralization conditions for 10 days. **a** A representative photograph of calcium deposition as assessed by Alizarin red S staining. **B** Box plot representations showing minima. first quartile. median. third quartile and maxima of the quantification of calcium deposition. Data are from 2 independent experiments with 4 matrices analyzed per experiment and per condition



Supplementary Fig. 3 U(VI) release from SAOS-2 extracellular matrices. De-cellularized Saos-2 matrices containing the indicated amounts of U(VI) were seeded with RAW 264.7 cells in the absence or presence of RANKL. On day 3 (D3). culture supernatants (supernatant D3) were harvested. The box plot shows U(VI) quantification in D5 supernatants of SAOS-2 matrix cultures. according to the presence of RANKL. n=3 independent experiments, each done in 2-4 replicates

 $[U(VI)] = 0 \ \mu M \ (0 \ \mu g/well)$



200 µm

 $[U(VI)] = 2 \mu M (\approx 1 \mu g/well)$



200 µm

Osteoclast on biological matrices

Supplementary Fig. 4 Osteoclasts behavior on Saos-2 matrices containing U(VI). RAW 264.7 cells were cultured with RANKL onto de-cellularized Saos-2 extracellular matrix. prepared in the presence of the indicated concentration (μ M) of U(VI) and containing. on average. the indicated amount (μ g/well) of U(VI). Representative micrographs of TRAPpositive osteoclasts (> 3 nuclei) obtained after 5 days of culture in the absence or presence of U(VI)





b



Supplementary Fig. 5 TEM and HRTEM analyses of osteoclasts resorbing Saos-2 matrix containing U(VI). **a** A representative TEM image of an osteoclast with several vesicles (white arrows) containing needle (black arrowheads) and platelet-like structures (blue arrowheads). **b** HRTEM-EDX analysis of needles and platelets seen in vesicles was performed. Representative micrographs are shown with X-ray microanalyses results. including weight and atomic percentage of O. P. Ca and U elements