Supporting Information

Study of Osteoclast Adhesion to Cortical Bone Surfaces: A Correlative Microscopy Approach for Concomitant Imaging of Cellular Dynamics and Surface Modifications

*Michal Shemesh[a], Sefi Addadi[b], Yonat Milstein[b], Benjamin Geiger[c] and Lia Addadi[a]**

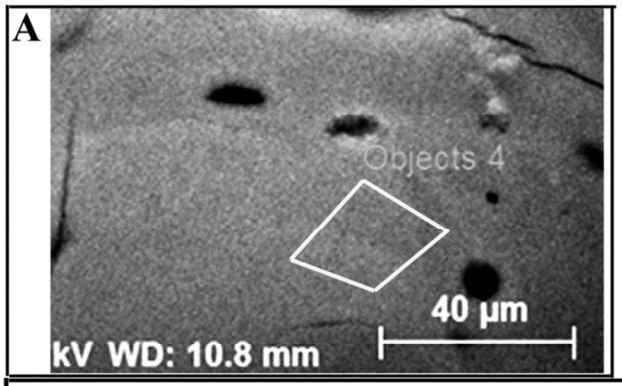
[a]Department of Structural Biology and [c]Department of Molecular Cell Biology, Weizmann Institute of Science, 76100 Rehovot, Israel

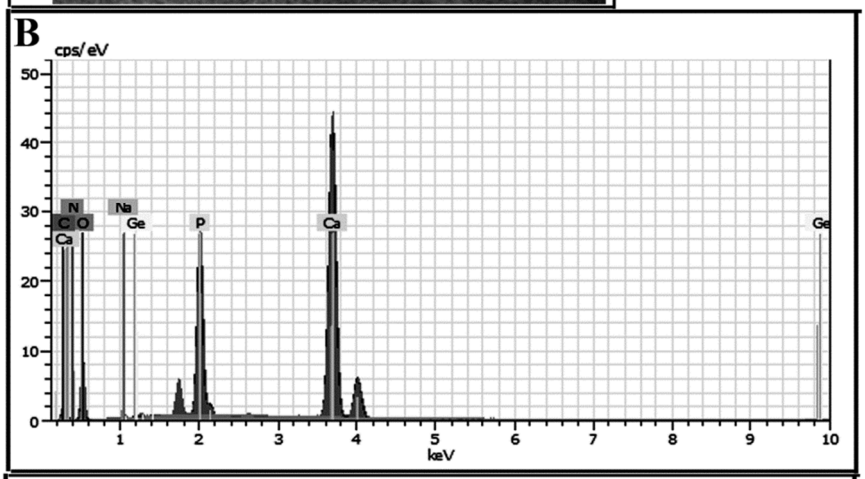
[b]B-Nano Ltd., 76326, Rehovot, Israel

**To whom correspondence should be addressed: Lia Addadi*

E-mail: Lia Addadi@weizmann.ac.il;

Phone: +972-8-934 2228.





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Element	Series	Unn.C (wt. %)	Norm.C (wt. %)	Atom.C (wt. %)	Error (wt. %)
Oxygen	K-series	52.67	46.13	54.92	19.74
Calcium	K-series	28.62	25.06	11.91	2.60
Carbon	K-series	12.41	10.87	17.24	5.77
Phosphorus	K-series	11.50	10.07	6.20	1.47
Nitrogen	K-series	7.48	6.55	8.91	4.48
Germanium	L-series	0.56	0.49	0.13	0.20
Sodium	K-series	0.94	0.83	0.69	0.29
	Total:	114.18	100.00	100.00	

Figure S1. EDX data obtained from a polished bone surface.

(A) airSEM image of a polished, cortical bone surface. (B) EDX spectra taken from the marked area in (A). (C) Corresponding EDX elemental analysis of the bone surface in the marked region.

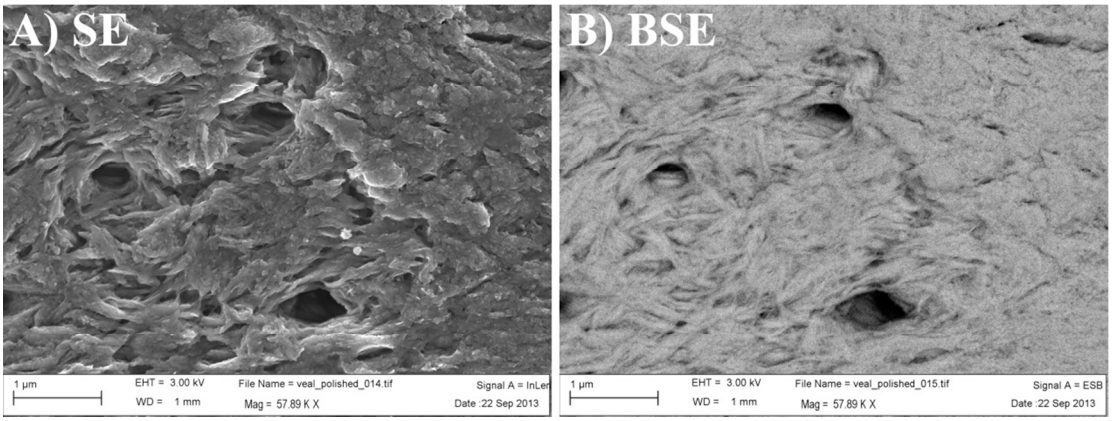

Figure S2. High resolution SEM images of bone resorption

(A) Secondary electrons image of bone area that was resorbed, exposing an osteocyte lacuna. The arrangement of mineralized collagen bundles is evident. (B) Back scattered image (resembles more the air-SEM signal) of the same area as in (A) showing the homogenous composition of bone surface.

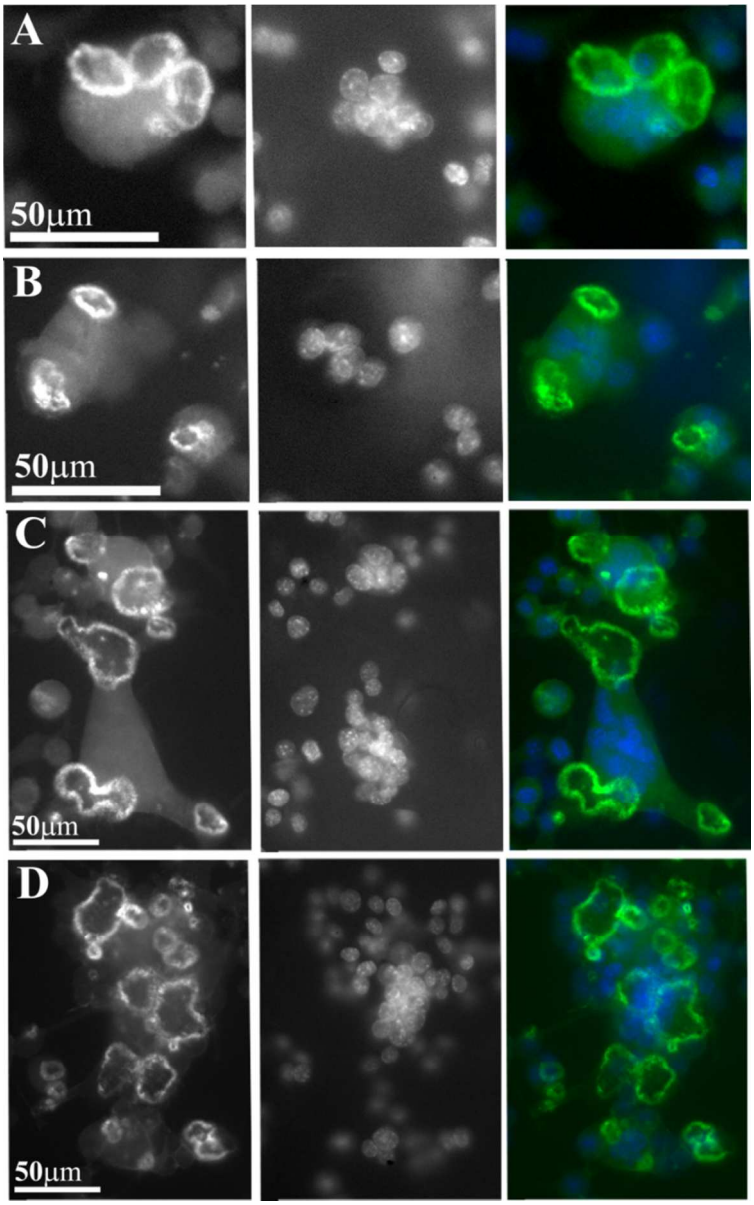

Figure S3. Fluorescence images of osteoclasts fixed at different times after transfer to bone surface.

Osteoclasts stained for actin (left panels) and nuclei (middle panels). Right panels show an overlay of SZ rings (green) and nuclei (blue). (A) One hour post-transfer, multinuclear cells with developed SZ rings are seen. (B) Three hours post-transfer, the cell size and SZ rings are similar to those seen one hour post-transfer. (C) Six hours post-transfer, some of the osteoclasts have grown in size. The SZ rings do not differ in size or shape, compared to images of rings fixed for shorter periods of time. (D) Twenty-four hours post-transfer, large multinuclear cells are seen, each containing several SZ rings. Notice that small and large rings are present in close proximity, underneath the same cell body. A comparison of images taken at the different time points shows that the adhesive apparatus of osteoclast cells (SZ rings) is formed within the first hour of cell plating on bone.

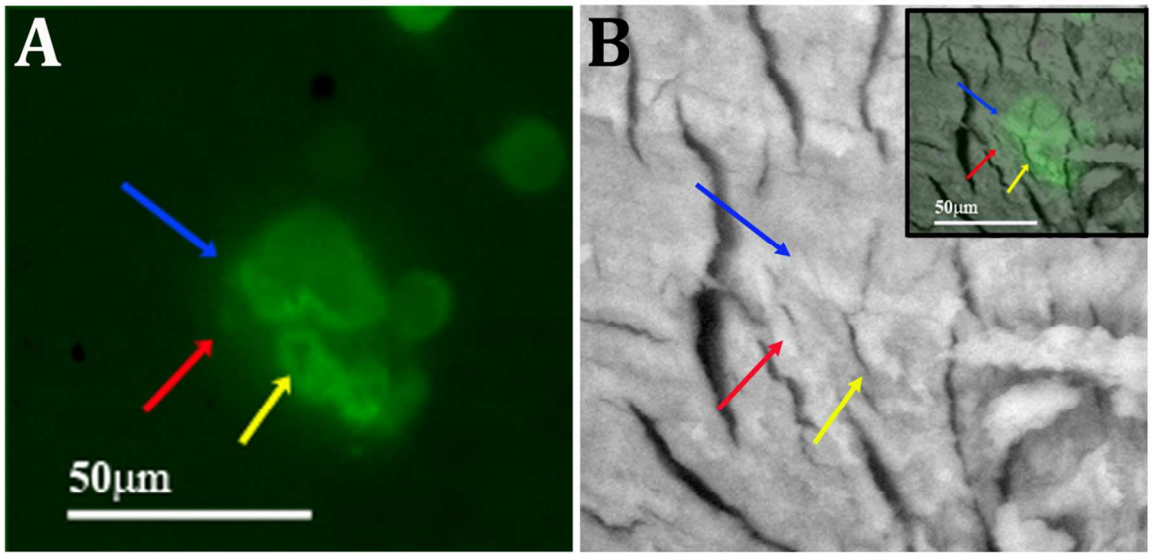

Figure S4 Correlation between SZ rings at the end of the time-lapse movie shown in Figure 5, and the bone surface after cell removal.

(A) Fluorescence image of SZ rings (green) at the end of the movie. Rings showing varying dynamics are denoted by different-colored arrows (see **Fig.** 5, and text for more details). (B) Bone surface following cell removal with NaOCl. Insert shows rings overlaid on the bone surface. The effect of NaOCl on bone surface results in widening of cracks that were previously present, possibly due to bone dehydration. No additional alterations in bone surface were noted.

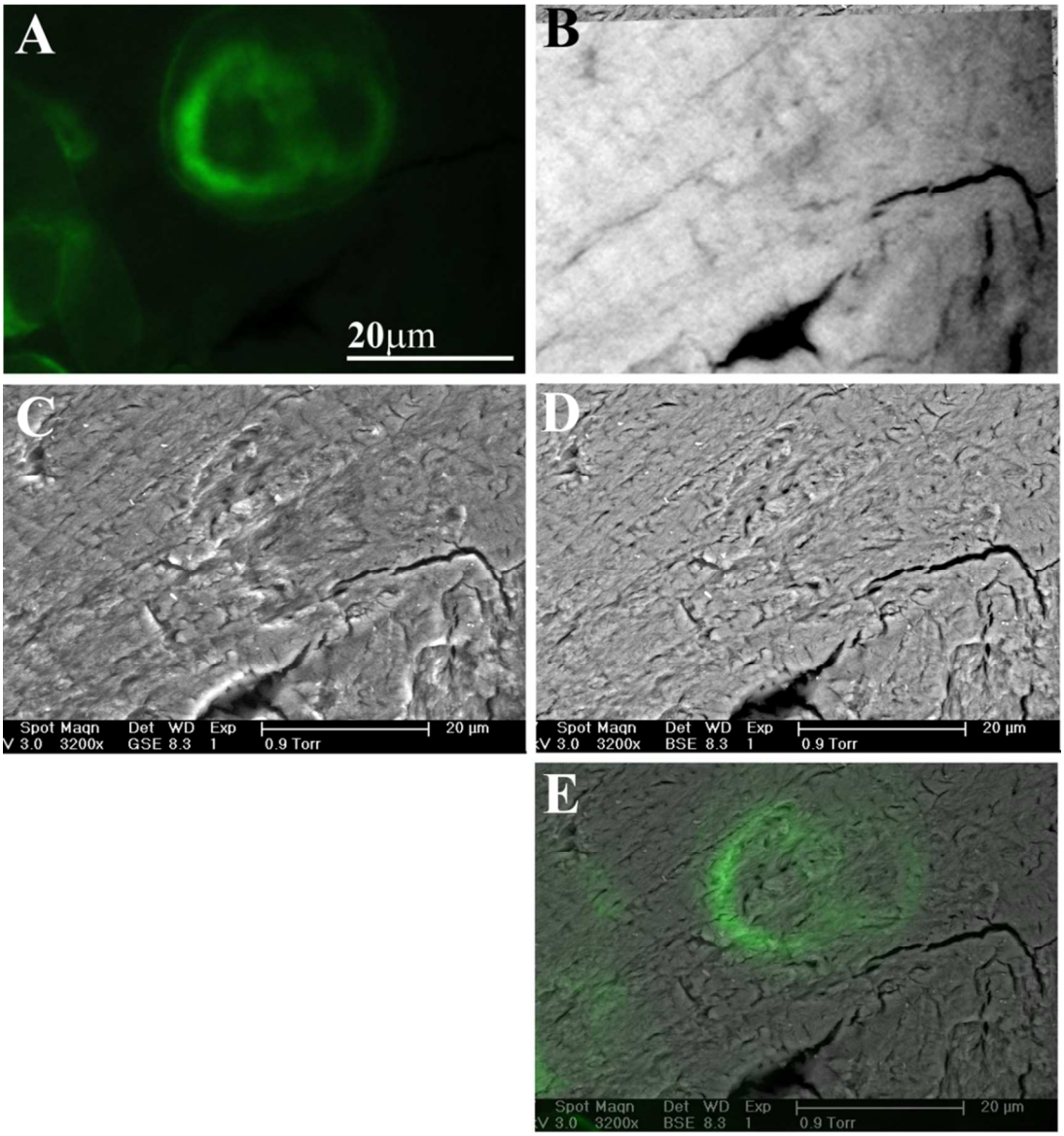

Figure S5. Bone surface area of interest, imaged by the *airSEM* and ESEM, following osteoclast removal.

(A) Fluorescence image of SZ rings in the cell. (B) The corresponding bone area imaged with the *airSEM*. The area related to SZ rings seems darker than the surrounding surface, but its morphological details are not clear. (C) ESEM SE image, giving rise to signals from surface topography, showing the morphology of the area juxtaposed onto SZ rings, in greater detail. (D) ESEM BSE image, comparable to the *airSEM* image (also based on BSE detection), but at higher resolution. (E) Overlay of an image of SZ rings, with that of the bone surface. The rings are placed above the lower area, suggesting cellular functionality in the area enclosed by them.