Bone Cell Proliferation on Carbon Nanotubes

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SUPPORTING INFORMATION PARAGRAPH

Light microscopy: For cell counts, we used a phase contrast inverted Olympus XI50 microscope. Cell numbers were counted manually per field of observation at a magnification of x200.

Fluorescence microscopy: Cell counts were also performed with fluorescence microscopy on a Leica SM confocal microscope. ROS 17/2.8 cells were stained with 3 µM octadecyl-rhodamine for 5 min. The lipophylic dye staines cell membranes, thus facilitating observation and counting.

Alkaline Phosphatase (ALP) staining: For this qualitative assay, ROS 17/2.8 cells were stained with 10 µM naphtol-AS-BI-phosphate (Sigma) in 100 mM Tris-base buffer, pH 8.5, with the addition of 0.06 % fast blue RR (Sigma) for 20 min at 37º C. The formation of a blue precipitate indicates ALP activity.

Von Kossa staining: ROS 17/2.8 cells were rinsed with PBS, and fixed with 10 % neutral-buffered formalin (Sigma) for 10 min. After rinsing 3 times with distilled water,

cells were incubated with 5 % silver nitrate in the dark for 30 min. Culture plates were then exposed to ambient light for color development. A dark/brown precipitate is indicative of positive staining for deposited mineral.