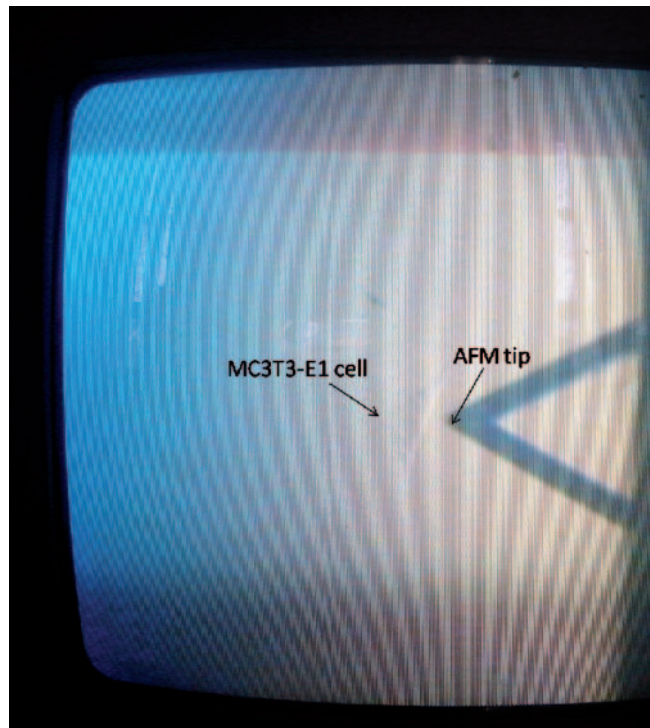
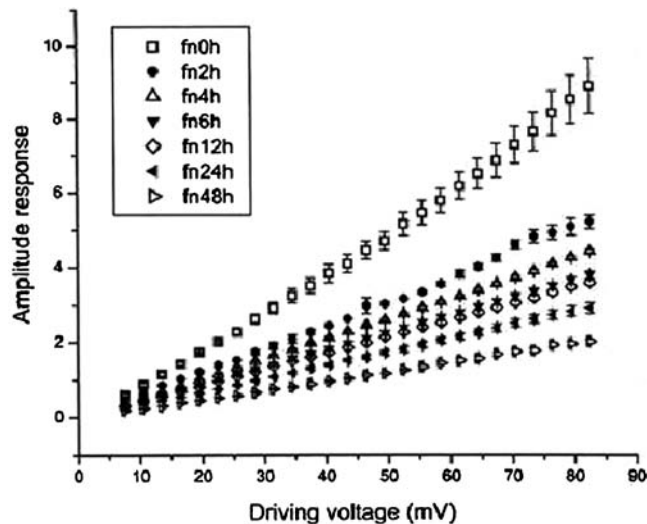


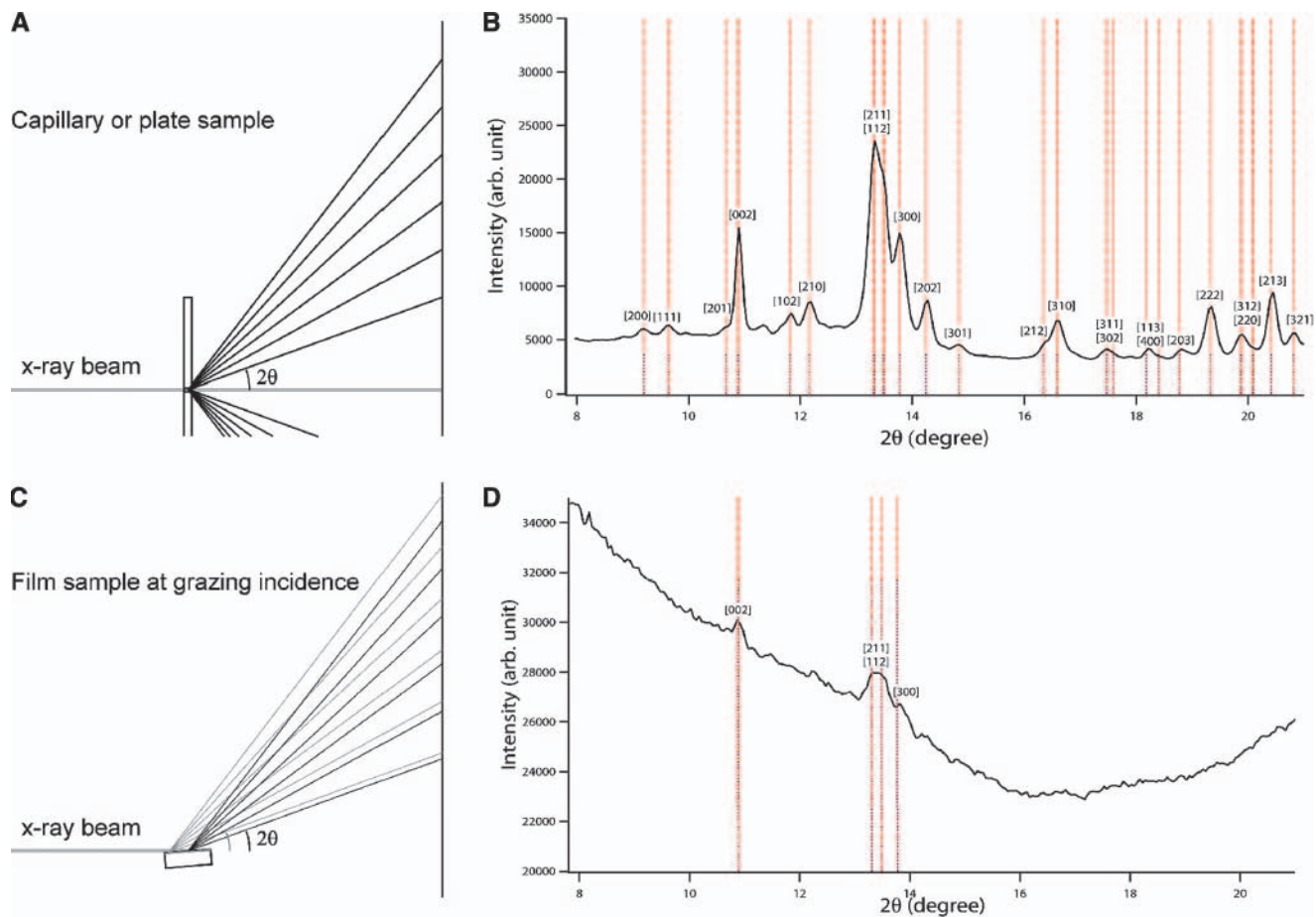
SUPPLEMENTAL FIG. 1. Figure 2 of Pernodet *et al.*³³ Adsorbed fibronectin (Fn) morphology as a function of surface charge density. After 70h of incubation with a 100 $\mu\text{g}/\text{mL}$ Fn/PBS solution at 37°C, 100% humidity: (a) $-0.08 \text{ C}/\text{m}^2$ surfaces demonstrate a thin globular layer of proteins with peak heights of 80 nm; (b) $-0.08 \text{ C}/\text{m}^2$ surfaces demonstrate a fusion of adsorbed protein globules achieving peak heights of 150 nm; (c) $-0.12 \text{ C}/\text{m}^2$ surfaces demonstrate the initiation of fibril formation with peak heights of 280 nm; and (d) $-0.15 \text{ C}/\text{m}^2$ surfaces demonstrate an extensive Fn network with peak heights reaching 1400 nm. Surfaces were imaged by contact mode AFM in PBS solution; each image is $20 \times 20 \mu\text{m}$. Reprinted with permission of John Wiley & Sons, Inc.



SUPPLEMENTAL FIG. 2. This is a view of the optical microscope that we used in our SMFM setup to select which cell and region to scan using the atomic force microscope. When an MC3T3-E1 cell is identified on the monitor, we slowly bring the atomic force microscope tip to its vicinity. For an ECM scan, we collect the AFM image in the region surrounding that particular cell. To measure the mechanical properties of the cell, we position the tip in the middle of the cell and then lower it just enough to maintain contact with the cell membrane.



SUPPLEMENTAL FIG. 3. Figure 7.5 (c) of DiMasi *et al.*⁴⁹ Series of raw amplitude response data obtained in one SMFM relative modulus measurement (fibronectin fibers mineralized in a flow cell, described in Hollinger *et al.*²⁰ and Liu and Ma²³). Each curve represents a series of measurements of the amplitude response as a function of driving voltage applied to oscillate the tip laterally. The slope of these curves drops after each time step, indicating that the material is stiffer after being exposed to CaCO_3 mineralizing conditions. Reproduced with permission from © Wiley-VCH Verlag GmbH & Co.



SUPPLEMENTAL FIG. 4. To demonstrate the difference in X-ray scattering between a powder sample in a glass capillary versus a thin film sample (as in the case of our cultured cells that were dried on SPS-coated Si), we here show schemes depicting the scattering and detector geometry along with experimental data from these two systems. The X-ray wavelength is 0.65255 Å.

In a transmission X-ray diffraction measurement from a capillary containing a powder sample of synthetic HAP (A), scattering from a very small volume is collected on an area detector screen, depicted by the plane at the right. After calibration with an alumina powder standard, the relationship between position of detected intensity on the detector and scattering angle 2θ is known. The HAP powder peaks are broader than those of the standard, and allow us to estimate a particle size of 25 nm, as opposed to 33 nm or greater for the resolution limited standard. The indexed experimental HAP powder data from the capillary are azimuthally averaged over the area detector and shown with Bragg peak indices in (B).

Our synchrotron GIXD setup, appropriate for thin film samples, is illustrated in (C). The grazing angle prevents excessive background scattering from the substrate by minimizing bulk X-ray penetration. The illuminated spot extends to nearly the full length of the sample due to the finite X-ray beam height: in this case, the 0.25-mm-high beam incident at 1° creates an illuminated stripe 14-mm long, which illuminates as much of our very small sample volume as possible. The offset of the origin of the scattering causes the diffracted beams to hit the detector in a position displaced from the nominal, which appears as a broadening of the peaks when analyzed [see light and heavy rays in (C)]. The experimental diffraction pattern in (D) is of the same commercial HAP powder as (B), but dusted onto a blank SPS-Si wafer. There is an enormous scattering background from the polymer material, and the very thin powder coating shows only the strongest Bragg peaks, the [002] and the [211]/[112] that slightly overlap each other and the [300]. The geometry-induced broadening suggests a particle size of 12 nm, but this is a lower bound since the higher-resolution capillary measurement tells us that the particles are of order 25 nm. This type of thin-film diffraction pattern is what we have access to, for the tiny particles deposited on the surfaces by our mineralizing osteoblasts after at least 14 days of incubation in osteogenic medium. Hence our reported particle sizes in the manuscript represent a lower bound, and can only be analyzed at the well-separated [002] peak.