Supporting Information

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SI Text

Image Reconstruction. Reconstructed high-resolution microcomputed tomography (μCT) images from the specimens were analyzed with CTAn. A region of interest (ROI) was hand drawn, selecting the fibrous cap in each atheroma as the visible layer of soft tissue separating the lumen from the necrotic core. These ROIs were then binarized, segmenting the calcified particles from the soft tissues in the atheroma. The size, volume, surface, and centroid of each individual 3D object was calculated automatically using CTAn analysis software.

Microcalcification Aspect Ratio. An equivalent spherical diameter, $D = (6V/\pi)^{1/3}$, was calculated for each particle along with its sphericity S_{ph}, which is defined by $S_{ph} = S_{eq}/S$, where S is the measured surface area and $S_{eq} = \pi D^2$. If the particle is assumed to be an ellipsoid of revolution, its sphericity can be approximated by $S_{ph} = (r/l)^{1/3}$, provided that $S_{ph} > 0.5$ or $l < 8r$. Nearly all microcalcifications (μCalcs) that we have observed satisfy this approximation. Using the volume of an ellipsoid, $V_{ell} = 4\pi r^2 l/3$, one can now solve for r and l.

Histology and Transmission EM. A tissue processing protocol was developed for high-fidelity histological analysis of nondecalcified coronary arteries (1) in Fig. S3. Histology and transmission electron microscopy images were manually segmented, and the 2D contours of the μCalc were approximated with overlapping ellipses of different size and aspect ratio. The ellipses were then revolved along their major axis to construct a 3D shape. This realistic complex 3D μCalc geometry was then placed virtually in the center of an idealized fibrous cap used in previous finite element analysis models and a stress concentration factor calculated when the particle was oriented along the tensile axis, the worst case scenario.

Stenosis. Minimum luminal diameter at the target lesion site is compared with the lumen at a healthy reference site that is either proximal or distal to the lesion. Diametric values of lumen are calculated from measurements of the perimeter of the lesion and the reference site. The percentage of stenosis is calculated as $(1 - D_{stenosis}/D_{healthy}) \times 100$, where $D_{stenosis}$ and $D_{healthy}$ are the diameter of the lesion and reference site, respectively.

1. Maldonado N, et al. (2012) A mechanistic analysis of the role of microcalcifications in atherosclerotic plaque stability: Potential implications for plaque rupture. *Am J Physiol Heart Circ Physiol* 303(5):H619–H628.

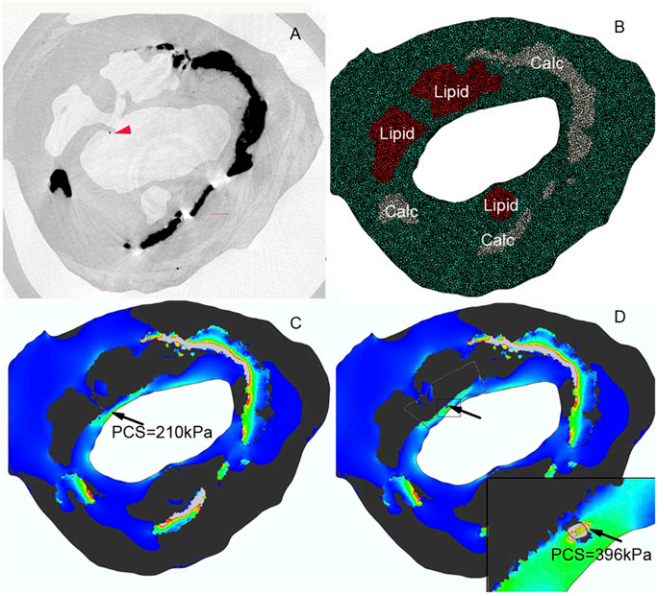

Fig. S1. Finite element model of a ruptured plaque. (*A*) High-resolution μCT image of ruptured plaque with a μCalc present at the rupture site. (*B*) Material assignment in reconstructed nonruptured state. (*C*) Circumferential stress distribution without μCalcs and location of peak circumferential stress (PCS) in the cap. (*D*) Magnified view of the ROI indicating PCS in the vicinity of the μCalc.

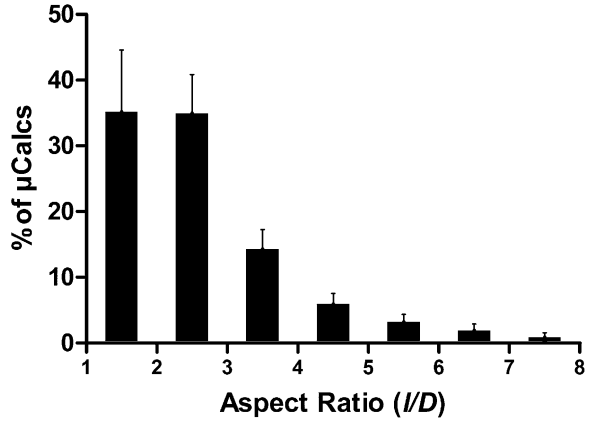

Fig. S2. Relative distribution (in percentage) of μCalcs in the fibrous cap by aspect ratio.

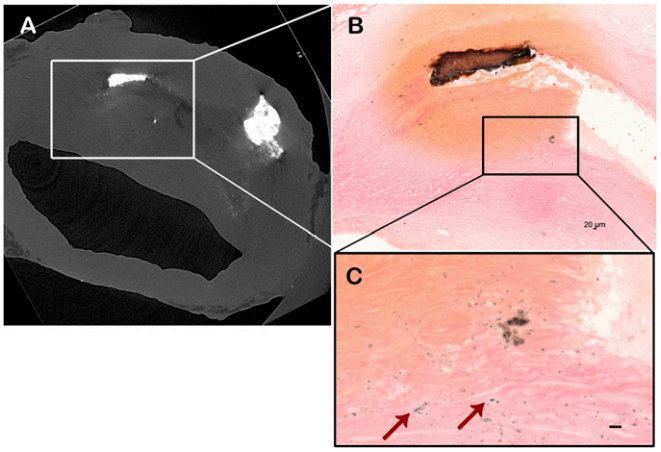

Fig. S3. μCT vs. histological image of an atheroma. (*A*) A μCT image of an atheroma at 2.1-μm resolution containing a ~20-μm μCalc in the fibrous cap region. (*B*) A histological image of the same slice shows that this μCalc is actually a conglomerate of many even smaller μCalcs that are <5 μm in size. (*C*) A magnification of the cap area in *B* shows μCalcs as small as 0.5 μm that are indicated with red arrows that cannot be seen in HR-μCT. (Scale bar: 20 μm.)