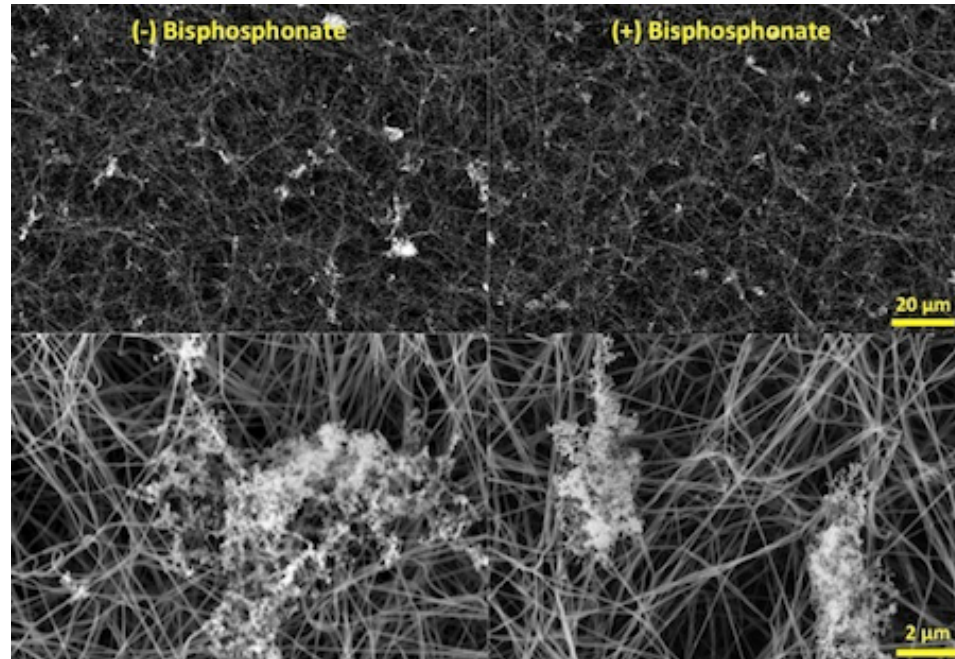


Presentation: 658 - Unraveling the Controversy of Bisphosphonates as Vascular Calcification Therapy Using a Nanoanalytical Approach

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Abstract: Vascular calcification significantly predicts atherosclerotic plaque rupture and cardiovascular events. Retrospective studies of women taking bisphosphonates, a proposed therapy for vascular calcification, paradoxically indicated increased risk in patients with prior acute events. We recently demonstrated that calcifying extracellular vesicles (EVs) released by cells within the plaque aggregate and nucleate calcific mineral, but the underlying mechanism and the potential for pharmacological intervention remain poorly understood. We hypothesize that bisphosphonates block EV aggregation and arrest existing mineral growth, freezing calcifications in a high-risk morphology that hastens plaque rupture. This study visualized for the first time EV aggregation and calcification at single-EV resolution, via scanning electron microscopy. Three-dimensional (3-D) collagen hydrogels incubated with calcifying EVs modeled fibrous cap calcification, serving as an in vitro platform to image mineral nucleation and test candidate drugs for the potential to inhibit or reverse vascular calcification. EVs aggregated along and between collagen fibrils. Energy-dispersive x-ray spectroscopy (EDS) confirmed that EV aggregates contained calcium and phosphorous, the building blocks of calcific mineral (vs. internal collagen control,  $p < 0.001$ ). The addition of the bisphosphonate ibandronate decreased the EDS-detected amount of calcium (4.32% by weight (wt%) vs. 2.36 wt%,  $p < 0.001$ ) and phosphorous (4.26 wt% vs. 1.94 wt%,  $p < 0.001$ ) comprising EV aggregates. Further, ibandronate reduced the size ( $21.5 \mu\text{m}^2$  vs.  $14.2 \mu\text{m}^2$ ,  $p = 0.012$ ) and changed the morphology of calcific EV aggregates (Figure). These findings agree with our hypothesis that bisphosphonates alter EV-driven calcification, and confirm that our 3-D collagen hydrogel system is a viable platform to study EV-mediated mineral nucleation and evaluate potential therapies for cardiovascular calcification.



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