Supplemental data

Detection of Hydroxyapatite in the Calcified Cardiovascular Tissues

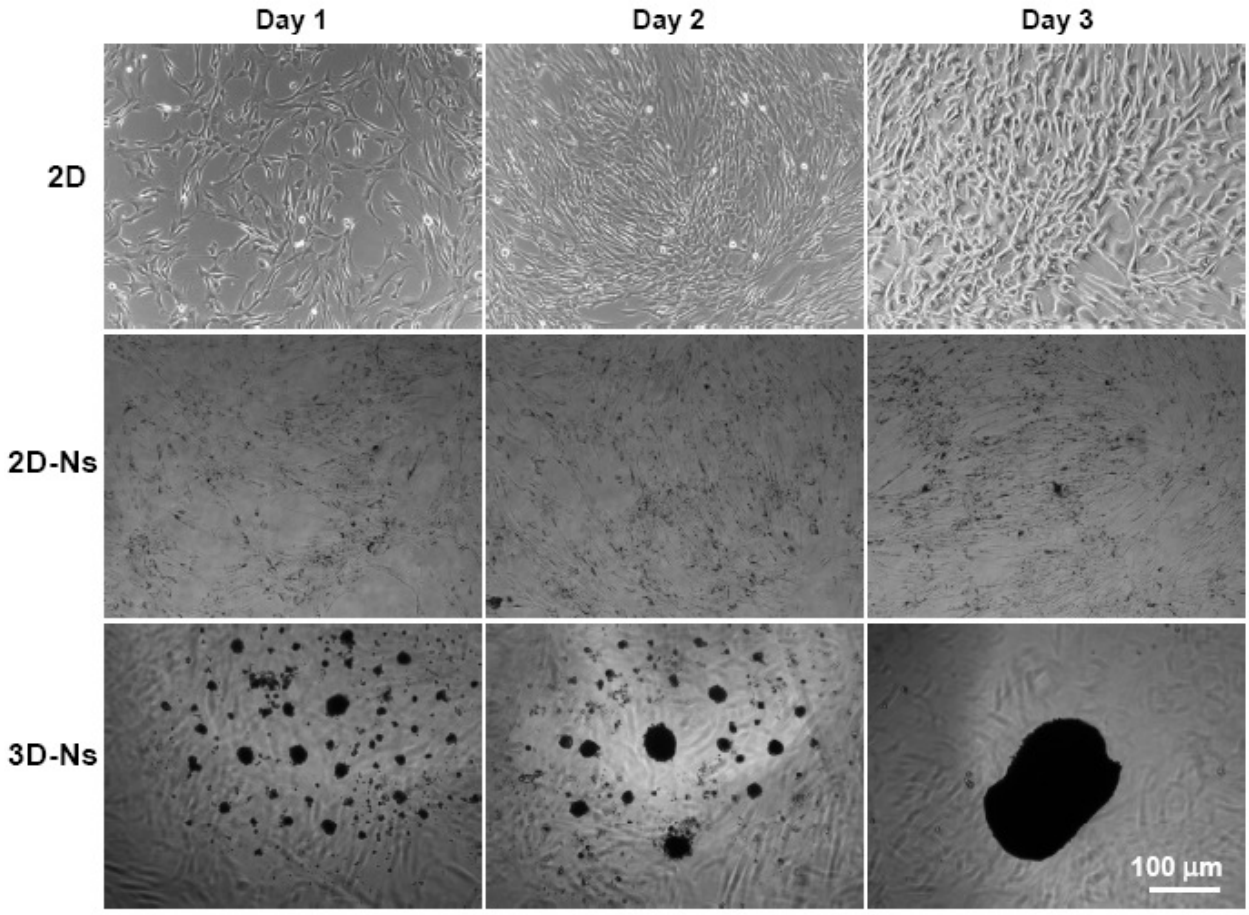
Jae Sam Lee[1], Joel D. Morrisett[2], Ching-Hsuan Tung[1]*

[1]Department of Translational Imaging, The Methodist Hospital Research Institute, Weill Cornell Medical College, Houston, TX

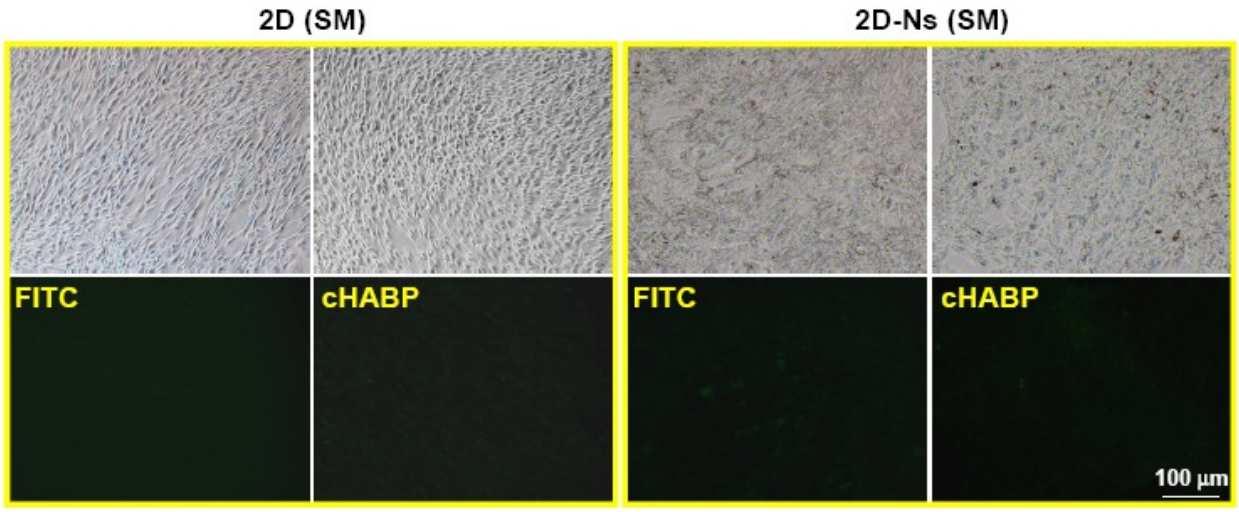
[2]Department of Medicine, Atherosclerosis and Vascular Medicine Section, Methodist DeBakey Heart Center, Baylor College of Medicine, Houston, TX

*Correspondence to
Ching H. Tung,
Department of Translational Imaging,
The Methodist Hospital Research Institute,
6565 Fannin Street, #B5-009,
Houston, TX 77030.
E-mail: ctung@tmhs.org

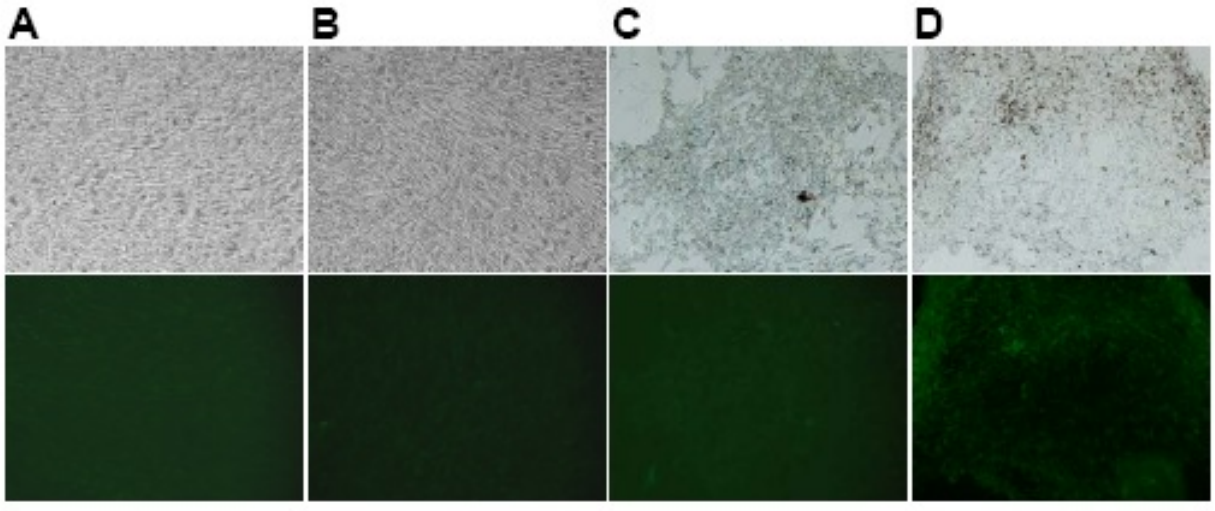
Supplemental Figures



Supplemental Figure I. Morphological changes of VSMCs during mineralization. Cells were incubated under control media (CM) for 3 days. Developed mineral layers from 2D, 2D-Ns, and 3D-Ns cultures observed by a light microscopy.



Supplemental Figure II. HA calcification of VSMCs induced by extrinsic stimuli. Cells cultured in 2D SM or 2D-Ns SM for 4 d were stained with FITC or cHABP and observed by a fluorescent microscopy, bright field (top) and fluorescence images (bottom) from mineral layers of VSMCs.



Supplemental Figure III. HA calcification of VSMCs induced by extrinsic stimuli. For corroborative visualization of the extent of HA calcification, cells cultured in CM or SM for 4 d were stained with HABP-19 and observed by a fluorescent microscopy. (A-B) Bright field (top) and fluorescence images (bottom) from mineral layers of VSMCs stained with HABP-19. Cells were cultured in 2D CM (A) or 2D SM (B). (C-D) Calcium deposition of VSMCs induced by 2D-Ns CM (C) or 2D-Ns SM (D) were stained with HABP-19.

Supplemental Movie Legend

Supplemental Movie I. A 3 dimensional μCT movie of surgically excised aortic valves. Cleaved aortic valves were incubated in the solution of PBS (1), FITC (2), cHABP-19 (3), or HABP-19 (4) for 1 h and then further incubated in PBS for 24 h. μCT imaging was performed at medium resolution and real time images were reconstructed. White color in the μCT images represents calcium depositions on the aortic valve surfaces. Scanner was operated in a 3 D volume imaging acquisition mode. Aortic valves were aligned at the center of the scanner FOV for subsequent imaging. μCT was acquired in approximately 3 min, and concurrent image reconstruction was achieved using a COBRA (Siemens). Invenon Research Workplace software (Siemens) was used to view and adjust imaging.