Electronic Supplementary Information

Hydroxyapatite-binding micelles for the detection of vascular calcification in atherosclerosis

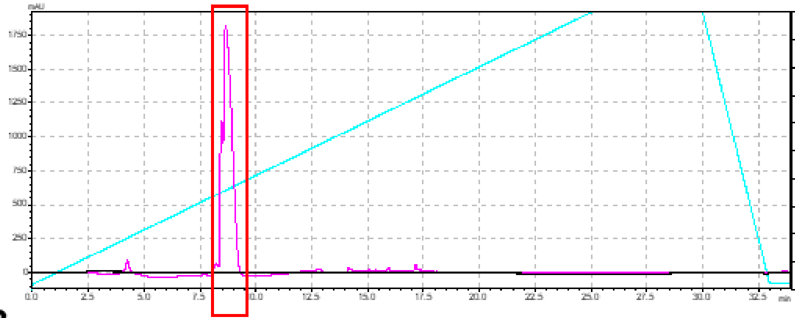
Deborah D. Chin[a], Jonathan Wang[a], Margot Mel de Fontenay[a], Anastasia Plotkin[b], Gregory A. Magee[b], Eun Ji Chung[a,b,c,d,e,f]

[a]Department of Biomedical Engineering, University of Southern California, Los Angeles, CA, USA
[b]Department of Surgery, Division of Vascular Surgery and Endovascular Repair, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA
[c]Eli and Edythe Broad Center for Regenerative Medicine and Stem Cell Research, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA
[d]Norris Comprehensive Cancer Center, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA
[e]Department of Chemical Engineering and Materials Science, University of Southern California, Los Angeles, CA, USA
[f]Department of Medicine, Division of Nephrology and Hypertension, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA

*Corresponding author: Eun Ji Chung
University of Southern California
Department of Biomedical Engineering
1042 Downey Way, 140
Los Angeles 90089, CA, USA
Tel: 1 213 740 2925
Email: eunchung@usc.edu

Figures and Figure Legends

A



B

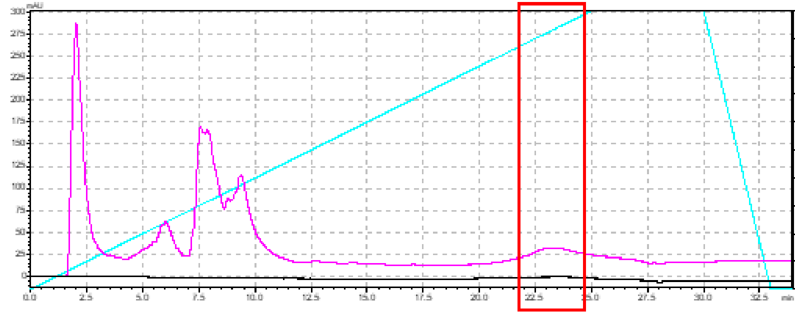


Fig. S1. HPLC chromatograms of A) HABP and B) DSPE-PEG(2000)-HABP show elution times of 8 min and 23 min, respectively.

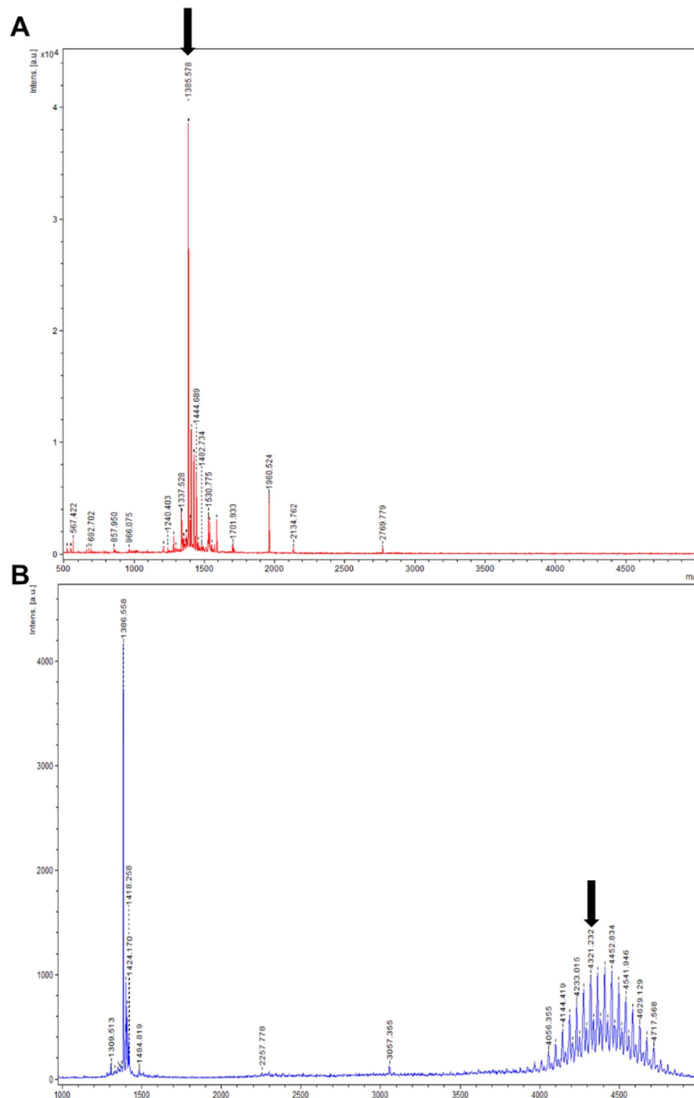


Fig. S2. MALDI mass spectrometry confirm correct molecular weight of A) purified HABP (1386 m/z, arrow) and B) purified DSPE-PEG(2000)-HABP (4321 m/z, arrow). The broad peak at 4321 m/z is characteristic of PEG products.

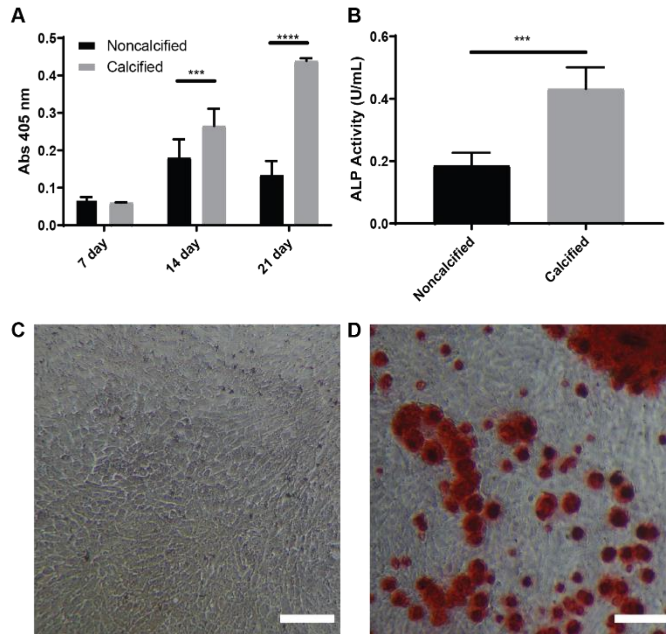
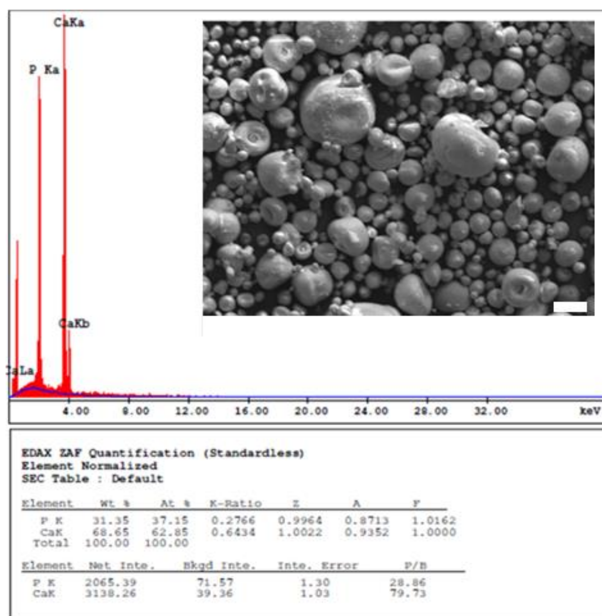


Fig. S3. MOVAS cells in osteogenic and growth media at days 7, 14, and 21 show calcifications. The amount of calcium mineralization was determined by fixing and staining cells with ARS dye. Calcification from MOVAS cells is detected from day 14 to 21. *** $p \leq 0.001$, **** $p \leq 0.0001$. B) At day 21, ALP activity of cells in osteogenic media is enhanced by more than 2-fold compared to cells in growth media. *** $p \leq 0.001$. Brightfield micrographs of ARS stained MOVAS cells at day 21 of C) growth media and D) osteogenic media show calcifications in red. Scale bar 100 µm.



EDAX ZAF Quantification (Standardless)
Element Normalized
SEC Table : Default

Element	Wt %	At %	K-Ratio	Z	A	F
P K	31.35	37.15	0.2766	0.9964	0.8713	1.0162
CaK	68.65	62.85	0.6434	1.0022	0.9352	1.0000
Total	100.00	100.00				

Element	Net Inte.	Bkgd Inte.	Inte. Error	P/B
P K	2065.39	71.57	1.30	28.86
CaK	3138.26	39.36	1.03	79.73

Fig. S4. SEM images show the spherical structure of commercial HA microcrystals and EDAX quantification verified the expected HA calcium phosphate ratio (1.69 ± 0.01). Scale bar 10 µm.

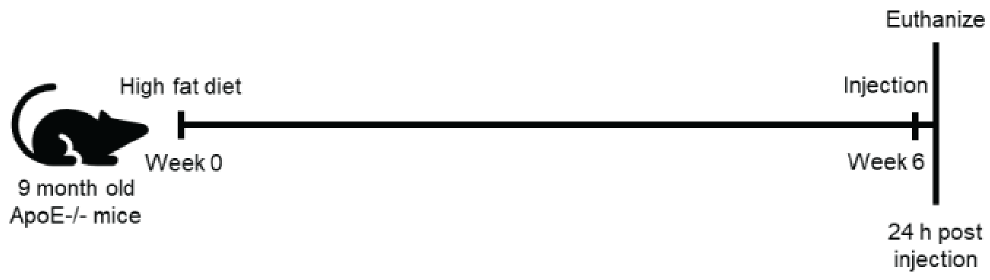


Fig. S5. Design of *in vivo* animal studies.

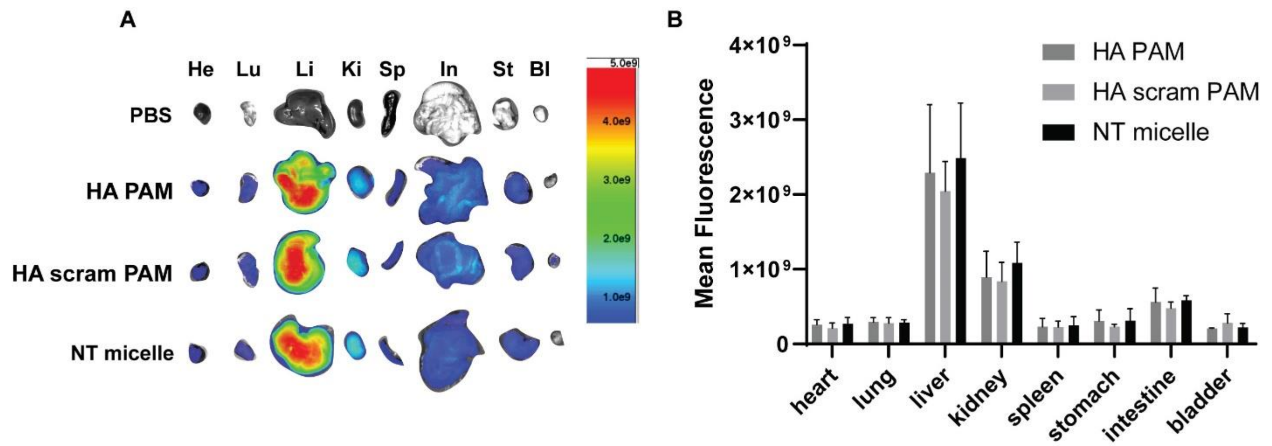


Fig. S6. A) Biodistribution of HA PAMs, HA scram PAMs, and NT micelles in ApoE -/- mice 24 hours post-administration was assessed from A) *ex vivo* fluorescence images and B) quantified.

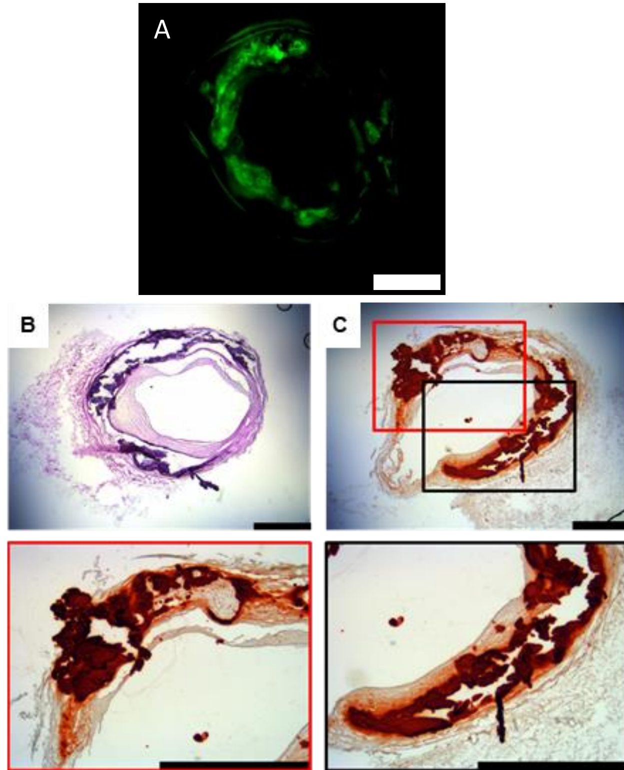


Fig. S7. Fluorescence, top down images of diseased human tibial arteries show A) HA PAMs (green) bind to bulk regions of calcifications. B) H&E staining of diseased human tibial arteries. C) Sections were stained with ARS (2%) to show areas of calcium (red). Insets show microcalcification nodules near the lumen and bulk calcification in the media. Scale bars 1 mm.

Table and Table Legend

Table. S1. Size and zeta potential measurements of HA scrambled PAM via DLS.

Particle	Radius (nm)	PDI	Zeta Potential (mV)
HA scram PAM	8.9 ± 1.1	0.37 ± 0.0	-0.1 ± 0.6