Quantitative molecular imaging of atherosclerotic endothelial dysfunction with perfluorocarbon (19F) nanoparticle magnetic resonance imaging and spectroscopy

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Introduction: Heart attack and stroke account for the great majority of deaths worldwide, and most occur without premonitory warning signs or symptoms. Disturbed endothelial barrier function in atherosclerosis has been detected by MRI by imaging gadolinium leakage into the vascular interstitium but not yet quantified. Alternatively, we propose that the unique, no background ^{19}F signal from crown ether perfluorocarbon-core nanoparticles (NP: ~250 nm) might both visualize *and* quantify endothelial disruption in advanced atherosclerosis.

Materials and Methods: Five NZW rabbits were fed a high fat diet for 9-12 months (cholesterol: 1200-1700 mg/dL). Fluorescently-labeled, nontargeted NP were injected (2 ml/kg) intravenously into rabbit ear vein. After circulation *in vivo* for 1, 6 or 24 hours, aortas were excised for ^{19}F MRI and spectroscopy (Varian 11.7 T scanner); and whole mount fluorescence imaging (Xenogen IVIS system). Two human carotid endarterectomy tissues were collected from operation room. After pretreatment with plasmin to digest fibrin on the endothelial surface and incubation with nontargeted NP for 6 hours, tissues were rinsed and formalin fixed for ^{19}F MRI and spectroscopy. A 1cm single turn solenoid coil was used for both ^{19}F MR spectroscopy and ^{1}H / ^{19}F MR imaging. A multi-slice spin-echo sequence was used for ^{19}F imaging for both rabbit aorta and human plaque tissues. Imaging parameters were: TR, 1s; TE, 14ms; resolution: 1*1*1 mm^3; imaging time: 4.5hour. After scanning, ^{19}F MR images were coregistered with ^{1}H images using MATLAB software to show the locations of the NP. An internal perfluorooctyl bromide standard was used in ^{19}F spectroscopy to quantify tissue bound NP numbers and concentrations in each imaged voxel.

Results: In rabbit aortas, MRI (^{19}F/^{1}H overlay) revealed abundant ^{19}F signal from intact NP that were localized heterogeneously in the plaque interstitium (Fig.1A) but not in unaffected areas. The average tissue concentration of NP calculated from MR spectroscopy (Fig.1B) was 2.36 ± 0.42 × 10^9 /g aorta. The accumulation of NP is distinct from macrophage uptake, as demonstrated by high resolution fluorescence microscopy (Fig.1C). Fluorescence imaging (Fig.1D) also confirmed the presence of NP. In human carotid arterectomy tissues, the detected ^{19}F signals were primary located on the endothelial/luminal side (Fig.2). Quantitative MRS-based analysis showed that average NP concentration in carotid arterectomy tissue was 47.1 ± 18.3 × 10^9/g tissue.

Conclusions: For both advanced experimental atherosclerosis and native human atherosclerosis tissues, nontargeted NP rapidly penetrate the leaky endothelial barrier, which can be visualized and quantified *ex vivo* with the use of "no background" ^{19}F MRI and MRS. This experimental strategy offers a potential new approach for quantification of endothelial dysfunction employing both *in vivo* and *ex vivo* incubation with nanoparticle tracers.

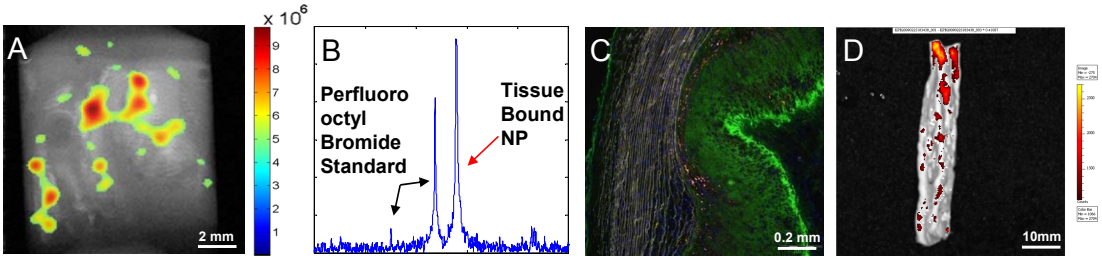

Figure 1. Rabbit aortic atherosclerotic tissue ^{19}F and ^{1}H MRI coregistered image (A), MRS (B), fluorescence microscopy (C) and whole-mount IVIS fluorescence image (D). The number of NP in each voxel in color bar in (A) was calculated based on MRS quantifications shown in (B).

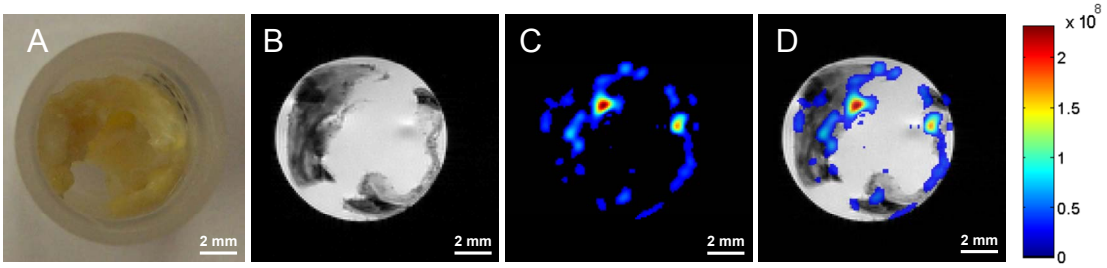

Figure 2. Human carotid endarterectomy tissue photo (A), ^{1}H and ^{19}F MR image (B,C) and ^{1}H, ^{19}F co-registered image (D). Number of NP in each voxel was calculated based on MRS quantifications and ^{19}F MR images were color coded. Color bar shows the number of NP in each imaging voxel.