Method For Specific Detection of Plaque Angiogenesis in the Cholesterol-fed Apolipoprotein-E Deficient Mouse With MR Fluorine Spectroscopy of Integrin-Targeted Perfluorocarbon Nanoparticles at 11.7T

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Introduction Angiogenesis plays a critical role in both atherosclerotic plaque development and plaque destabilization. To definitively detect plaque angiogenesis, we developed a novel method for targeting angiogenesis in atherosclerotic plaques of Apo-E -/- mice with the use of high field spectroscopy of $\alpha_v\beta_3$-integrin targeted perfluorocarbon nanoparticles that bind to endothelial molecular epitopes on the vasa vasorum, which expands dramatically under cholesterol drive as part of the vascular inflammatory process..

Methods Perfluorocarbon nanoparticles were prepared in two formulations according to methods standard in our laboratory: 1) $\alpha_v\beta_3$-targeted perfluoro-octylbromide (**PFOB**) particles, and 2) $\alpha_v\beta_3$-targeted crown ether (**CE**) particles. The targeting ligand, a vitronectin antagonist, is highly specific for $\alpha_v\beta_3$ with a dissociation constant in the low nanomolar range versus micromolar for other integrins. Two Apo-E -/- mice were maintained on a high cholesterol Western diet for approximately five months, after which each mouse was injected intravenously with 200 uL of nanoparticles. One mouse received targeted PFOB particles and the other received targeted CE particles. Nanoparticles were allowed to circulate for 2 hours post-injection so targeted particles could bind to the angiogenic vessels. Mice were then euthanized and perfused with saline solution to wash out any unbound nanoparticles, followed by 10% buffered formalin to fix the tissue. The heart and aorta were injected with dye for visualization purposes, excised, rinsed again, and spectroscopy was performed on a Varian 11.7T MR scanner with a custom-designed four-turn solenoid volume coil constructed in-house. General 19F spectroscopy was performed on both samples, and localized spectroscopy on the CE-containing sample. Localized spectra were acquired by selecting an imaging voxel on gradient echo scout images, then acquiring the spectrum using a PRESS sequence. All spectra were acquired with 128 signal averages (acquisition time ~5 min.); 200Hz line broadening was applied to the nonlocalized spectra, and 20Hz line broadening to the localized spectra.

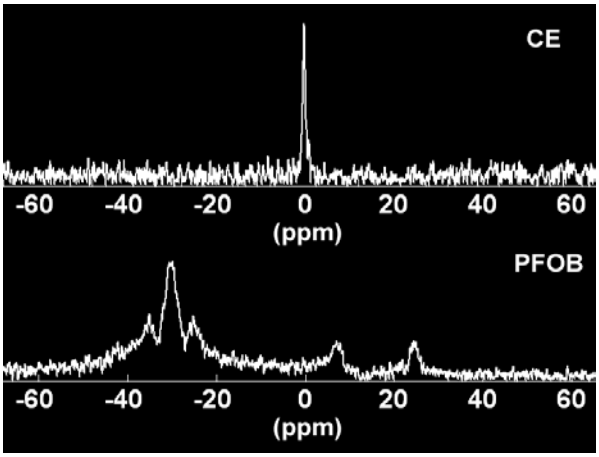

Figure 1: Nonlocalized fluorine spectra acquired from entire aortic specimens. Top, CE nanoparticle signal showing characteristic single peak. Bottom, PFOB nanoparticle signal showing characteristic triplet and two singlet peaks.

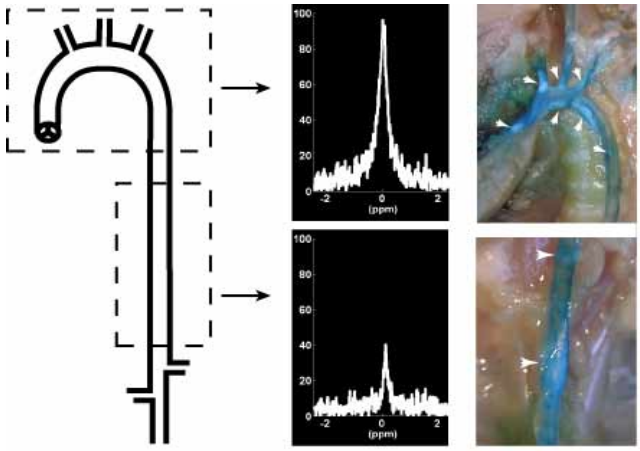

Figure 2: Crown ether nanoparticle signal from localized aortic segments: Localized spectra of CE nanoparticles and corresponding aortic specimen. Spectra were acquired in the regions of the aortic arch and renal arteries. Arrowheads on spectra indicate the single crown ether peak. Arrowheads on dissection photos indicate atherosclerotic plaques, visible as opaque white areas against the dye used for visualization. Note the larger peak at the aortic arch corresponding to a more extensive distribution of disease than in the abdominal aorta.

Results Fluorine signal was readily apparent in the aortas treated with targeted particles. Because the CE spectrum exhibits only a single fluorine peak, as compared with five PFOB peaks (Fig. 1), the CE particles yielded a higher signal-to-noise ratio, as expected. Localized spectroscopy in the aorta treated with the targeted CE particles manifested a higher signal in the aortic arch than in the proximal or distal descending aorta, which is consistent with the greater extent and severity of disease in the arch as confirmed by gross pathological inspection, as shown in Fig. 2.

Discussion In this pilot study, we report the development and implementation of a novel method for definitively detecting plaque angiogenesis associated with atherosclerosis , in a well-described murine model of atherosclerosis. Because fluorine is not abundant in living tissue, perfluorocarbon-based targeted nanoparticles represent an excellent candidate agent for characterizing pathological molecules causative of atherosclerosis with the use of MRI and MRS. We have demonstrated that it is possible to detect angiogenesis with two different perfluorocarbon particle formulations, and further, that the method is sensitive enough to detect spatial variations in disease extent based on fluorine signal strength from targeted particles (Fig. 3). Because the fluorine spectrum is theoretically quantitative with respect to the number of nanoparticles bound (and thus molecular epitopes present)[1], this method may permit localized fluorine spectroscopy to delineate the extent of plaque angiogenesis in different regions of the mouse aorta in vivo.

References [1]Morawski AM et al. *Mag Res Med* 2004 Dec; 52(6): 1255-62