

## Identification of vulnerable plaque by MRI and fluorescence imaging in a rabbit model

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**Introduction:** Atherosclerotic plaques progress slowly and frequently remain asymptomatic (stable plaque). However, disruption of vulnerable plaque and subsequent thrombosis can lead to rapid occlusion of the lumen and a potentially fatal event. Therefore, it is important to identify vulnerable plaques prior to acute events *in vivo*. Because of the sudden and unpredictable disruption of vulnerable plaques, this event is impossible to study in a controlled manner in humans. We have established a rabbit model of atherosclerosis and pharmacologically controlled plaque disruption and recently shown that *in vivo* MRI can reliably predict plaque vulnerability<sup>1</sup>.

To gain both morphological and functional information about the plaques, we have combined two imaging modalities; (i) *in vivo* MRI at 3T and (ii) *ex vivo* optical imaging using activatable cell-penetrating peptides (ACPPs) activated by either matrix metalloproteinase-2 and 9 (MMP 2/9) or thrombin. These markers have been shown to be increased in vulnerable plaques<sup>2,3</sup>. Our hypothesis was that localized regions of higher fluorescence signal seen *ex vivo* would correlate with vulnerable plaques identified *in vivo* by MRI based on the presence of positive vessel wall remodeling and/or the higher uptake of Gd<sup>3+</sup>. All assignments were confirmed by histological analysis.

**Methods:** Atherosclerotic lesion was induced in 2 male New Zealand White rabbits by endothelial denudation, 8 weeks of 1% cholesterol diet and 4 weeks of normal diet. At the end of the 12-week dietary protocol, disruption of plaques was induced by pharmacological triggering<sup>1</sup>. *In vivo* MRI was performed before and after the pharmacological triggering using a 3T Philips Intera System and a synergy knee coil with 6 elements. Cardiac gated axial T1- black-blood (T1BB) images were acquired in the abdominal aorta before (Pre-CE) and 10-15 minutes after Gd-DTPA (Post-CE) injection using a dual inversion recovery spin echo sequence. The parameters were: TR=2 cardiac cycles, TE=10ms, TSE=15, axial slice thickness = 4mm, inversion recovery delay = 350 ms, NEX=2, MTX= 384x362 (in-plane resolution=0.23x0.23 mm), scan time= 8 min. Pre-CE T1BB images were used to calculate the remodeling ratio [RR = vessel area<sub>lesion</sub>/ vessel area<sub>reference</sub>] after tapering correction<sup>4</sup>. Plaques with vulnerable features were defined as those with positive remodeling (RR>1.05). After the MRI experiments, the two rabbits were injected (300nm, IV) with two different Cy5 labeled cell-penetrating peptide probes. These probes contain an activation sequence, which is rapidly cleaved by either MMP2/9 (PLGLAG) or thrombin (DPRSFL). Such cleavage jettisons a polyanionic segment and unmasks the adhesiveness of a fluorescently labeled polycationic peptide. After 6 h the animals were sacrificed, and the abdominal aorta was extracted, extended to its anatomical length and pinned down on a dissection tray. *Ex-vivo* fluorescence imaging was acquired at 640/700nm excitation/emission with a Xenogen system. Transverse cryo-sections were collected and stained with Masson's trichrome to identify plaque components and thrombi. Vulnerable plaques were defined as those with attached platelet and fibrin-rich thrombi.

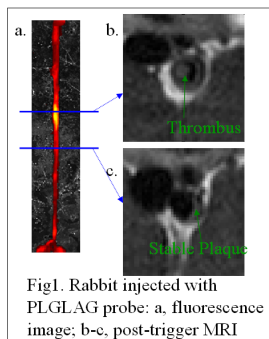


Fig1. Rabbit injected with PLGLAG probe: a, fluorescence image; b-c, post-trigger MRI

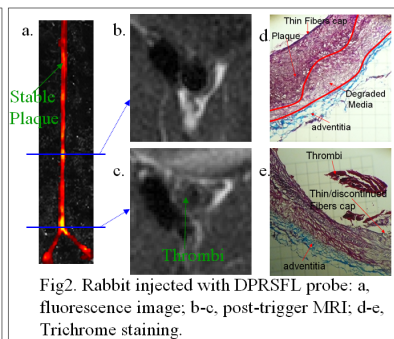


Fig2. Rabbit injected with DPRSFL probe: a, fluorescence image; b-c, post-trigger MRI; d-e, Trichrome staining.

**Results and Discussion:** Atherosclerotic plaques and thrombi were identified in the abdominal aorta by *in vivo* MRI. A rabbit injected with PLGLAG-ACPP showed significant enhancement of the fluorescence signal (yellow, Fig. 1a) at the region of a disrupted plaque with an overlying thrombus that had been identified by *in vivo* MRI to exhibit positive remodeling with an RR=1.10 (Fig 1b). In contrast, the stable plaque (Fig. 1c) showed low uptake of the fluorescent probe (Fig. 1a). In the rabbit injected with the DPRSFL-ACPP probe (Fig. 2), two plaques with positive arterial remodeling showed an enhanced fluorescent signal *ex vivo*, one 3.0-3.5 cm above the iliac bifurcation (Fig. 2a and 2b) and another one around iliac bifurcation (Fig. 2a

and 2c). Complimentary histology showed that both plaques had histological features of plaque vulnerability: the top plaque had a thin fibrous cap and degraded media (Fig. 2d), and the bottom plaque had a thin fibrous cap and a large lipid core and an overlying thrombus (Fig 2e). Stable plaques present elsewhere in the aorta (e.g., green arrow in Fig. 2a) showed low uptake of each probe in comparison to the vulnerable plaques.

**Conclusions:** We demonstrated that positively remodeled vulnerable plaques identified by *in vivo* MRI exhibited increased uptake of fluorescence probes targeting MMP2/9 and thrombin as assessed by *ex vivo* optical imaging. The combination of these techniques may help to understand the pathophysiology of plaque vulnerability and lead to the rational design of future targeted contrast agents for MRI applications and therapeutic approaches.

**References:** 1.Phinikaridou A et. al, Circ Cardiovascular Imaging. 2010 May; 3(3):323-32; 2.Newby AC, Trends Cardiovascular Med. 2007 Nov; 17(8):253-8;3.Galis ZS et. al, Arterioscler Thromb Vasc Biol. 1997 Mar; 17(3):483-9;4.Pasterkamp G et. al, Arterioscler Thromb Vasc Biol. 1997 Nov; 17(11):3057-63.