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Intravascular Molecular-Structural Assessment of Arterial Inflammation in Preclinical Atherosclerosis Progression

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Coronary atherosclerosis progression is a key determinant of future clinical events (1). Although prospective intravascular ultrasound (IVUS) imaging studies have shown that high plaque burden (PB) is associated with coronary events, molecular imaging of plaque pathobiology is a new approach that may enhance identifying plaques prone to progression (2). We investigated the relationship between in vivo plaque inflammatory protease activity, as detected by near-infrared fluorescence-optical coherence tomography (NIRF-OCT) molecular structural imaging, and experimental plaque progression in coronary-sized arteries.

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Inflamed aortic atherosclerotic plaques were induced in rabbits by cholesterol feeding and balloon injury (Massachusetts General Hospital Institutional Animal Care and Use Committee approval #2013N000015) (3). Serial 2-timepoint in vivo multimodal imaging with x-ray angiography, IVUS, and NIRF-OCT was performed at baseline (8 weeks after balloon injury) and then at follow-up (4 or 8 weeks after baseline imaging). Rabbits (N = 16) received ProSense VM110 (PerkinElmer; 4 mg/kg intravenously 24 hours earlier), a cathepsin protease activity NIRF reporter, at 8 weeks to assess baseline NIRF inflammation. At follow-up, inflammation was re-evaluated by NIRF-OCT after readministering ProSense in an identical fashion. Atheroma progression was quantified on per animal and per slice bases. Per animal changes were quantified as the change in percent atheroma volume (PAV), where: $PAV (\%) = [\Sigma(EEM_{CSA} - Lumen_{CSA}) \div \Sigma EEM_{CSA}] \times 100\%$. Per slice changes were quantified as change in PB (PB) of co-registered IVUS data sets manually segmented every 0.4 mm, with a rabbit atheroma defined as 10% PB over 3 consecutive frames, where: $PB (\%) = [(EEM_{CSA} - Lumen_{CSA}) \div EEM_{CSA}] \times 100\%$. NIRF concentration was quantified by automated correction on the basis of the distance between the NIRF-OCT catheter and the lumen surface. IVUS and NIRF-OCT images were manually co-registered using arterial side branches as fiducial markers. Following sacrifice of the animals, aortas underwent ex vivo fluorescence imaging and histopathologic examination. Unadjusted and multivariable regression models were performed. To account for clustering of observations for each animal, we used a linear mixed-effects model with random intercepts.

Atheroma inflammation was detected by in vivo NIRF-OCT imaging (Figure 1A). Comparatively, the flanking uninjured aorta exhibited low NIRF inflammation signal. Over time, plaque NIRF inflammation increased in 15 of 16 animals, with the average NIRF 225% and 168% higher at weeks 12 (36.1 ± 13.2 nM) and 16 (26.8 ± 9.5 nM), respectively, than at the week 8 baseline (16.0 ± 4.2 nM; $P < 0.05$ for both) (Figure 1B and 1C). Univariable regression analysis demonstrated that the baseline NIRF signal at 8 weeks was strongly associated with per plaque progression (PAV, $r = 0.70$; pooled 12-week + 16-week follow-up groups; $P = 0.003$) (Figure 1D). In comparison, no significant relationship was observed between PAV and baseline cholesterol or IVUS-derived structural measures (plaque length, minimal luminal area, maximum PB, or remodeling index; all $P > 0.05$).

Next, a detailed slice-based analysis of 2,609 axial images was performed every 0.4 mm between matched NIRF-OCT and IVUS images. The baseline NIRF inflammation signal was associated positively with per slice plaque progression (PB, 8 weeks-12 weeks (N = 867 slices): $r = 0.35$; $P < 0.001$; and 8 weeks-16 weeks (1,742 slices): $r = 0.27$; $P < 0.001$). In multivariable analyses, NIRF inflammation per slice also predicted the PB (8 weeks-12 weeks: $\beta = 0.16$; SE = 0.03; $P < 0.001$; and 8 weeks-16 weeks: $\beta = 0.29$; SE = 0.02; $P < 0.001$), including after adjustment for 8-week total cholesterol, PB, minimal luminal area, and remodeling index (8 weeks-12 weeks: $\beta = 0.19$; SE = 0.03; $P < 0.001$; and 8 weeks-16 weeks: $\beta = 0.48$; SE = 0.02; $P < 0.001$).

This serial molecular-structural NIRF-OCT and IVUS imaging study of coronary artery-sized plaques provides several new insights. First, in vivo cathepsin protease inflammatory activity, known to destabilize coronary plaques, is associated with atheroma progression

in coronary-sized arteries independent of PB. Second, a single baseline measurement of NIRF plaque inflammation is associated with experimental plaque progression on both a per plaque level and a per slice level. Third, changes in atherosclerosis inflammatory protease activity parallel changes in plaque progression in vivo. Clinical translation of this approach appears feasible because both intravascular NIRF-OCT imaging catheters (4) and a similar NIRF cathepsin agent (5) have been tested clinically. Overall, this in vivo study provides a foundation to assess the role of coronary artery inflammation in predicting plaque progression in patients and to guide personalized anti-inflammatory therapy of coronary artery disease.

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Abbreviations:

FRI	fluorescence reflectance imaging
IVUS	intravascular ultrasound
MLA	minimal lumen area
NIRF	near-infrared fluorescence microscopy
OCT	optical coherence tomography
PAV	change in percent atheroma volume
PB	change in plaque burden
RI	remodeling index

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FIGURE 1. Intravascular Molecular-Structural NIRF-OCT Arterial Plaque Inflammation Imaging in Vivo

(A) Representative multimodal images at 12 weeks after balloon injury. X-ray angiography demonstrates mild atherosclerosis in the balloon-injured zone (**white line**). Longitudinal optical coherence tomography (OCT) and 1-dimensional (1D) average near-infrared fluorescence (NIRF) inflammation image overlay (**yellow/green is high; blue is low**). In vivo 2-dimensional (2D) near-infrared fluorescence maps reveal heterogeneous near-infrared fluorescence inflammation across the catheter pull back, corroborated by ex vivo fluorescence reflectance imaging (FRI) near-infrared fluorescence. (Scale = 5 mm.)

(B) Serial intravascular near-infrared fluorescence–optical coherence tomography imaging. The **color bar** indicates regions of high (**yellow/green**) and low (**blue**) near-infrared fluorescence inflammation. Quantitative 1-dimensional near-infrared fluorescence and near-infrared fluorescence ratio (integrated near-infrared fluorescence signal at 16 weeks divided by 8 weeks) and 1-dimensional plaque burden (PB) plot and percent atheroma volume (PAV) ratio (16 weeks/8 weeks). (C) Baseline near-infrared fluorescence inflammation is associated with change in plaque burden plaque progression. Representative axial slice at 8 weeks showing high near-infrared fluorescence inflammatory protease activity followed by high intravascular ultrasound (IVUS) plaque progression from 8 weeks–12 weeks (v = vessel, fiducial). External elastic membrane (**yellow**) minus lumen (**white**) contours define the atheroma cross-sectional area (CSA). (Scale = 1 mm.) (D) Baseline near-infrared fluorescence inflammation is associated with change in percent atheroma volume plaque progression. Univariable regression per animal comparing 8-week near-infrared fluorescence inflammation and intravascular ultrasound change in percent atheroma volume plaque progression between 8 weeks and 12 or 16 weeks. $^{**}P < 0.01$.