Atherosclerotic plaque rupture and subsequent thrombotic complications are a leading cause of myocardial infarction and sudden cardiac death. The majority of these cardiovascular events occur in coronary arteries with <50% stenosis, indicating that information about luminal stenosis is not sufficient to predict plaque vulnerability. Despite a progressive understanding of the crucial role of inflammation in plaque rupture, conventional intracoronary imaging modalities including intravascular ultrasound and optical coherence tomography (OCT) have focused on the morphological changes and are insufficient to provide information about inflammatory activity. Noninvasive positron emission tomography/computed tomography imaging is feasible for estimating vascular inflammation in large arteries such as the carotids and the aorta, but it

**Background**—Lipid-rich inflamed coronary plaques are prone to rupture. The purpose of this study was to assess lipid-rich inflamed plaques in vivo using fully integrated high-speed optical coherence tomography (OCT)/near-infrared fluorescence (NIRF) molecular imaging with a Food and Drug Administration–approved indocyanine green (ICG).

**Methods and Results**—An integrated high-speed intravascular OCT/NIRF imaging catheter and a dual-modal OCT/NIRF system were constructed based on a clinical OCT platform. For imaging lipid-rich inflamed plaques, the Food and Drug Administration–approved NIRF-emitting ICG (2.25 mg/kg) or saline was injected intravenously into rabbit models with experimental atheromata induced by balloon injury and 12- to 14-week high-cholesterol diets. Twenty minutes after injection, in vivo OCT/NIRF imaging of the infrarenal aorta and iliac arteries was acquired only under contrast flushing through catheter (pullback speed up to ≤20 mm/s). NIRF signals were strongly detected in the OCT-visualized atheromata of the ICG-injected rabbits. The in vivo NIRF target-to-background ratio was significantly larger in the ICG-injected rabbits than in the saline-injected controls (P<0.01). Ex vivo peak plaque target-to-background ratios were significantly higher in ICG-injected rabbits than in controls (P<0.01) on fluorescence reflectance imaging, which correlated well with the in vivo target-to-background ratios (r=0.85; P<0.01). Cellular ICG uptake, correlative fluorescence microscopy, and histopathology also corroborated the in vivo imaging findings.

**Conclusions**—Integrated OCT/NIRF structural/molecular imaging with a Food and Drug Administration–approved ICG accurately identified lipid-rich inflamed atheromata in coronary-sized vessels. This highly translatable dual-modal imaging approach could enhance our capabilities to detect high-risk coronary plaques. (Circ Cardiovasc Interv. 2014;7:560-569.)

**Key Words:** indocyanine green • molecular imaging • optical coherence tomography • plaque, atherosclerotic
WHAT IS KNOWN

- Plaque rupture mainly occurs in lesions with a large lipid core and an overlying thin fibrous cap with evidence of macrophage infiltration within the cap.
- Current intracoronary imaging modalities, such as intravascular ultrasound or optical coherence tomography, are not able to estimate the risk of future plaque rupture.

WHAT THE STUDY ADDS

- An integrated optical coherence tomography/near infrared fluorescence imaging catheter with a clinically available near-infrared fluorescence-emitting dye was used to detect macrophage infiltration and lipid-rich components of atheromata simultaneously in vivo in coronary-sized vessels.
- This fully integrated dual-modality technology leveraged 2 major approaches to intravascular atherosclerosis imaging and has implications for the clinical detection of high-risk coronary plaques.

is still challenging to estimate coronary plaque inflammation, primarily attributable to the small imaging target volume and multiple sources of image degradation, including blood flow and respiratory movement. Therefore, it is urgently needed to develop a more accurate imaging strategy for detection of high-risk atherosclerotic plaques in coronary arteries.

Recently, near-infrared fluorescence (NIRF) imaging has emerged as an ionizing radiation-free high-resolution imaging approach to detect plaque inflammation. An NIRF imaging platform can be created for an intravascular NIRF imaging catheter and even fully integrated with OCT structural imaging. In the feasibility study, the integrated OCT/NIRF intravascular imaging was able to detect plaque protease NIRF signals, but the slow pullback speed (2.5 mm/s) and substantial background noise because of cross-talk between different properties in double-clad fiber were still obstacles to clinical application, which ultimately requires imaging only under flushing without proximal occlusion, to eliminate blood in the segment. Furthermore, the prosense/VM110 used in the previous study is not yet clinically available as an NIRF agent and needs to be validated for clinical safety.

In contrast, indocyanine green (ICG), an amphipathic NIRF agent that is approved by the Food and Drug Administration (FDA) for ophthalmologic NIRF imaging, has been shown to bind to lipoproteins and also to be accumulated in inflamed tissues. Recently, Vinegoni et al have reported that ICG could bind to lipid-rich macrophage-harboring atheromata. Intriguingly, these NIRF signals were detected by a stand-alone NIRF fiber sensing with an offline intravascular ultrasound data fusion after separate data acquisition. Despite the potential implications of this study, the 1-dimensional (1D) NIRF approach with slow pullback capability (0.5 mm/s) could image only one third of the entire 360° arterial wall where NIRF signals were captured.

To solve these limitations and answer the unmet needs of clinically available intracoronary structural/molecular imaging of high-risk plaques in coronary arteries, herein we have extended the previous OCT/NIRF study to advance a high-speed, fully integrated OCT/NIRF imaging catheter system and have investigated the feasibility of a novel strategy of combined OCT/NIRF imaging with an FDA-approved NIRF-emitting ICG to image high-risk plaques in coronary-sized rabbit arteries.

Methods

Integrated OCT/NIRF System
See Methods in the Data Supplement.

Briefly, a laboratory-built dual-modality imaging system (Figure 1) enabled both OCT and NIRF imaging from a single pullback scanning through a custom-made catheter with an outer diameter of 0.87 mm. The OCT system with a center wavelength of 1290 nm provided high-resolution cross-sectional images at an A-line rate of 120 kHz. The NIRF system was integrated with the OCT system through a dual-modality small form factor fiberoptic rotary junction (FRJ). The rotary part of the FRJ was constructed with a custom-built collimator using a short-length double-clad fiber. The OCT images and the NIRF signal plot were generated and displayed by custom-built real-time visualization software.

Phantom Studies (Distance Calibration)
Briefly, the intensity of the NIRF signal decreases exponentially with increasing distance from the catheter to the fluorochrome. Thus, for quantitative analysis, it is necessary to correct for NIRF signal intensity with distance. See Methods in the Data Supplement for the distance calibration methods.

ICG NIRF Molecular Imaging Agent
We used ICG (C43H47N2O6S2Na; molecular weight, 775 Da) as the molecular imaging agent. ICG is an amphipathic NIR fluorochrome approved by the FDA. It has peak absorption at 805 nm and peak emission at 830 nm in blood.

Experimental Protocol
These animal experiments were approved by the Institutional Animal Care and Use Committee of Korea University (KUIACUC-2013–88) and were performed in accordance with the recommendations of the national and institutional guidelines. Detailed protocols are shown in Methods in the Data Supplement.

In Vivo Intravascular Imaging
After 12 (for lipid-rich atheroma model) and 3 weeks (for fibrotic plaque model), the experimental rabbits were anesthetized and in vivo imaging was performed. Detailed imaging methods are described in Methods in the Data Supplement.

Ex Vivo Imaging
NIR fluorescence reflectance imaging (FRI) of ICG using an imaging system consisting of a 300-W Xenon arc lamp (MAX-302; Asahi Spectra Co, Ltd, Japan) and a cooled charge-coupled device camera (PIXIS 1024BR, Roper Scientific) with an excitation bandpass filter of 749 to 790 nm and an emission bandpass filter of 814 to 851 nm (Semrock, Rochester, NY) for detecting ICG was performed for the resected aortoiliac vessels. We took images with multiple exposure times (0.01–16 s) for each wavelength. White light images were also obtained with an exposure time of 500 ms. Because of vessel shrinkage after resection, the aorta and the iliac vessels were manually elongated to in vivo measured lengths. The regions of interest were traced manually after visual identification of atherosclerotic plaque, normal...
vessel, and background, respectively, with a length of ≈5 to 10 mm for each subsegment (ImageJ 1.43j, Wayne Rasband, US National Institutes of Health). Target-to-background ratios (TBRs) were calculated for each region of interest by dividing the signal of the target region of interest by normal vessel background.

Fluorescence Microscopy

The 20-μm cryosections were examined by fluorescence microscopy of atherosclerotic plaque and normal vessel sections using a Nikon Eclipse TE2000-U fluorescence microscope (Nikon Instruments, Melville, NY) with filter sets for ICG NIRF (excitation/emission, 768/807 nm; exposure time, 30 s) and autofluorescence (excitation/emission, 495/519 nm; exposure time, 2 s). Windowing of images was identical, and all images were captured with a charge-coupled device camera (ProgRes MF cool, JENOPTIK, Germany) and analyzed using ImageJ software (ImageJ 1.43j, Wayne Rasband, US National Institutes of Health).

See the Methods in the Data Supplement for detailed information on immunohistochemistry and in vitro validation test of ICG.

Statistical Analysis

Data are presented as mean±SE. Nonparametric Mann–Whitney test was used to compare the peak plaque TBR between the ICG injection group and the saline injection group for both in vivo and ex vivo fluorescence studies. Linear regression was performed with Prism version 5.0 (GraphPad Software, San Diego, CA). Values of P<0.05 were considered statistically significant. The agreement between in vivo and ex vivo NIRF signal values was evaluated through the use of Bland–Altman analysis by calculating the bias (mean difference) and the 95% limits of agreement (2 SD around the mean difference).

Results

High-Speed Dual-Modal Catheter Imaging System

We developed a custom-built dual-modality system that can simultaneously generate images of microstructure and provide fluorescent molecular information (Figure 1). Notably, the catheter is mechanically and structurally identical to the OCT catheter that is currently used in the cardiac cath laboratory. Our system has several advantages compared with a previous OCT/NIRF imaging system.7 First, our catheter system permits stable high-speed rotation (100 revolutions per second) because it has a miniaturized rotary optical fiber coupling system that is made with 1.8-mm diameter gradient index lenses. Second, the high-speed swept source laser (117.2 kHz) used by our system permits a rapid pullback speed of 20 mm/s, which is comparable with commercialized OCT systems, and allows nonocclusive flushing, an ability that is essential for clinical application of the system. OCT image quality is dependent on optical coupling between stationary and rotary fibers in the FRJ, because cross-talk between single-mode core and multimode cladding on double-clad fiber increases background noise. In this study, we fabricated simple and
robust FRJ using gradient index lens collimators and small rotary parts that enhanced mechanical stability. The resultant coupling loss of single-mode coupling was <1 dB. Thus, background noise attributable to the use of the double-clad fiber was significantly lower compared with the previous system.

Distance Calibration of In Vivo NIRF Signals
The 2D NIRF map of the tube phantom filled with homogeneous ICG in milk was processed using Matlab (Matlab 2013b, The MathWorks, Inc), and the background noise resulting from autofluorescence of the optical fiber was eliminated from the 2D NIRF map. The NIRF signals showed nonhomogeneous intensity mainly attributable to the distance effect (Figure IIIA in the Data Supplement). Hence, distance calibration was conducted on the background-subtracted 2D NIRF map using the calibration function. After correction, the 2D NIRF map showed homogeneous intensity over the entire imaging pullback (Figure IIIB in the Data Supplement), demonstrating that distance calibration enabled quantitative measurement of the NIRF signals.

In Vivo OCT/NIRF Imaging With ICG Targeting Lipid-Rich Inflamed Plaque
To determine whether the ICG could generate a suitable signal for in vivo detection of atherosclerosis, we conducted OCT/NIRF to simultaneously image atherosclerotic plaque and the associated inflammation. The integrated OCT/NIRF imaging was performed 20 minutes after injection of ICG (n=6) or saline (n=3). The dual-modality intra-arterial catheter allowed us to collect simultaneous OCT and NIRF images of normal and atherosclerotic portions of the iliac arteries and the aorta. We acquired the dual-modality images from a total of 22 arteries, 8 aortas, 7 injured right iliac arteries, and 7 noninjured left iliac arteries. A total of 3 pullbacks in each artery were obtained. In 1 aorta and 2 of both iliac arteries, we were unable to obtain the image because of the vascular tortuosity.

Longitudinal OCT images of the atherosclerotic rabbit arteries demonstrated highly scattering thickened arterial wall (versus noninjured, normal-looking areas; Figure 2A, see Movies I and II in the Data Supplement). The lower panel of Figure 2A shows the representative cross-sectional OCT/NIRF images from the aorta (Figure 2A, a and b). OCT revealed the microstructure of atherosclerotic plaque including the signal-poor lipid-rich regions (Figure 2A-b, white arrows). Simultaneously obtained NIRF images showed the strong signals at the atherosclerotic portion (b, outer color spectrum), whereas NIRF signals from noninjured normal segment were almost negligible except ICG-stained guidewire (Figure 2A-a; also see Movie I in the Data Supplement for atheroma segment and Movie II for normal segment). Intriguingly, the NIRF imaging at the lipid-rich areas identified by OCT showed much stronger signals compared with those at other plaque portions (Figure 2A-b white arrows, Movie I in the Data Supplement).

In control atheroma rabbit without injection of ICG, NIRF signal was undetectable even in the large atheromatous areas.
identified by OCT images (Figure 2C). The in vivo peak plaque TBR was nearly 3.9× greater than control in aorta (6.28±1.10 versus 1.60±0.15; P=0.01; Figure 2B) and 3.6× greater than control in iliac artery (6.35±1.41 versus 1.77±0.07; P=0.01; Figure 2B). A color-coded 3-dimensional reconstruction cutaway view (Figure IV in the Data Supplement) and a flythrough movie (Movie III in the Data Supplement) of the OCT/NIRF imaging demonstrated that strong NIRF signals existed in the atheroma segments.

**Ex Vivo FRI for Validation of In Vivo NIRF**

Ex vivo FRI showed strong focal NIRF signals within plaques and colocalized with atherosclerotic plaques visible in white light vessel images of atheroma-bearing rabbits (Figure 3A). In contrast, there were no signals in the saline-injected atheroma rabbit (Figure 3B) and little NIRF signals in an ICG-injected normal rabbit without plaques (Figure 3C). We quantitated the ICG NIRF signals from in vivo and ex vivo NIRF 2D mapping images. The ex vivo peak plaque TBR was >3.9× greater than control (8.38±0.94 versus 2.16±0.41; P<0.01; Figure 3D) in aorta and 3.2× greater than control in iliac artery (6.30±0.87 versus 2.21±0.12; P<0.01; Figure 3D). Correlation between the in vivo and ex vivo peak plaque TBRs was statistically significant (r=0.85; P<0.01; Figure 3E), and Bland–Altman analysis resulted in no significant bias (0.41; Figure V in the Data Supplement). The simultaneously acquired in vivo NIRF data were fitted to 2D geometric NIRF maps and compared with the ex vivo FRI of the resected vessels (Figures 4 and 5A). There was a good visual correspondence between the in vivo intravascular NIRF signals and the ex vivo FRI signals in the aortoiliac vessels (Figure 4A and 4B). Also, to further validate the colocalization between ex vivo FRI and in vivo NIRF images, we directly compared ex vivo FRI and in vivo NIRF mapping from a single representative rabbit aorta (Figure 5A). We calculated a longitudinal average intensity profiles (Figure 5B) from the images using the analytic method of Ripplinger et al.16 The average intensities from ex vivo FRI and in vivo NIRF mappings along the longitudinal direction at an interval of 0.4 mm showed a good colocalization (R²=0.89; P<0.01; Figure 5C).

**Colocalization of Microscopic ICG NIRF Signals With Lipid-Rich Atheromata Containing Abundant Macrophages**

Fluorescence microscopy revealed strong NIRF signals in diffuse and focal plaques (Figure 6A and 6B), suggesting that the ICG binding is possible in advanced lesions and in relatively early-stage plaques as well. As compared with the ICG group, NIRF signals at fluorescence microscopy in the saline group were nearly negligible even in the plaque areas, and only the autofluorescence signals that originated from elastin fibers in the medial layer (Figure 6C) were noted. In addition, the immunostaining analysis showed that plaque NIRF colocalized with lipid-rich areas in the rabbit atheroma, as confirmed by oil red O (ORO) staining (Figure 6A and 6B). The ICG also colocalized with a subset of RAM 11-positive plaque macrophages (Figure 6A and 6B), particularly lipid-associated foam cells, as evidenced by ORO staining. Endothelial cells stained with CD31 antibody were present at the luminal surface of the intimal layer, but were not so strongly colocalized with the ICG fluorescence signals (Figure 6A and 6B, black arrowheads).

**Figure 3.** Ex vivo fluorescence reflectance images (FRIs) of rabbit arteries and corresponding white light images. A, Artery from atherosclerotic rabbits injected with indocyanine green (ICG). B, Artery from atherosclerotic rabbits injected with saline. C, Normal rabbit injected with ICG. D, Ex vivo peak plaque target-to-background ratios (TBRs) in ICG- and saline-injected rabbits of aorta and iliac artery. E, Linear regression and Spearman rank correlation of the ex vivo and in vivo peak pTBRs. FRIs were equally processed and windowed. Scale bars, 10 mm. Em indicates emission; Ex, excitation; and pTBRs, peak target-to-background ratios.
ICG Generates NIRF Signals in Nonlipid Inflamed Lesion

To determine whether ICG could provide applicable signal for in vivo detection of nonlipid inflamed lesion, only balloon-denudated rabbits (n=3) with normal diet underwent in vivo OCT/NIRF imaging and ex vivo FRI and immunostaining analysis (Figure 7). Ex vivo FRI demonstrated focal NIRF signals within balloon-injured aortic lesion (Figure 7A), which colocalized well with NIRF signals of in vivo OCT/NIRF longitudinal image (Figure 7B). Intriguingly, only balloon-denudated

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**Figure 4.** Comparison of ex vivo fluorescence reflectance imaging (FRI) and in vivo near-infrared fluorescence (NIRF). Left, The entire ex vivo FRI image from a resected atherosclerotic rabbit artery. A, High-magnification FRI image from the atherosclerotic aorta and the corresponding 2-dimensional in vivo NIRF signals. B, High-magnification FRI image from the atherosclerotic iliac artery and the corresponding 2-dimensional in vivo NIRF map. Scale bars, 10 mm. Em indicates emission; and Ex, excitation. Equally windowed.

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**Figure 5.** Ex vivo fluorescence reflectance imaging (FRI) vs in vivo near-infrared fluorescence (NIRF) mapping signal colocalization. A, Comparison of ex vivo FRI image and in vivo 2-dimensional NIRF map from the single atherosclerotic rabbit aorta. B, Line-by-line comparison of longitudinal intensity profiles of average ex vivo FRI and in vivo NIRF signals. C, Correlation between average ex vivo FRI and in vivo NIRF signals. Ex vivo and in vivo NIRF signals were accurately colocalized along the longitudinal direction (R²=0.89; P<0.01). Scale bars, 5 mm. Equally windowed.
nonlipid inflamed lesions showed much weaker peak target-to-background ratios compared with those of the lipid-rich inflamed rabbits (3.49±0.09 versus 8.38±0.94; \( P < 0.01 \); Figure 7C). Also, there was a significant difference in peak target-to-background ratios between nonlipid inflamed lesions and saline-injected controls (3.49±0.09 versus 2.16±0.17; \( P < 0.01 \); Figure 7C). The corresponding cross-sectional OCT/NIRF image demonstrated focal weak NIRF signals existing in fibrotic mild plaque (Figure 7D). Fluorescence microscopy and immunohistological analysis from the sister sections revealed that ICG fluorescence colocalized with RAM-11–positive macrophages even in ORO-negative nonlipid plaque, which represents that ICG is able to demonstrate the signals of plaque macrophages per se without respect to lipid accumulation (Figure 7E). On the contrary, we noted that there were no ICG signals in nonlipid, noninflamed plaque (Figure 7F).

**In Vitro ICG Binding in Murine Macrophages**

We incubated murine macrophage cells, RAW 264.7, with different ICG concentrations (0–50 \( \mu \)mol/L) to determine the binding affinity of ICG to macrophages. Confocal laser scanning microscope of macrophages revealed strong NIRF signals in a concentration-dependent manner as compared with control which shows only background signals (Figure 8).

**Discussion**

In the present study, we demonstrated the feasibility of in vivo imaging of lipid-rich inflamed plaque via a fully integrated high-speed OCT/NIRF structural/molecular imaging combined with an FDA-approved NIRF signal-enhancing ICG agent. These findings extend a previous OCT/NIRF integration technical feasibility demonstrating the ability of simultaneous imaging of plaque morphology and nanoagent enhancing protease activity in rabbit vessels, whose size is similar to that of the human coronary artery. The novel imaging strategy described in current study is highly translatable and therefore can be expected to offer a new avenue for accurate estimation of the rupture-prone coronary plaques that are responsible for acute coronary syndrome.

Conventional vascular imaging for patients with atherosclerosis has focused on anatomic information such as severity of plaque burden or degree of luminal stenosis. However, acute coronary syndrome results from thrombotic complications that are often associated with noncritical stenosis. Positive remodeling through compensatory enlargement permits the development of a considerable plaque burden without flow limitation. Indeed, disruption of an atherosclerotic plaque, rather than a pre-existing high-degree stenosis, is thought to be the cause of most fatal coronary events. Although OCT is a recently developed imaging modality that provides outstanding resolution in comparison with intravascular ultrasound and can more clearly identify morphological features of rupture-prone plaque such as thin cap fibroatheroma, the Providing Regional Observations to Study Predictors of Events in the Coronary Tree (PROSPECT) trial suggested that morphological imaging was not sufficient to predict subsequent coronary plaque ruptures. Lately, there are accumulating evidences supporting inflammation as a major biological process responsible for fibrous cap weakening through production of collagen-destroying enzymes such as matrix metalloproteinases and cysteine protease. Accordingly, unique features of high-risk plaques such as the presence of abundant macrophages in a thin fibrous cap and a large lipid core should be key targets of high-risk plaque imaging.
Molecular imaging using 700 to 900 nm NIRF has emerged as a sensitive high-resolution approach for in vivo imaging of atherosclerotic lesions without ionizing radiation, and a recently developed integrated intravascular dual-modality OCT/NIRF imaging system provided precise colocalization of microstructural and molecular pathway information including thrombus and cathepsin B. The catheter imaging system had been constructed on a clinical OCT catheter platform, but there were many limitations to clinical application, including low pullback speed (2.5 mm/s), which would inevitably require proximal arterial occlusion to eliminate blood within the target segment and significant cross-talk between 2 fiber...
and lipid-rich plaque areas. Moreover, consistent with an earlier NIRF imaging colocalized well with both abundant macrophages and membrane stained with CellMask orange plasma (pseudocolor, green; excitation, 561 nm; emission, 563–630 nm). ICG signals were evident on the near-infrared fluorescence channel (red; excitation, 633 nm; emission, 650–800 nm). Scale bars, 20 μm. DAPI indicates 4′-6-diamidino-2-phenylindole.

Another important challenge to be addressed before clinical application of OCT/NIRF imaging is the identification of NIRF signal-enhancing agents that prove safety for humans. Although the previous OCT/NIRF feasibility study demonstrated the capability of the dual-modal catheter system to simultaneously image plaque morphology and protease activity, an activatable NIRF signal amplifying nanoagent should be required to enhance NIRF signals in atheromata. Although prosence VM110, a cathepsin B–activatable NIRF signal producing nanoagent, used in that study, the unique FRJ design of OCT/NIRF platform in current study minimized the cross-talk in double-clad fiber to a negligible level.

The advanced dual-modal OCT/NIRF catheter platform described herein was able, instead, to offer a rapid pullback speed (≤20 mm/s), which could permit nonocclusive imaging under contrast flushing within just a few seconds. In addition, as compared with the earlier system, the unique FRJ design of OCT/NIRF platform in current study minimized the cross-talk in double-clad fiber to a negligible level.

Although ICG-based OCT/NIRF intravascular molecular imaging is a clinically feasible approach for detection of inflamed lipid-rich atherosclerotic plaques, there are several limitations. First, the mechanistic details regulating ICG accumulation in plaques, such as factors determining its uptake by macrophages and binding to lipid, still require further exploration. Second, our dual-modal imaging system is based on an optical imaging, which has low depth penetration. Thus, designed catheter does not allow accurate detection of the plaque burden and positive remodeling. Third, the ICG fluorescence signal in atheromas was more prominent near the luminal area, which suggests that ICG is localized in the intimal areas rather than deep tissue portions. The uneven distribution of ICG within the plaque may be caused by several factors including (1) lower vascular permeability of ICG and preferential ICG binding to lipoproteins; (2) larger molecular size of ICG compared with other plasma proteins such as albumin; and (3) the short half-life, 2 to 4 minutes, of ICG. We euthanized the rabbits 90 minutes after in vivo OCT/NIRF imaging, and the ICG signals may have been diminished from the plaque areas during the preparation. To answer these remaining issues and establish the optimal dose, administration route, and imaging time after ICG injection, more research should be required. In addition, this imaging strategy needs to be further validated in beating coronary atheroma such as in a porcine plaque model, which phenotype closely resembles clinical thin cap fibroatheroma.

Conclusions

Although ICG-based OCT/NIRF intravascular molecular imaging is a clinically feasible approach for detection of inflamed lipid-rich atherosclerotic plaques, there are several limitations. First, the mechanistic details regulating ICG accumulation in plaques, such as factors determining its uptake by macrophages and binding to lipid, still require further exploration. Second, our dual-modal imaging system is based on an optical imaging, which has low depth penetration. Thus, designed catheter does not allow accurate detection of the plaque burden and positive remodeling. Third, the ICG fluorescence signal in atheromas was more prominent near the luminal area, which suggests that ICG is localized in the intimal areas rather than deep tissue portions. The uneven distribution of ICG within the plaque may be caused by several factors including (1) lower vascular permeability of ICG and preferential ICG binding to lipoproteins; (2) larger molecular size of ICG compared with other plasma proteins such as albumin; and (3) the short half-life, 2 to 4 minutes, of ICG. We euthanized the rabbits 90 minutes after in vivo OCT/NIRF imaging, and the ICG signals may have been diminished from the plaque areas during the preparation. To answer these remaining issues and establish the optimal dose, administration route, and imaging time after ICG injection, more research should be required. In addition, this imaging strategy needs to be further validated in beating coronary atheroma such as in a porcine plaque model, which phenotype closely resembles clinical thin cap fibroatheroma.

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Disclosures
None.

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