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Localization and Quantification of Platelet-Rich Thrombi in Large Blood Vessels With Near-Infrared Fluorescence Imaging

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Background—Imaging of thrombus formation in vivo has been limited by the inability to directly visualize and measure thrombi in large blood vessels in real time. Near-infrared light, with its superior tissue penetration and reduced scatter, could potentially solve this problem.

Methods and Results—Platelets were labeled with the near-infrared fluorophore IR-786. Optimal total fluorescence yield occurred at 6 attomoles of IR-786 per platelet. IR-786–labeled platelets were tested for their ability to detect thrombus formation in large animal model systems relevant to common human vascular procedures. Invisible near-infrared light did not distort the surgical field in any way, and after optimization of per-platelet fluorescence yield, platelets remained fully functional. Intravenous infusion of just $3.6 \times 10^{10}$ labeled platelets into a 35-kg Yorkshire pig permitted thrombus visualization, with a signal-to-background ratio $\geq 2$, for at least 2 hours in coronary, carotid, and femoral vessels. Platelet-rich, actively growing clots were monitored in real time after injury to vessels, cutaneous incisions, intravascular stent insertion, or introduction of embolic coils. Similarly, formed clots were monitored in real time during thrombolysis with streptokinase and heparin. Vessel patency was assessed independently with a second near-infrared fluorescent blood pool agent.

Conclusions—IR-786–labeled platelets provide sensitive, specific, and real-time visualization of thrombi in thick-walled blood vessels. In addition to immediate application in cardiac, transplant, and vascular surgery, the mechanisms that underlie thrombus formation in large blood vessels can now be investigated. (Circulation. 2007;115:84-93.)

Key Words: imaging • platelets • thrombosis • fluorescence

Among the emerging imaging techniques in cardiovascular medicine is the use of near-infrared (NIR) light (700 to 900 nm) to monitor the circulatory system.1–5 The fundamental advantage of imaging in the NIR range is that photon penetration into living tissue is higher because of lower photon absorption and scatter.6 An additional advantage is that tissue emits limited intrinsic fluorescence (ie, autofluorescence) in the 700- to 900-nm range. Therefore, fluorescence contrast agents that emit in the NIR range demonstrate a favorable signal-to-background (SBR) ratio when used in animal models or for patient care.

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Real-time in vivo visualization of thrombus formation is an important goal in both research and clinical settings but is problematic because the vasculature is a closed system. In small animal models, thrombi have been visualized with probes such as fluorophore-conjugated antibodies or platelets labeled with fluorescent dyes.7–10 The fluorophores used in these approaches emit light in the visible spectrum. Because of high absorption and scatter, visible wavelengths cannot penetrate large vessels and, thus, this approach has been largely limited to small vessels such as those within the mesenteric and cremaster microvasculatures of rodents.7–10 Dyes that emit NIR light have been used to assess perfusion of cardiac vessels intraoperatively.2,4,11–13 Although these dyes are useful for detecting stenosis and obstruction, they have only modest sensitivity for thrombus detection and cannot distinguish evolving clots from stabilized clots. New approaches that can detect thrombus formation in vessels that approximate the size of human coronary, carotid, and femoral vessels are required to study thrombus formation in large animal models and to detect thrombi during vascular procedures.

We hypothesized that platelets loaded with a dye that emits NIR light could be used to image thrombus formation after injury of large muscular vessels. To evaluate this possibility, we labeled platelets with IR-786, a lipophilic, cationic,

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heptamethine indocyanine-type NIR fluorophore. We now show that IR-786–labeled platelets used in conjunction with an integrated NIR fluorescence imaging system are able to quantitatively monitor thrombus formation and dissolution in real time. This method enables visualization and measurement of thrombus formation in major vessels of large animals. Use of IR-786–labeled platelets in conjunction with an NIR fluorescence imaging system demonstrates several favorable features compared with current approaches of thrombus detection.

Methods

Preparation of IR-786–Labeled Washed Platelets

Washed human platelets were prepared by differential centrifugation of fresh blood obtained from aspirin-free donors as described previously.10,14 Washed pig platelets were isolated from fresh blood obtained from anesthetized Yorkshire pigs. Platelet-rich plasma was prepared by centrifugation at 200g for 20 minutes. Platelets were then isolated from platelet-rich plasma by centrifugation at 1400g for 10 minutes in the presence of 50 ng/mL prostaglandin E1 and 10% (v/v) acid citrate/dextrose, pH 4.6, and resuspended at a concentration of 4 × 10^7 cells/mL in Tyrode’s-HEPES buffer. The percolate salt of IR-786 (CAS #102185-03-5) was purchased from Sigma-Aldrich (St. Louis, Mo).

Quantification of IR-786 Uptake Into Platelets

Platelet counts were measured with the HEMAVET Multispecies Hematology Analyzer (Drew Scientific, Oxford, Conn). For platelet-loading experiments, 1-mL samples of washed platelets (~4 × 10^10 total) were incubated for 0, 15, 30, 60, 90, or 120 minutes at room temperature with gentle rocking in Tyrode’s-HEPES supplemented with 5, 2.5, 1.25, 0.625, or 0 μmol/L IR-786. For measurements, platelets were pelleted for 5 minutes at 2000 x g in the presence of prostaglandin E1. Pellets were lysed with 500 μL absolute methanol by repeated pipetting and sonication for 1 minute at a 50% duty cycle. Sample fluorescence was measured by comparison to IR-786 calibration standards in methanol (pellets) or Tyrode’s-HEPES (supernatant).

Spectral Measurements and NIR Fluorescence Microscopy

Absorbance and fluorescence measurements were performed as described previously.15 For measurement of relative fluorescence yield, IR-786 samples in Tyrode’s-HEPES, methanol, or concentrated in washed platelets were matched for absorbance (0.1 A units) and area under the fluorescence emission curve calculated after excitation with a 5-mW 655-nm laser diode. NIR fluorescence microscopy was performed as previously described.16

Platelet Aggregation Studies

Platelets resuspended at a density of 4 × 10^7 cells/mL in modified Tyrode’s-HEPES buffer were stimulated with agonists in siliconized glass tubes in an optical aggregrometer (Chronolog, Haverton, Pa). Assays were performed at 37°C and with constant stirring. Aggregation was monitored by measurement of optical density of the platelet suspension.17

Animal studies were performed in accordance with an approved institutional protocol. Yorkshire pigs (E.M. Parsons and Sons, Hadley, Mass) that weighed 35 kg were anesthetized with 4.4 mg/kg IM tiletamine/zolazepam (Telazol, Fort Dodge Labs, Fort Dodge, Iowa). Animals were intubated with a 7-mm cuffed endotracheal tube and anesthesia was maintained with oxygen and isoflurane 0.5 to 5.0% to effect. Animals were repositioned and draped in the usual sterile fashion, and the indicated vessels were exposed with standard surgical techniques.

Vessel Injury Models

Platelets (3.6 × 10^10 total) in Tyrode’s-HEPES were labeled with 2 μmol/L IR-786 for 30 minutes at room temperature and infused intravenously before induction of thrombi with FeCl3, embolic coil, intravascular stent, or cutaneous incision. For induction of thrombus formation with FeCl3, a 0.5 × 1-cm swatch of grade 413 Whatman filter paper was saturated with a 50% solution of FeCl3 (Sigma-Aldrich) and applied beneath the vessel so as not to impede visualization of IR-786–labeled platelet accumulation. For induction of thrombi with either embolic coils or stents, a 5F Pinnacle sheath introducer (Terumo Medical, Elkton, Md) was inserted into the vessel and used to deploy devices. Thrombus formation was then imaged continuously until fluorescence signal stabilized. Streptokinase was from Sigma-Aldrich and heparin was from American Pharmaceutical Partners (Schaumburg, Ill).

Intraoperative NIR Fluorescence Imaging System

The imaging system has been described in detail previously,18 with the following modifications. Three wavelength-isolated excitation sources were utilized: one that generates 400- to 680-nm “white” light (0.5 mW/cm²); a second that generates 680- to 700-nm low-NIR fluorescence excitation light (1 mW/cm²) with model number L-660-66-60-550 high-power light-emitting diodes (Marubeni Epitex, New York, NY) and custom excitation filters; and a third that generates 725- to 775-nm NIR fluorescence-excitation light (5 mW/cm²). All sources operated over a 15-cm diameter field of view. Photon collection is achieved with custom-designed optics that maintain the separation of the white light and NIR fluorescence emission (ie, 700 to 725 nm versus >795 nm) channels. With custom LabVIEW software (National Instruments, Austin, Tex), anatomic (white light) and functional (NIR fluorescence light) images can be displayed separately and merged. To create a single image that displays both anatomy (color video) and function (NIR fluorescence), the NIR fluorescence image was pseudocolored (eg, in lime green) and overlaid with 100% transparency on top of the color video image of the same surgical field. All images are refreshed up to 15 times per second. The entire apparatus is suspended on an articulated arm over the surgical field, which thus permits unobtrusive imaging.

Vessel patency was assessed by intravenous injection of 1 mL of 1% (10 mg total) methylene blue (Mayne Pharma, Paramus, NJ) with continuous imaging of NIR fluorescence (700 to 725 nm) emission (E.T., unpublished data, 2006).

Quantification of In Vivo Thrombi

NIR fluorescence excitation fluence rate and field of view were held constant for all quantitative comparisons. Regions of interest of a defined shape and pixel number could be moved anywhere within the field of view to quantify NIR fluorescence emission signal intensity. SBR was assessed by quantification of fluorescence signal from a region of interest that encompasses the thrombus compared with an intravascular region of interest of the same size proximal to the thrombus.

Statistics

A Wilcoxon rank-sum test was used for statistical comparison of SBR of thrombi. The number of thrombi used for each comparison is indicated in the text. Two femoral thrombi (right and left) were formed in each animal.

The authors had full access to the data and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

Incorporation of IR-786 Into Human Platelets

To develop a contrast agent with the capability to detect thrombi in major vessels of large animals, we labeled platelets with IR-786. IR-786 is a highly hydrophobic nonsulfonated heptamethine that emits NIR light (Figure 1A). Initial ex vivo studies were designed to determine the optimal fluores-
cence yield after platelet loading. Time course studies demonstrated maximal incorporation of IR-786 into platelets after 30 minutes of incubation (Figure 1B). A dose-response curve showed that platelet loading occurred in a linear manner until 2.5 μmol/L (Figure 1C). At concentrations >2.5 μmol/L, the linearity of dose-dependency was lost, which indicates self-quenching and/or dye aggregation. All subsequent experiments were therefore performed with 2 μmol/L IR-786. At
this concentration, platelets incorporated ≈6 attomoles (≈3 600 000 molecules) of IR-786 per platelet.

Spectral analysis demonstrated that platelet incorporation of IR-786 resulted in a characteristic red shift. The excitation and emission maxima of IR-786 in aqueous buffer were 769.3 and 788.8 nm, respectively (Table 1). In contrast, its excitation and emission maxima after incorporation into platelets were 788 and 804 nm, even redder than emission found in neat methanol (Table 1).

We next characterized the subcellular location of IR-786 in labeled platelets. Although there was a small degree of homogeneous staining of lamellipodia and pseudopodia consistent with plasma membrane staining, the majority of fluorescence was punctate and localized in the central granulomere (Figure 1D). This pattern of fluorescence suggests incorporation of dye into intracellular structures and is consistent with staining patterns observed in other cell types.16

To ensure that platelets labeled with 2 μmol/L IR-786 retained function, we assessed the ability of IR-786–labeled platelets to aggregate in response to agonists. IR-786–labeled platelets demonstrated normal aggregation in response to either thrombin or collagen-related protein (Figure 1E). Evaluation of resting platelets labeled with 2 μmol/L IR-786 showed no significant P-selectin surface expression, which demonstrates that incubation with IR-786 does not activate platelets (data not shown). These data indicate that platelets remain functionally intact after labeling.

### Clearance of IR-786–Labeled Platelets In Vivo

A porcine model was used to test the clearance of platelets labeled with IR-786. Washed pig platelets (3.6×10¹⁰) were labeled with 2 μmol/L IR-786 for 30 minutes. IR-786–labeled platelets were then infused through a cannula in the internal jugular vein of the pig and the line was extensively flushed with saline. Blood samples were obtained at the indicated times after infusion, and platelet-rich plasma was analyzed (Figure 2A).

Evaluation of platelet-associated fluorescence demonstrated a rapid increase in fluorescence after infusion of the IR-786–labeled platelets. After this increase, there was a period of sharp decline in fluorescence until ≈20 minutes. A slow decline in fluorescence then followed. Clearance of IR-786–labeled platelets was also analyzed by manually counting labeled and unlabeled platelets in platelet-rich plasma. Microscopic analysis

<table>
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<tr>
<th>Buffer/Cell</th>
<th>Peak Absorbance, nm</th>
<th>Peak Emission, nm</th>
<th>Relative Fluorescence Yield</th>
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<td>Tyrode’s-HEPES</td>
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<td>789</td>
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<tr>
<td>Methanol</td>
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<td>797</td>
<td>9.5</td>
</tr>
<tr>
<td>Platelets</td>
<td>788</td>
<td>804</td>
<td>1.0</td>
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</tbody>
</table>
showed that 2.0±0.4% of platelets were labeled at 15 minutes after infusion and that 2.6±0.6% (n=3, P=0.4) were labeled at 150 minutes after infusion. Based on these data, we conclude that a majority of IR-786–labeled platelets remain in circulation 150 minutes after infusion into pigs. The decline in fluorescence observed in Figure 2A may not be primarily the result of platelet clearance. We suspect that free IR-786 is rapidly cleared by the liver after infusion\(^\text{16}\) and that the fluorescent signal in the platelets slowly declines over time as demonstrated in Figure 1B. Overall, these data indicate that the majority of IR-786–labeled pig platelets are not rapidly cleared from the circulation.

We also tested the ability of pig platelets to aggregate after incubation with IR-786. Washed pig platelets incubated for 30 minutes with 2 \(\mu\)mol/L IR-786 aggregated normally in response to thrombin or collagen-related protein (Figure 2B). Evaluation of aggregometry of pig platelets obtained 2 hours after infusion of IR-786–labeled platelets demonstrated that labeling with 2 \(\mu\)mol/L IR-786 did not affect platelet aggregation. Furthermore, NIR microscopy of platelets after aggregation studies demonstrated that IR-786–labeled platelets incorporate into aggregates (data not shown). Evaluation of erythrocyte and platelet counts after infusion of IR-786–labeled platelets demonstrated that infusion of this contrast media had no significant effects on circulating numbers of these blood cells (data not shown).

Figure 3. Real-time detection and quantification of thrombus formation after FeCl\(_3\)-induced injury. A, One hour after treatment of the femoral artery with FeCl\(_3\). Shown are color video (left), NIR fluorescence (middle; 67 ms exposure time), and a pseudocolored (lime green) merge of the color video and NIR image (right). Arrow indicates location of intravascular thrombus. A representative image from 8 independent femoral artery thrombi is shown. B, One hour after treatment of the femoral vein with FeCl\(_3\), with imaging as described in 3A. C, Hemotoxylin and eosin (H&E) histology and NIR fluorescence from the same tissue section of a FeCl\(_3\)-induced thrombus in the femoral artery. Note characteristic changes to the vessel wall exposed to FeCl\(_3\). A representative image from 8 independent femoral artery thrombi is shown. D, IR-786–labeled platelets were infused 30 minutes after application of FeCl\(_3\) to femoral arteries. The image shows H&E histology and NIR fluorescence from a longitudinal section of an injured femoral artery. The image represents 1 of 4 independent femoral artery thrombi that were evaluated under these conditions.
IR-786–Labeled Platelets Detect ThrombusFormation In Vivo

We next determined whether we could detect thrombi in live pigs and in real time with IR-786–labeled platelets. Thrombus formation after oxidant injury induced by exposure of vessels to filter paper saturated with FeCl₃ is a widely used and reliable method for in vivo induction of thrombus formation.⁷,¹⁹,²⁰ We hypothesized that the high fluorescence yield of IR-786 in platelets and the enhanced tissue penetration of NIR light would enable us to visualize thrombi in large, thick-walled vessels. FeCl₃-induced injuries to the femoral arteries were studied first. Imaging demonstrated the accumulation of platelets at the site of injury as represented by increased fluorescence signal (Figure 3A). Development of platelet-rich thrombi could also be visualized after FeCl₃-induced injury of the femoral vein (Figure 3B). Quantification of images demonstrated that platelet accumulation began 25 to 35 minutes after application of FeCl₃ (see Applications of IR-786–Labeled Platelets). This delay may represent the time required for diffusion of the FeCl₃ through the vessel wall or the time required for oxidative denudation of the endothelium. Thrombus formation began after this delay and continued to increase over the 150-minute experiment. These studies demonstrated that IR-786–labeled platelets accumulate at sites of thrombus formation, which thereby provides precise localization of thrombi within large, thick-walled vasculature.

Hematoxylin and eosin staining of these vessels demonstrated large thrombi oriented toward the portion of the vessel exposed to FeCl₃ (Figure 3C). NIR microscopy showed that IR-786–labeled platelets are diffusely incorporated throughout the body of the thrombus (Figure 3C). There is no evidence for incorporation of IR-786 into the underlying vasculature, which indicates that IR-786 remains platelet-associated.

To determine whether IR-786–labeled platelets can accumulate at sites of preexisting thrombi, IR-786–labeled platelets were infused 30 minutes after exposure of femoral arteries to FeCl₃. Under these conditions, fluorescence accumulated at the injury site within minutes of infusion of IR-786–labeled platelets. Histology of the injured artery demonstrated a component of the thrombus devoid of fluo-
rescence directly apposed to the inferior surface of the vessel, adjacent to the site of FeCl$_3$ application. Only the more luminal component of the thrombus contained IR-786–labeled platelets (Figure 3D). These results indicate that IR-786–labeled platelets adhered to and accumulated at the site of a preexisting thrombus.

Applications of IR-786–Labeled Platelets

Although the FeCl$_3$-induced injury is widely used to model thrombus formation in vivo, we sought to determine whether IR-786–labeled platelets could detect thrombi formed under circumstances encountered during surgical or vascular procedures. We observed that, after cutaneous incision and wound irrigation, a rim of thrombus formation could be visualized at the edge of wounds (Figure 4A). Occasionally, a thrombus would form at the site of electrocautery, as is shown in Figure 4A for the carotid artery. Reproducible thrombus formation occurred after insertion of intravascular devices into major vessels. Placement of an embolic coil into the iliac artery in an unheparinized animal resulted in rapid thrombus formation (Figure 4A). Similarly, a thrombus developed in the iliac artery after placement of a stent in an unheparinized animal (Figure 4A). The onset of thrombus formation after surgical manipulation of vessels or placement of intravascular devices was significantly more rapid than that after FeCl$_3$ exposure (Figure 4B). The average maximal SBR after FeCl$_3$ exposure was 4.4±1.7 (n=4) compared with an average maximal SBR of 3.3±1.9 (n=4) after embolic coil placement. These examples demonstrate that IR-786–labeled platelets constitute a versatile and quantitative contrast medium for detection of thrombi formed after a variety of vascular manipulations.

Monitoring Thrombolysis With IR-786–Labeled Platelets

We next asked whether IR-786–labeled platelets could be used to monitor the dynamics of thrombus growth and dissolution. As shown in Figure 5A, placement of an embolic coil in the femoral artery resulted in rapid formation of an intravascular thrombus, the extent of which could be quantified with NIR fluorescence. By 40 minutes, the thrombus had stabilized in size, at which point streptokinase and heparin were infused and dissolution of the thrombus was monitored in real time. Thrombolysis resulted in a 22.5% (P=0.0312, n=6) decrease in fluorescence signal. A second pattern of thrombus behavior after streptokinase and heparin infusion is shown in Figure 5B for a femoral artery treated with FeCl$_3$. In this case, thrombolytics triggered embolization of the thrombus, which then reformed slowly in the vessel. These data demonstrate that IR-786–labeled platelets can be used to monitor the efficacy of thrombolytic therapy in vivo and in real time.

Assessment of Vascular Patency During Thrombus Formation

An intravascular thrombus, even a large one, does not necessarily result in cessation of blood flow. One of the many advantages of NIR light is that the “NIR window” (wavelength range 700 to 900 nm) is 200 nm wide. This permits >1 NIR fluorophore to be used simultaneously. In recent work (E.T., unpublished data, 2006), we have characterized the NIR fluorescent properties of methylene blue, an agent already approved by the US Food and Drug Administration as a blue dye for surgery. Because methylene blue fluorescence peaks at ~700 nm, its fluorescence is well separated from that of IR-786. As shown in Figure 6, methylene blue can thus be used to assess vessel patency simultaneously with IR-786–labeled platelets used to monitor thrombus size and location. In the example shown, a thrombus is seen growing in the vessel until vascular occlusion occurs, at which point the vessel is supplied only by back-fill through a small collateral vessel.
The present study demonstrates that IR-786–labeled platelets are a sensitive reagent for the optical detection of thrombi in the major vessels of large animals. Previously described molecular probes designed to detect thrombi in vivo with NIR fluorescence have typically been engineered with a single fluorophore molecule per probe.22–24 The intracellular probe described in the present study concentrated to \(\sim 3 \times 10^6\) molecules of fluorophore per platelet, which corresponds to an intracellular concentration of \(\sim 700 \mu\text{mol/L}\). This enormous concentration of probe compensates for the fact that the fluorescence yield from IR-786 incorporated into platelets is significantly lower than that of free IR-786 in methanol or aqueous buffer (Table 1) as a result of quenching and internal absorption.16 In addition to producing a concentrated dye, the platelet is uniquely adapted as a probe to detect blood clots because of its ability to adhere to sites of vascular injury and incorporate into thrombi. This biological signal amplification enables thrombi to be imaged while circulating labeled platelets remain undetectable.

IR-786–labeled platelets demonstrate several benefits compared with previously described fluorescent contrast agents and probes used to detect thrombi in vivo. This reagent can detect thrombus formation in femoral, carotid, and coronary vessels of large animals. Thrombi formed in these thick-walled arteries and veins cause clinically important occlusion. There is considerable interest in both research and clinical settings to develop reagents capable of real-time imaging of thrombus formation in this aspect of the vasculature. Sensitivity is a second advantage of this reagent. Typical cardiac contrast agents produce only a negative image of the thrombus. IR-786–labeled platelets incorporate directly into thrombi, and thus produce a positive signal. These platelets appear to be a more sensitive indicator of thrombus formation than blood pool agents, as thrombi that fail to cause a filling defect are detected by IR-786–labeled platelets (Figure 6). In addition, IR-786–labeled platelets provide precise localization of the thrombus. Localization of thrombi with standard contrast agents can be confounded if, eg, an occlusion results in altered blood flow to tributaries and misrepresents the thrombus location (Figure 6). The fact that NIR fluorescent platelets actively incorporate into growing thrombi enables evaluation of the kinetics of thrombus growth. In this manner, an actively evolving thrombus can be distinguished from a stable thrombus because only the former will actively incorporate platelets. Because IR-786 remains associated with platelets and does not transfer to underlying structures (Figure 5), thrombolysis can also be followed with this reagent. These characteristics represent meaningful advantages over presently available probes and contrast agents used in cardiovascular imaging.

Some limitations of IR786–labeled platelets are evident from the present study. Although the tissue penetration of NIR light is considerably improved over that of visible light, NIR light emitted from within a vessel cannot penetrate the dermis. Thus, an invasive procedure, such as surgical exposure or angioscopy, is required to visualize IR-786–labeled platelets in vivo. IR-786–labeled platelets are not as sensitive as blood pool agents for evaluation of vessel patency. For this reason, we have developed a method that uses methylene blue...
in conjunction with IR-786–labeled platelets to monitor both thrombus formation (IR-786–labeled platelets emitting at 800 nm) and vessel patency (methylen blue as a blood pool agent emitting at 700 nm). This method provides direct visual assessment of the relationship between thrombus formation and vessel patency.

We envision several applications for IR-786–labeled platelets. Presently, there are no large animal models in which the kinetics of platelet accumulation into thrombi can be imaged in real time. This model will offer new opportunities for study of thrombus formation in vessels that approximate the size of human vessels affected in myocardial infarction, stroke, and peripheral vascular disease. The model will be useful for testing stents and other vascular devices in which thrombus formation is a known complication. In addition, testing antiplatelet drugs and other antithrombotic pharmaceuticals with real-time imaging of vessels with blood flow velocities and shear rates similar to those of critical human vessels will now be possible. In clinical medicine, IR-786–labeled platelets could enable improved visualization of thrombus formation during vascular surgery. For example, intraoperative graft occlusion occurs as a complication of cardiac bypass surgery during ~1.5 to 5.0% of procedures. Enhanced detection of intraoperative thrombi with IR-786–labeled platelets along with an integrated NIR fluorescence imaging system could provide useful information that can direct required interventions during surgeries.

Another potential application of IR-786–labeled platelets is in the characterization of atherosclerotic plaque in conjunction with NIR fluorescence angiography. There is increasing evidence that platelets function at multiple stages in atherogenesis. Platelets incorporate and deposit cytokines into early atherosclerotic lesions, adhere to eroded plaque, and initiate thrombus formation at sites of ruptured plaque. Techniques to distinguish vulnerable plaque from quiescent plaque with near-infrared angiography are currently being developed. Interaction of platelets with plaque may provide further prognostic information regarding plaque stability. Future studies will evaluate potential uses of IR786–labeled platelets to study the interaction of platelets with atherosclerotic plaque in animal models and in the clinical setting.

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Disclosures

All intellectual property for the intraoperative NIR fluorescence imaging system used in the present study is owned by the Beth Israel Deaconess Medical Center, although, as inventor, Dr Frangioni may someday receive royalties if the system is commercialized. Dr Frangioni also received a sponsored research grant from GE on the intraoperative imaging system, and has a collaborative research agreement with GE funding. The authors report no conflicts.

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**CLINICAL PERSPECTIVE**

The intact vasculature is opaque to the human eye. Hence, the formation of intravascular thrombi cannot currently be detected in real time, especially in the intraoperative setting, where occult thrombi can lead to significant patient morbidity. Invisible near-infrared (NIR) light, in the wavelength range of 700 to 900 nm, is capable of penetrating deeply into living tissue, and NIR fluorescence can provide highly sensitive detection of labeled targets. In the present report, we describe highly NIR fluorescent autologous platelets that retain full bioactivity. When injected intravenously at low doses, they circulate for hours, yet concentrate and become detectable in the setting of intravascular thrombi. When used in conjunction with an intraoperative NIR fluorescence imaging system, NIR fluorescent platelets permit sensitive detection of thrombi in muscular arteries and veins such as coronary, carotid, and femoral vessels. This technology has immediate application for detection of thrombus formation during common surgical procedures such as coronary artery bypass surgery, endarterectomy, and peripheral vascular surgery. NIR fluorescent platelets could also be used in conjunction with NIR fluorescence angioscopy for characterization of atherosclerotic plaque and may ultimately be used to distinguish quiescent from vulnerable plaque.