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RESEARCH LETTER

Fluorine-19 Magnetic Resonance Imaging of Activated Platelets

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The central role of platelets in thrombosis is well characterized. Recently, evidence has emerged that platelets also play crucial roles in inflammation and cancer. Therefore, reliable and sensitive imaging of activated platelets holds promise to improve diagnosis in numerous diseases and addresses a significant clinical need.[1] Here, we describe an innovative method for imaging activated platelets using advanced fluorine-19 (^{19}F) magnetic resonance imaging (MRI).

MRI provides high-resolution imaging, even in deep tissues, without harmful ionizing radiation. Visualization of thrombi, inflammation, early cancer, and metastases is difficult since these structures are often not well differentiated against surrounding tissues. MRI contrast based on gadolinium or iron oxide has been employed to improve tissue differentiation. However, these contrast agents are imaged using conventional hydrogen (^{1}H)-based MRI and are sometimes difficult to differentiate from anatomical structure. An alternative, ^{19}F-based MRI holds promise for greater specificity. ^{19}F is essentially absent from biological tissue, allowing for background-free localization. The merge of ^{1}H and ^{19}F data sets allows for exact anatomical localization and molecular characterization. Furthermore, the linear relationship between fluorine concentration and signal intensity permits direct quantification of contrast agent accumulation.

^{19}F MRI contrast agents are typically generated using perfluorocarbons, which are chemically and biologically inert, rendering them safe for in vivo use.[2] Perfluorocarbons are emulsified with phospholipids to form stable, biocompatible, and intravenously administrable nanoemulsions (PFCs). Non-conjugated PFCs are phagocytosed by monocytes/macrophages, a method utilized for imaging of inflammation in mouse models of myocardial and cerebral ischemia, myocarditis, and pneumonia.[1] Direct targeting of PFCs, independent of phagocytosis, using small antiplasmin peptide–conjugated PFCs were successfully employed to detect the early phase of venous thrombosis via ^{19}F MRI.[3]

To achieve selective targeting of activated platelets, we chose to target the activated conformation of integrin glycoprotein IIb/IIIa (GPIIb/IIIa; $\alpha_{IIb}\beta_3$, CD41/CD61). GPIIb/IIIa is highly specific for platelets and is the most abundantly expressed receptor on the platelet surface (≈60 000 receptors per platelet). Upon platelet activation, GPIIb/IIIa undergoes a conformational change with the selective exposure of specific epitopes.[4] We generated a unique human single-chain antibody (scFv), that binds specifically to the activated conformation of GPIIb/IIIa (scFv^{Targ}), and exhibits the same binding profile on human and mouse platelets, providing a distinct advantage for cross-species testing.[4,5] The scFv^{Targ} does not bind to nonactivated circulating platelets or to other proteins or cells in the blood.[4] This scFv^{Targ} has been successfully used for molecular imaging of diseases across a range of imaging technologies, including ultrasound, positron emission tomography,

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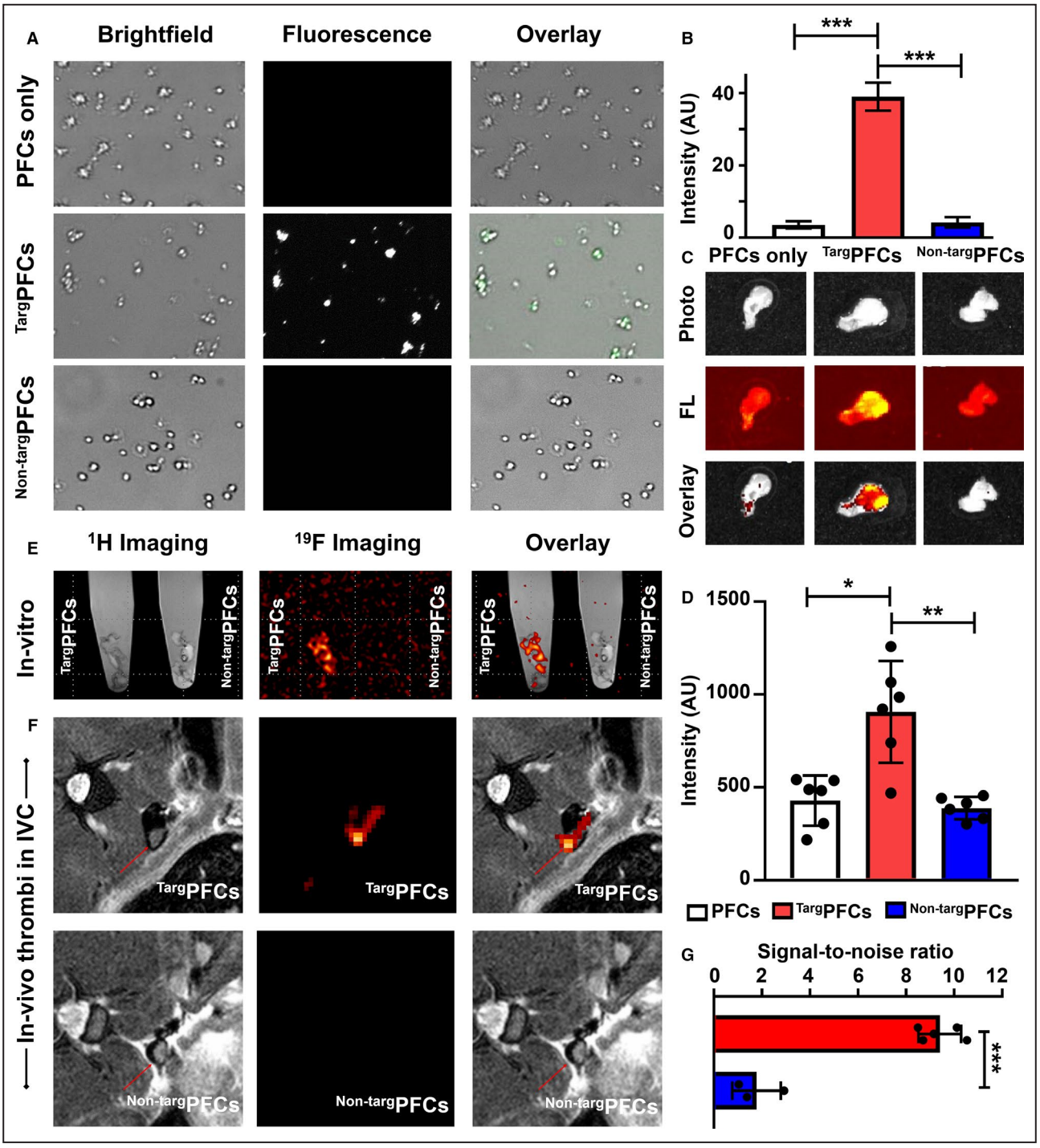

fluorescence emission computed tomography, and conventional MRI.[1,4,5] We have now established an antibody-targeted, ^{19}F-based MRI approach that enables selective imaging of activated platelets, with a large scope of potential applications, such as in the diagnosis of atherothrombotic, inflammatory, and malignant diseases.

For imaging of activated platelets by ^{19}F MRI, we generated dual-modality PFCs, which were functionalized with a biotin-lipid for conjugation with the scFv and a fluorescence dye. PFCs containing 0.01 mol% biotin-DHPE were coated with neutravidin and washed by centrifugation. Biotinylated scFv^{Targ} was then coupled to the neutravidin moiety of the perfluorocarbons (^{Targ}PFCs). As a control, we conjugated a non-targeted, nonbinding scFv$^{Non\text{-}targ}$ to the PFCs ($^{Non\text{-}targ}$PFCs). Targeting properties of these PFCs were confirmed using a flow-chamber adhesion assay using

Figure. Flourine-19 (^{19}F) magnetic resonance imaging (MRI) of thrombosis by targeting to the activated form of glycoprotein IIb/IIIa on platelets.

A, Representative microscopy images demonstrate the successful attachment of FITC-labeled targeted perfluorocarbon nanoemulsions (^{Targ}PFCs) to microthrombi in a flow-chamber adhesion assay, generated with human blood. **B**, Quantification of fluorescence intensity demonstrates a significant increase in intensity for ^{Targ}PFCs (red) binding to human microthrombi, as compared with non-targeted PFCs ($^{Non\text{-}targ}$PFCs; blue) and non-conjugated PFCs (white; PFCs only) (***P<0.001). **C**, In vitro formation of human thrombi demonstrates targeting of ^{Targ}PFCs using fluorescence imaging on in vivo imaging system (IVIS). **D**, Quantification of IVIS fluorescence intensity of rhodamine on perfluorocarbons (**P<0.01; *P<0.05). **E**, In vitro formation of thrombi demonstrates targeting of ^{Targ}PFCs using ^{19}F MRI. **F**, In vivo imaging of activated platelets using ^{Targ}PFCs in a mouse model of ferric chloride–induced thrombosis of the inferior vena cava (IVC); red arrows indicate the location of thrombi, with representative MRI (hydrogen [^{1}H] and ^{19}F) images of thrombi in the IVC using ^{Targ}PFCs (upper) or $^{Non\text{-}targ}$PFCs (lower). **G**, A significant increase in the ^{19}F signal was observed for ^{Targ}PFCs, as compared with $^{Non\text{-}targ}$PFCs, for signal-to-noise ratio (***P<0.001). Male C57Bl/6J mice (20–25 g), sourced from the ZETT (central animal facility) of the Heinrich Heine University (Düsseldorf, Germany), were randomized into different experimental groups and thrombosis was then induced surgically in the IVC. All procedures for animal studies were performed in accordance with institutional guidelines. PFCs injected intravenously 15 minutes post injury and ^{19}F MRI was performed immediately post administration. Assays with 2 groups were analyzed using unpaired *t* test, and all assays with ≥3 groups were analyzed with 1-way ANOVA followed by the Bonferroni post hoc test.

human blood[4,5] (Figure—Panels A and B). ^{Targ}PFCs displayed significantly increased binding to microthrombi compared with $^{Non\text{-}targ}$PFCs and unmodified PFCs (39.0 versus 4.2 versus 3.5 fluorescence intensity, respectively; n=15, P<0.05). Additionally, we incubated in vitro–generated human thrombi with ^{Targ}PFCs and $^{Non\text{-}targ}$PFCs, followed by imaging via an in vivo imaging system and ^{19}F MRI (Figure—Panels C through E). Thrombi exposed to ^{Targ}PFCs displayed a significantly increased fluorescence intensity, as well as ^{19}F signal (907 versus 389 versus 478 fluorescence intensity, respectively; n=6, P<0.05).

To explore the feasibility of imaging activated platelets in vivo, we used a murine model of ferric chloride–induced thrombosis of the inferior vena cava (Figure—Panels F and G). Male C57Bl/6J mice (20–25 g), sourced from the ZETT (central animal facility) of the Heinrich Heine University (Düsseldorf, Germany), were randomized into different experimental groups. All procedures for animal studies were performed in accordance with institutional guidelines. Perfluorocarbons (1 µg/g of body weight) were injected 15 minutes post injury and ^{19}F MRI was conducted immediately. Intravenous administration of ^{Targ}PFCs revealed a strong, background-free ^{19}F MRI signal at the thrombus, whereas injection of $^{Non\text{-}targ}$PFCs showed only a low background signal (9.4 versus 1.8 signal-to-noise ratio, P<0.001). Injection of ^{Targ}PFCs led to significantly increased ^{19}F MRI intensity compared with $^{Non\text{-}targ}$PFCs (8×10^5 versus 2.4×10^5 intensity, P<0.001).

Overall, our data demonstrate that ^{Targ}PFCs are an ideal contrast agent for background-free molecular ^{19}F MRI of activated platelets, in both humans and mice. ^{Targ}PFCs bind specifically to activated GPIIb/IIIa receptors in vitro and in vivo; therefore, they only accumulate at sites of platelet activation. Early diagnosis of diseases characterized by involvement of activated platelets, such as thrombotic, atherosclerotic, inflammatory, and malignant diseases, facilitates early therapeutic intervention and ultimately improves outcomes for patients. While this is a proof-of-concept study, the use of well-tolerated PFCs and low-antigenicity human scFvs, along with background-free ^{19}F MRI, strongly supports the translational prospect of early diagnosis of numerous diseases with a potentially broad health impact.

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Disclosures

Wang and Peter are inventors on patents describing activated platelet–targeting recombinant antibodies.

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