Contrast to Labeled Rehydrated, Lyophilized Platelets Using Magnetomotive OCT

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Abstract: Rehydrated, lyophilized platelets for hemostatic therapy are incorporated with commercial MRI iron oxide contrast agents. We demonstrate that magnetomotive OCT contrasts the platelets and propose this system for monitoring hemopathic sites targeted by platelets.

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1. Introduction

Rehydrated lyophilized (RL) human platelets (Stasix™, Entegrion, Inc.), are chemically stabilized infusion agents for treating platelet-responsive bleeding [1,2], and are currently in non-human primate preclinical trials. Stasix™ retain many normal platelet functions [3] and overcome logistical difficulties of platelet storage and harvesting [4]. In order to monitor their efficacy as a therapeutic, we have incorporated similar RL platelets with a dextran-coated superparamagnetic iron oxide (SPIO) imaging agent FDA-approved for liver contrast in MRI (Feridex™, Advanced Magnetics, Inc.) The resulting SPIO-RL platelets are highly responsive to magnetic field gradients, and, as we will show below, provide strong contrast using magnetomotive optical coherence tomography (MMOCT). This may enable monitoring of the adherence of SPIO-RL platelets to sites of bleeding and vascular damage, and may be particularly useful for assessing burn trauma.

MMOCT is an emerging technique for contrasting materials with a high magnetic susceptibility against a tissue background, by magnetically inducing nanoscale motions at a specific modulation frequency during acquisition of an OCT image [5,6]. The magnetic-specific image is obtained by bandpass filtering the phase of the interferometric OCT signal about this modulation frequency, while at the same time the traditional OCT image is obtained by lowpass filtering. In this way, a contrasted OCT image is obtained rapidly by acquiring only 2 frames of the object: one with the magnetic field modulated, and a control with the magnetic field off. In previous work a fiber-based spectral domain MMOCT was sensitive to SPIO concentrations as low as 27 μg/g [7]. Here we demonstrate a new, free-space spectral domain MMOCT system, and measure the dose-dependent contrast to SPIO-RL platelets in an agarose matrix.

2. Experimental Methods

SPIO-RL platelets were prepared by incubating platelet-rich plasma with SPIO (Feridex™) overnight. The Feridex™ nanoparticles internalized by platelets form clusters in the surface connected open canalicular system as observed in TEM (not shown). The amount of iron uptake was verified by three independent measurement techniques, resulting in measurements of 49 fg, 46 fg and 43 fg Fe per platelet, as measured respectively with mass spectrometry (Varian 820 MS), super-conducting quantum interference device (SQUID) magnetometry (5T MPMS, Quantum Design), and iron ferrocyanide colorimetric determination. This amount of uptake corresponds to nominally 0.19% volume loading of iron oxide in the platelet.

The spectral domain MMOCT system light source is a Ti:Sapphire laser (Griffin, KMLabs) producing 125nm of bandwidth centered near 810nm. The source light is coupled into a single-mode-fiber and directed into a free-space interferometer with a stationary reference and sample arm, with ~14mW of light power at the sample. Transverse scanning at the sample is accomplished with galvanometer-controlled mirrors. The output of the interferometer is again single-mode-fiber coupled and directed into a custom spectrometer with a Dalsa line camera operable up to 20kHz. The SNR of the system is nominally 90dB while imaging a mirror. A custom water-jacketed electromagnet supplies ~0.08T and 10T/m magnetic field and gradient at the sample, respectively.

The dose-dependent MMOCT signal was investigated by preparing 1% agarose samples gelled with premixed RL and SPIO-RL platelets, and 0.5 mg/mL TiO_2 micropowder added as an optical scatterer. The total platelet concentration was kept constant at $2.25\times10^6/\mu L$ in order to keep the overall sample elasticity constant, while the ratio of plain RL platelets to SPIO-RL platelets was varied. Each sample was imaged as follows: the magnetic field was modulated at 100Hz with a square-root sinusoid waveform and synchronized with the OCT axial scan at 1kHz. MMOCT images of 2500 × 1024 pixels were acquired over 2mm×1.5mm (transverse x × depth z). A total of 9 images stepped 250μm in the transverse y direction were obtained. The MMOCT images and the image-averaged MMOCT signals were computed as described previously [7].

4. Results and Discussion

The mean and standard deviation for each set of 9 images of each sample are displayed in Fig. 1. There is a small negative bias to the MMOCT signal that is obtained due to diamagnetic motion arising from the tissue background, which has a mechanical phase lag with respect to the driving magnetic field that is opposite that of the paramagnetic response of the SPIOs. This occurs because diamagnetic materials are forced away from the magnet, while paramagnetic materials are forced toward the magnet. The net balance between these opposing forces changes in favor of paramagnetic motion as the concentration of SPIO-RL platelets is increased to ~$1.5\times10^6/\mu L$ or above. Based upon the iron loading measurements this corresponds to ~70μg/g iron oxide, similar to the previously reported MMOCT sensitivity to pure SPIOs of 27 μg/g [7]. The previous result was based upon a different SPIO product (Ocean Nanotech, Inc.) that may account for this difference.

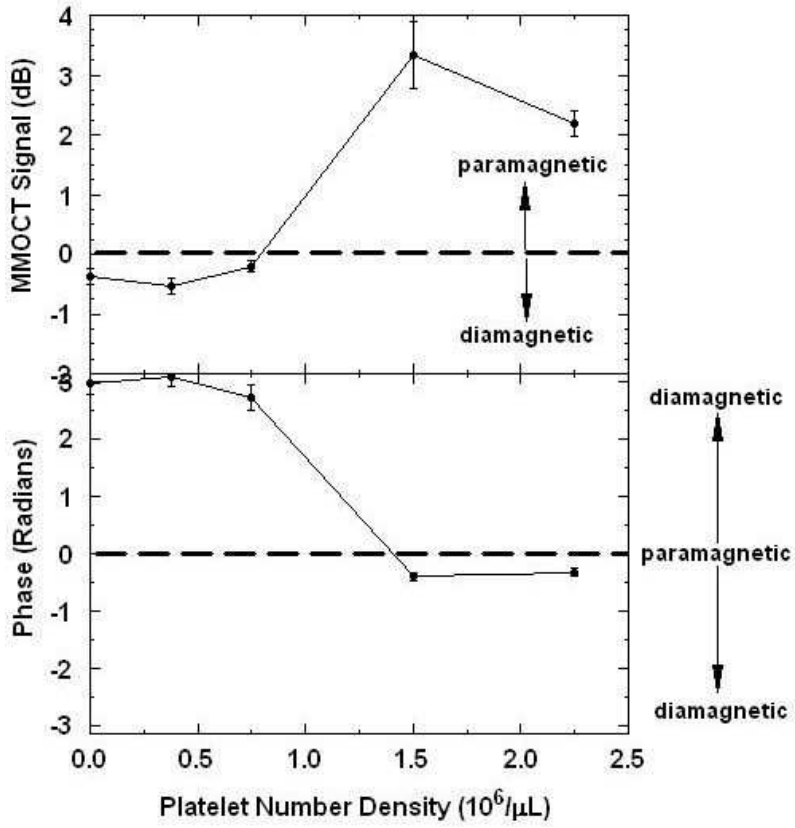

Fig. 1. Dose-dependent magnetomotive response of SPIO-RL platelets in agarose, showing a net paramagnetic response above $1.5\times10^6/\mu L$.

Representative MMOCT images are portrayed in Fig. 2. They demonstrate the high specificity of contrast to SPIO-RL platelets in comparison to control RL platelets, while the corresponding OCT images are nearly identical and non-specific.

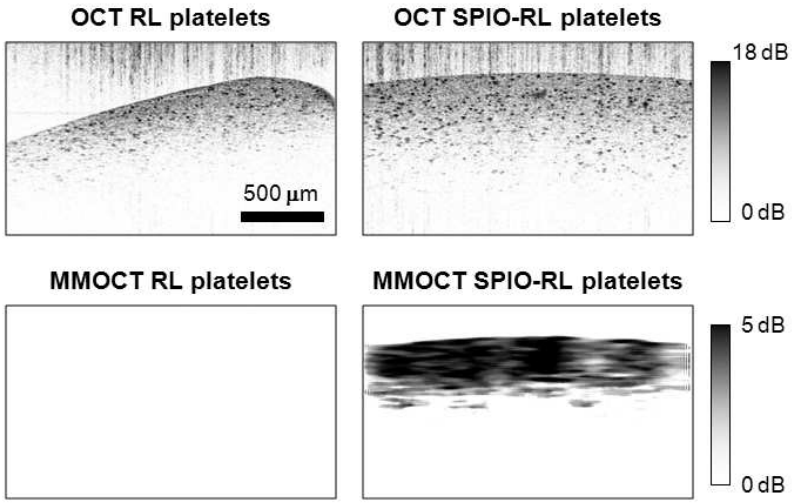

Fig. 2. Comparison between OCT and MMOCT images of SPIO-RL platelets and control RL platelets. The SPIO-RL platelets were at a concentration of $1.5\times10^6/\mu L$.

4. Conclusions and Future Outlook

The concentration threshold that provided sufficient contrast in MMOCT was nominally $1.5\times10^6/\mu L$. Many thrombi incorporate platelets at concentrations covering an area fraction of 35-70% [8] which would correspond to 20-60% by volume (assuming an isotropic distribution). Given an average platelet volume of 10 fL [9], this corresponds to a number density of $20\text{-}60\times10^6/\mu L$, more than an order of magnitude above our imaging contrast threshold. However, we do expect that SPIO-RL platelets, after infusion, will only represent a fraction of the total number of platelets in a thrombus or hemorrhagic site. Further investigation in animal models is needed to assess the potential of this technique for monitoring sites of vascular damage and thrombosis, which may lead to new ways of assessing vascular pathologies, detecting sites of hemorrhage or thrombosis, monitoring response to treatments for hemorrhage and thrombosis, and aid in the development of effective RL platelet therapeutics for providing hemostasis.

5. References

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