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T2 Magnetic Resonance: A Diagnostic Platform for Studying Integrated Hemostasis in Whole Blood—Proof of Concept

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BACKGROUND: Existing approaches for measuring hemostasis parameters require multiple platforms, can take hours to provide results, and generally require 1–25 mL of sample. We developed a diagnostic platform that allows comprehensive assessment of hemostatic parameters on a single instrument and provides results within 15 min using 0.04 mL of blood with minimal sample handling.

METHODS: T2 magnetic resonance (T2MR) was used to directly measure integrated reactions in whole blood samples by resolving multiple water relaxation times from distinct sample microenvironments. Clotting, clot contraction, and fibrinolysis stimulated by thrombin or tissue plasminogen activator, respectively, were measured. T2MR signals of clotting samples were compared with images produced by scanning electron microscopy and with standard reference methods for the following parameters: hematocrit, prothrombin time, clot strength, and platelet activity.

RESULTS: Application of T2MR methodology revealed conditions under which a unique T2MR signature appeared that corresponded with the formation of polyhedral erythrocytes, the dynamics and morphology of which are dependent on thrombin, fibrinogen, hematocrit, and platelet levels. We also showed that the T2MR platform can be used for precise and accurate measurements of hematocrit (%CV, 4.8%, $R^2 = 0.95$), clotting time (%CV, 3.5%, $R^2 = 0.94$), clot strength ($R^2 = 0.95$), and platelet function (93% agreement with light transmission aggregometry).

CONCLUSIONS: This proof-of-concept study demonstrates that T2MR has the potential to provide rapid and sensitive identification of patients at risk for thrombosis or bleeding and to identify new biomarkers and therapeutic targets with a single, simple-to-employ analytic approach that may be suitable for routine use in both research and diverse clinical settings.

Thrombosis and bleeding are among the foremost causes of morbidity and mortality, and the introduction of novel anticoagulants and antithrombotic and hemostatic drugs has increased the need for rapid and accurate assessment of their activities *(1, 2)*. However, laboratory assessment of hemostasis remains difficult for some common clinical situations. Contemporary clinical laboratory methods are based on measuring components of hemostasis (e.g., prothrombin time, activated partial thromboplastin time, platelet aggregometry) or global function as reflected in mechanical clot strength (thromboelastography, thromboelastometry) *(3–5)*. These methods successfully identify many, but not all, bleeding disorders. Additionally, existing methods provide little insight into the risk of thrombosis, lack sensitivity toward measuring fibrinolytic activity, and require complex mechanical instrumentation. Their use often requires specialized technical expertise not available in most hospital laboratories, blood draws of 1–25 mL, and the fact that sample processing and measurement can require 30–150 min *(3, 6, 7)*.

Clinical hemostasis involves the controlled rapid transformation of blood flowing under pressure to a highly localized, largely impermeable seal formed at sites of vascular damage followed by containment and then dissolution of the formed clot. These ordered sequential changes in clot structure are required to pre-

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vent untoward bleeding in vivo while limiting the risk of thrombotic vascular occlusion. We postulated that the transverse relaxation time of the nuclear magnetic resonance signal of water, referred to here as T2 magnetic resonance (T2MR),[8] could be used to probe microenvironments of water molecules in blood ex vivo, formed during hemostatic processes, without the need for reagents. Our results show that T2MR allows the physical states of blood to be monitored by continuously measuring the spin–spin (T2) relaxation times of water in a whole blood sample. Water is a sensitive and general magnetic resonance probe of the diverse and distinct microenvironments that develop during clot formation and the structural rearrangements that follow. For example, addition of an activator such as thrombin to whole blood initiates platelet aggregation and fibrin polymerization, generating a clot that subsequently undergoes platelet-mediated contraction *(8, 9)*. Contraction of the fibrin clot impacts microenvironments of water around the various components within the blood sample, including soluble proteins, erythrocytes, and the fibrin network itself, leading to the formation of multiple water compartments. These compartments and their formation over time can be discerned by applying an algorithm to resolve multiple time constants from a single T2MR relaxation curve. The sensitivity of the T2MR diagnostic platform to the hemostatic potential of blood arises from measuring these heterogeneities in the microenvironments of multiple water compartments that develop during clotting, clot contraction, and lysis.

Here we describe how T2MR reports on the integrated contributions to hemostasis of plasma, platelets, and other blood cells. This mix-and-read platform requires minimal sample volumes (0.04 mL) compared with conventional methods and enables the measurement of both established and newly described hemostatic parameters on a single, simple-to-use instrument using water to probe the hemodynamic behavior of blood. This methodology can be used to measure both individual hemostatic parameters and integrated hemostasis. Potential advantages over existing methods for measuring standard parameters include ease of performance by eliminating sample modification before analysis, data output in as short as 15 min with the option to monitor samples for hours, and volume requirements that are 10–100 times less than those for existing methodologies.

[8] Nonstandard abbreviations: T2MR, T2 magnetic resonance; PPP, platelet-poor plasma; tPA, tissue-type plasminogen activator; CPMG, Carr–Purcell–Meiboom–Gill.

Materials and Methods

BLOOD SAMPLE COLLECTION AND FRACTIONATION

Blood was obtained from healthy volunteers not taking aspirin, nonsteroidal antiinflammatory drugs, or other medications known to inhibit platelet function for least 7–10 days, with informed consent and approval by the Perelman School of Medicine–University of Pennsylvania Institutional Review Board. Blood was drawn via venipuncture into 3.2% trisodium citrate (9:1) following standard procedures that minimized platelet activation. Samples were kept at room temperature and were studied within 4 h after the blood draw. A complete blood count was performed on an automated hematology analyzer (HemaVet 950FS, Drew Scientific).

For experiments requiring fractionation and reconstitution of samples, 12 mL of blood was placed in 15-mL polypropylene tubes (Corning) and centrifuged for 15 min at 210*g* at ambient temperature (22 °C). Platelet-rich plasma was recovered from the upper layer in the tube following centrifugation and transferred to a new tube. The residual blood preparation was centrifuged again at 900*g* for 10 min at ambient temperature. The platelet-poor plasma (PPP) fraction was collected from the top layer and transferred to a new tube. Any remaining volume of PPP along with the buffy coat layer was removed, and the upper 1 mL of packed erythrocytes was aspirated and transferred to a new tube. To obtain concentrated platelets, platelet-rich plasma was centrifuged at 900*g* for 10 min at ambient temperature. To prevent platelet aggregation, prostaglandin E1 was added (final concentration, 5 μmol/L). The supernatant from this sample was aspirated and discarded, and the platelet pellet was resuspended in PPP not containing prostaglandin E1 to generate a concentrated platelet suspension. Reconstituted samples were prepared by mixing concentrated erythrocytes, concentrated platelets, and PPP at desired levels.

BLOOD CLOTTING, CONTRACTION, AND LYSIS WITH THROMBIN AND TISSUE PLASMINOGEN ACTIVATOR

Blood clotting was initiated by addition of 2 μL of a 0.2 mol/L $CaCl_2$ solution and 2 μL of thrombin (Sigma-Aldrich) (final concentration 0.1–3.0 U/mL) to 34 μL of blood in a 200-μL PCR tube (Eppendorf). All components were prewarmed for 1 min at 37 °C before mixing. Samples were mixed by 3 aspiration and dispersion cycles using a pipette, and then put into the T2MR reader for measurement. Typical run length was 30 min with a 10 s sampling rate. For some experiments, data collection time was extended to 1 h.

To establish T2MR signatures for fibrinolysis, tissue-type plasminogen activator (tPA) (Alteplase; Genentech) was added to samples clotted by thrombin.

Blood clotting was initiated as described above, and the sample was incubated for 60 min to allow for complete clot contraction. Then, 0.5–1 μmol/L tPA was added to clotted and contracted samples. Care was taken not to disturb clots, which were usually attached to the tube wall. The pipette tip was carefully placed into the visible serum layer on the tube side opposite to the contracted clot, and 3 μL of tPA solution was added with a single dispensing of the pipettor. The tPA solutions were made from a stock solution prepared according to manufacturer instructions using 0.15 mol/L sodium chloride, pH 7.4.

INSTRUMENT FABRICATION AND PULSE SEQUENCE PARAMETERS

A small, portable T2MR instrument (35 × 15 × 18 cm, 9 kg) was designed to measure the proton T2 relaxation times within blood samples *(10)*. The instrument consists of a 0.54 T (approximately 23 MHz) permanent magnet assembly, radiofrequency probe, single-board spectrometer, and peripheral electronics within a 37 °C temperature-controlled enclosure *(10)*. The radiofrequency probe accommodates 10–40 μL samples contained within a standard 0.2-mL polypropylene tube. A Carr–Purcell–Meiboom–Gill (CPMG) pulse sequence with an interecho spacing of 500 μs and repetition time of 2–10 s is applied to generate relaxation curves from which T2 values are extracted. This acquisition method removes the effects of static field inhomogeneities, enabling the use of a small, inexpensive magnet which is shimmed only once during manufacturing *(11)*.

For peak assignments, fibrinogen and platelet titrations, clotting reconstituted samples, scanning electron microscopy, clotting of mouse blood, method comparisons for hematocrit, prothrombin time, clot strength, and platelet activity, see the Supplemental Materials that accompany the online version of this article at http://www.clinchem.org/content/vol60/issue9.

Results

The relaxation mechanisms for magnetic resonance measurements of aqueous samples depend on chemical and diffusive exchange of water *(12–14)*. A single relaxation value is measured when exchange is rapid, but multiple relaxation values can be measured when there is a barrier to exchange between microscopic environments. Key to applying T2MR to monitor changes in the microenvironment is the ability to resolve specific T2 relaxation values of multiple water compartments within a sample. This is achieved by implementing an algorithm based on the inverse Laplace transform, which has been applied previously to estimate component decay constants in exponential decay curves *(12, 15, 16)*. Inverse Laplace transform processing of CPMG spectra produces a multiexponential fit of the relaxation data shown in equation 1:

$$S(t) = \sum_i A_i e^{-t/T2_i} + O \quad (1)$$

where $S(t)$ is the relaxation signal acquired over time t with the CPMG sequence, A_i is the amplitude corresponding to the relaxation time constant, $T2_i$, and O is the offset term. Fig. 1 shows how kinetic spectra can be formed from a numerical inverse Laplace transform.

The precision and reproducibility of multicomponent relaxation measurements across 3 T2MR instruments were characterized using mineral oil, which generates a 2-component signal. Average T2 relaxation times (30 min measurements at a sampling rate of 10 s) were 278 ms and 116 ms; mean within-run precision (%CV) values were 2.94% and 5.07% for the higher and lower component, respectively; day-to-day reproducibility (34 runs spanning 6 months) values were 3.4% and 7.6% for the higher and lower component, respectively.

T2MR SENSITIVITY TO HEMATOCRIT

Having established a method for precise measurements of water within multiple microenvironments, we evaluated the dependence of T2MR signals on hematocrit by measuring samples reconstituted with erythrocytes and plasma isolated from 5 individuals across a range of hematocrit values (20%–85%). We observed an inverse dependence of T2 on hematocrit (see online Supplemental Fig. S1a) or a linear dependence of 1/T2 on hematocrit levels (see online Supplemental Fig. S1b) generated by the paramagnetic properties of hemoglobin within erythrocytes, consistent with previous studies *(17)*.

REAL-TIME MONITORING OF CLOT FORMATION, CONTRACTION, AND FIBRINOLYSIS

We then measured the dependencies of the T2MR signals during clotting of recalcified citrated blood samples from healthy donors initiated by adding 3 U/mL thrombin. Thrombin activates platelets and cleaves fibrinogen to form a 3-dimensional fibrin network stabilized by factor XIIIa *(18)*. Addition of thrombin led to rapid formation of a gelatinous meshwork that filled the sample volume, accompanied by a small, rapid decrease in the T2MR signal over tens of seconds due to the sample transitioning from a liquid to gel state. In the initial gel state, only 1 relaxation rate was observed (Fig. 2a), reflecting uniform distribution of erythrocytes and other blood components. Approximately 4 min after thrombin addition, the T2MR signal split into 2 peaks representing distinct water populations in slow exchange. One peak decreased in T2 value (Fig. 2b), indicating increasing erythrocyte concentration in this compartment, whereas the T2 value of the other

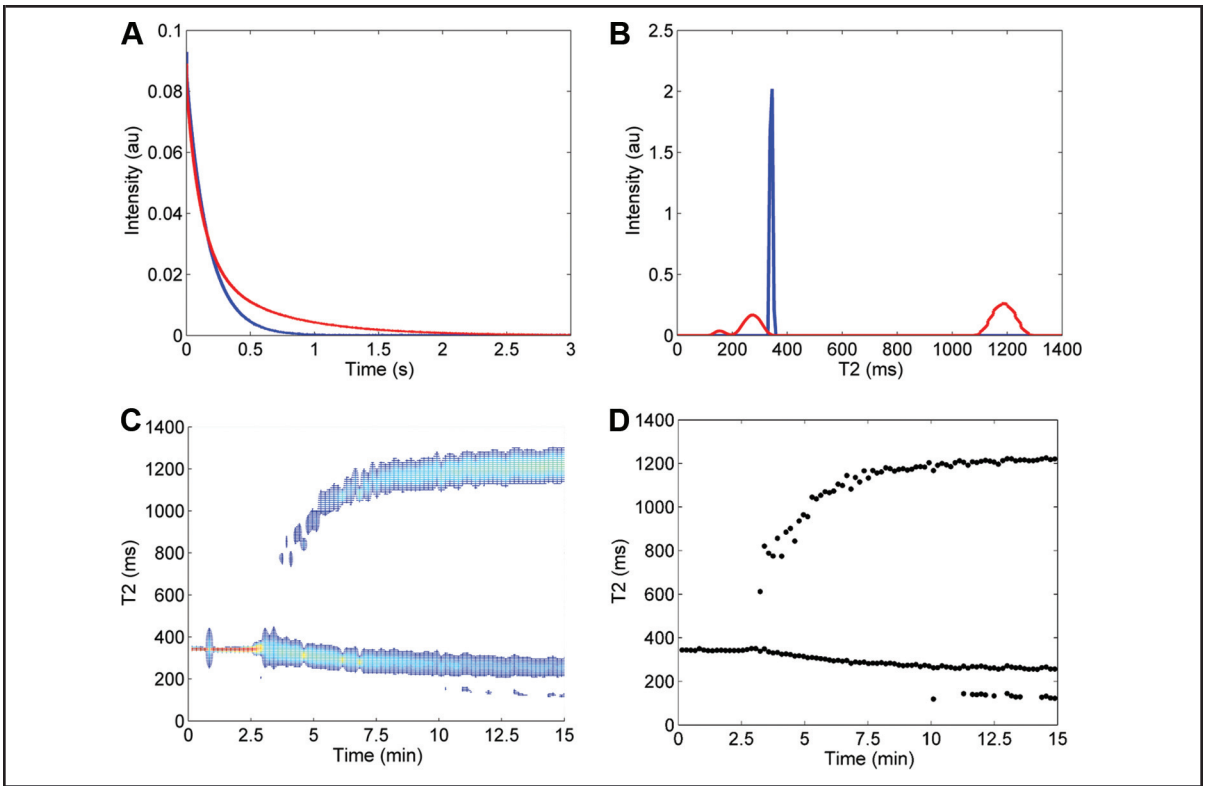

Fig. 1. Formation of kinetic spectra by numerical inverse Laplace transform.

(A), Relaxation curves at a single point in time of unclotted (blue) and clotted (red) blood are fit with an inverse Laplace transform algorithm to generate (B) T2 vs intensity spectra of unclotted (blue) and clotted (red) whole blood. au, arbitrary units. (C), Spectra like these are assembled into a 3-dimensional plot to generate a time series of the T2 vs intensity spectra. (D), To simplify data analysis, the T2 value corresponding to the center of each peak in each T2 spectrum is calculated by averaging over the encompassed T2 values, which are then plotted as a function of time to create T2 relaxation signatures.

peak increased rapidly, consistent with depletion of erythrocytes (Fig. 2c). Approximately 20 min after addition of thrombin, the upper peak reached a plateau (Fig. 2d). The lower peak at approximately 300 ms decreased in T2 value, associated with visible clot contraction, until around 10 min when it reached a plateau at approximately 275 ms (Fig. 2e). A third peak first appeared at 6 min at a lower T2 value (approximately 100 ms) (Fig. 2f).

We then assessed the sensitivity of the T2MR platform to fibrinolysis by adding tPA to the clotted samples 30 min after thrombin *(19)*. After tPA addition, the T2 value of the upper peak decreased rapidly (Fig. 2g), that of the middle peak decreased from 250 to 175 ms (Fig. 2h), while that of the third peak persisted at approximately 100 ms (Fig. 2i).

PEAK ASSIGNMENT

To interpret the T2MR signals during clot formation, contraction, and lysis, the major biological components of the system were measured in isolation and upon recombination (Table 1). Consistent with relaxation theory, T2MR signals were highest for serum, intermediate for plasma, and lowest for whole blood and contracted clots. The range of T2 values for whole blood from healthy donors, 400–285 ms, corresponds to hematocrit values of 35%–55%, and the range for reconstituted samples, 575–189 ms, corresponds to hematocrit values of 21%–83%. The higher T2MR signals in serum relative to whole blood arise from the lack of erythrocytes (and associated hemoglobin), which accelerates relaxation of water protons. The T2MR signal of plasma is lower than that of serum due to the relatively higher concentration of proteins that increase relaxation rates by exchange between free and protein-bound water (Table 1).

We next measured the T2MR signals of isolated contracted clots. One hour after recalcified citrated whole blood was clotted with 2 U/mL thrombin, contracted clots were removed and washed with PPP, and

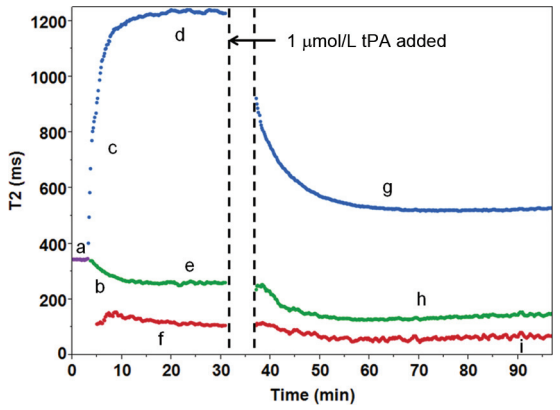

Fig. 2. Dynamic whole blood hemostasis monitoring with T2MR.

Clotting was initiated with 3 U/mL thrombin. (a) Single exchange-averaged water population, (b) initiation of clot contraction resolves the serum and erythrocyte water populations, (c) steep increase in the upper peak as serum is extruded from the clot, (d) completion of contraction and plateau of the upper peak, (e) plateau of the middle peak for loosely bound erythrocytes, and (f) a low T2MR signal corresponding to water trapped inside a tightly contracted clot. Fibrinolysis caused by the addition of tPA (30 min) (g) releases erythrocytes back into solution lowering the T2 value, (h) causes the middle peak to release erythrocytes and decrease in T2 value, leaving only (i) the signal associated with the more tightly bound erythrocytes.

T2MR signals were measured. Clots remained intact during manipulation, indicating tight contraction. The T2MR signals generated by isolated clots ranged from 100 to 150 ms (n = 6), consistent with this signal arising from a tightly contracted clot with a hematocrit approaching 100% based on equation S1 in the online Supplemental Materials.

Table 1. T2 values of isolated components of clotted blood samples as measured across n = 6 donor samples.

Isolated component	T2, ms
Serum	1000–1200
Plasma	800–1000
Homogenous whole blood	400–285 for 35%–55% hematocrit
Loosely bound or unbound erythrocytes	200–300
Contracted clot	75–175

The compartment generating the signal in Fig. 2b at 300 ms that dropped to 200 ms was assessed by testing 2 conditions: (*a*) recalcified citrated whole blood activated with thrombin to form a contracted clot and (*b*) recalcified citrated whole blood activated with thrombin followed by addition of tPA. After incubation, samples were analyzed before and after mixing with a pipette to resuspend unbound erythrocytes. In the sample clotted with thrombin, the 200–300-ms signal remained after mixing, but the T2 value of both the upper peak and this peak decreased as some unbound erythrocytes were dislodged by mixing (see online Supplemental Fig. S2a). In the sample clotted with thrombin then lysed with tPA, the 200–300-ms signal disappeared altogether after mixing. The upper T2 peak decreased in T2 value as the erythrocytes that were released from the fibrin network during clot lysis were resuspended by mixing (see online Supplemental Fig. S2b). These data support the conclusion that the T2MR signal at 200–300 ms originates from erythrocytes loosely bound to platelets and fibrin that is susceptible to tPA-induced fibrinolysis. The observation that the lowest T2MR signal in clotted samples persists after tPA addition is consistent with the signal emanating from a tightly compacted clot resistant to fibrinolysis.

SENSITIVITY TO THROMBIN ACTIVITY, FIBRINOGEN CONCENTRATION, AND PLATELET COUNT

We tested T2MR sensitivity to other hemostasis factors associated with clotting by activating recalcified citrated whole blood collected from healthy donors with 0.2 or 2 U/mL thrombin (Fig. 3). The higher thrombin concentration was chosen to generate more extensive clot formation and contraction. The dynamics of clot contraction and lysis by tPA of clots formed at lower thrombin concentrations more representative of normal physiology differed from higher thrombin concentrations in several ways, including a slower increase in the serum peak, slower time to appearance of the clot signals, and greater susceptibility to digestion by tPA (Fig. 3).

We also tested the effect of fibrinogen concentration on T2MR signals. Conversion of fibrinogen to fibrin by thrombin correlated with a decrease in T2MR signal, most likely due to decreased water diffusion. The magnitude of signal change depended on fibrinogen concentration and the rate depended on the amount of thrombin added to reconstituted samples in the absence and presence of erythrocytes (Fig. 3; also see Supplemental Fig. S3).

A similar dependence of the upper T2MR peak on platelet count alone was observed in reconstituted samples containing fibrinogen and platelets without erythrocytes (see online Supplemental Fig. S4). To explore the dependence of T2MR signals on the com-

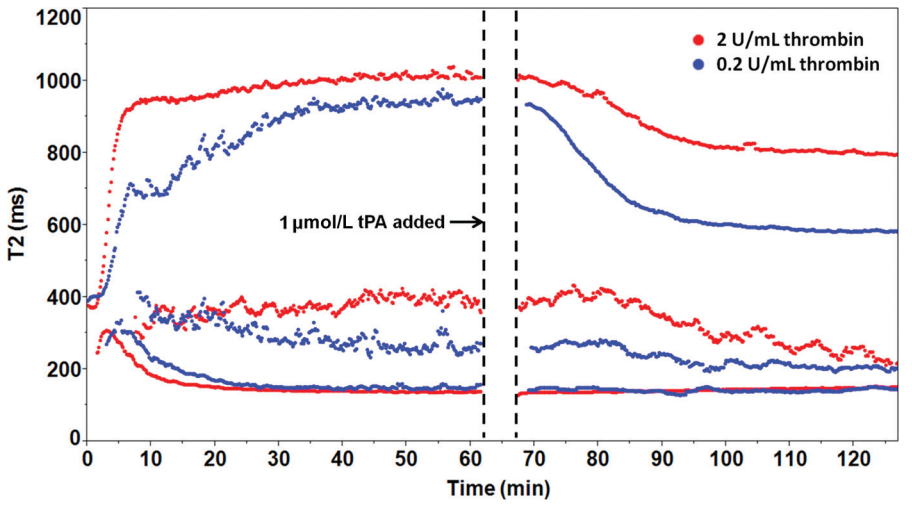

Fig. 3. Effect of thrombin concentration on clotting, clot contraction, and fibrinolysis.

At time zero citrated samples were activated with either (red curve) 2 U/mL thrombin and calcium or (blue curve) 0.2 U/mL thrombin and calcium. The signal emanating from serum rose more rapidly in the 2 U/mL thrombin sample and reached a higher T2 value than in the 0.2 U/mL sample. The 2 U/mL thrombin sample shows T2MR signals for loosely and tightly bound clots that appeared sooner than in the 0.2 U/mL thrombin sample. After addition of tPA at 60 minutes, the serum T2MR signal for the sample activated with 0.2 U/mL thrombin disappeared more rapidly along with disappearance of the signal from the loosely fibrin-bound erythrocytes.

bined effect of hematocrit and platelet level, we measured T2MR signals from 96 reconstituted blood samples ranging in platelet count from 20 000 to 550 000/μL and with hematocrit from 20% to 80% clotted by addition of calcium and kaolin. We calculated the percentage of these runs, showing the tightly contracted clot peak at $<$200 ms at low, normal, and high ranges of hematocrit and platelet count (see online Supplemental Table S1). For each low and normal hematocrit, the percentage of samples producing the contracted clot signal depended on platelet count. For all levels of platelet count (low, normal, and high), the percentage of samples with the contracted clot signal depended on hematocrit, suggesting cooperativity between erythrocytes and platelets, perhaps via thrombin generation *(20–22)*. Together, these studies show that the T2MR signal peak at approximately 100 ms, corresponding to the contracted clot, is generated under prothrombotic conditions, namely high thrombin, high fibrinogen, high hematocrit, and high platelet count.

STRUCTURAL CHARACTERIZATION OF CLOTS

To determine the relationship between the microscopic sample environments and corresponding T2MR signals, we examined clot structures using scanning electron microscopy *(23, 24)*. Clots formed by adding 0.2 or 2 U/mL thrombin were monitored with T2MR in real time; clots formed concurrently under identical conditions were isolated at different times during clotting and prepared for visualization by scanning electron microscopy. Clot contraction occurred later following activation with 0.2 U/mL thrombin (Fig. 4) than with 2 U/mL thrombin (see online Supplemental Fig. S5). Fibrin formed by activation with 0.2 U/mL thrombin was composed of thicker fibers with larger pores, consistent with previous studies *(25, 26)*. At early time points (5–10 min), scanning electron microscopy showed platelet aggregates associated with a fibrin network and trapped erythrocytes (Fig. 4B). After contraction, platelet aggregates were found predominantly on the exterior of the clot with tightly compressed red blood cells on the inside (Fig. 4B). Thus, the lower T2MR signal (approximately 100 ms) arose from water trapped within tightly compressed polyhedral erythrocytes, whereas the higher T2MR clot signal (approximately 200 ms) was generated by less compacted erythrocytes, which retained their normal biconcave shape. This observation is consistent with expectations from relaxation theory that restricted diffusion of water within the tightly compacted erythrocytes would lead to lower T2MR signals, and confirms previous findings *(27)*. Addition of tPA for 60 min digested the fibrin network on the outside of contracted clots with

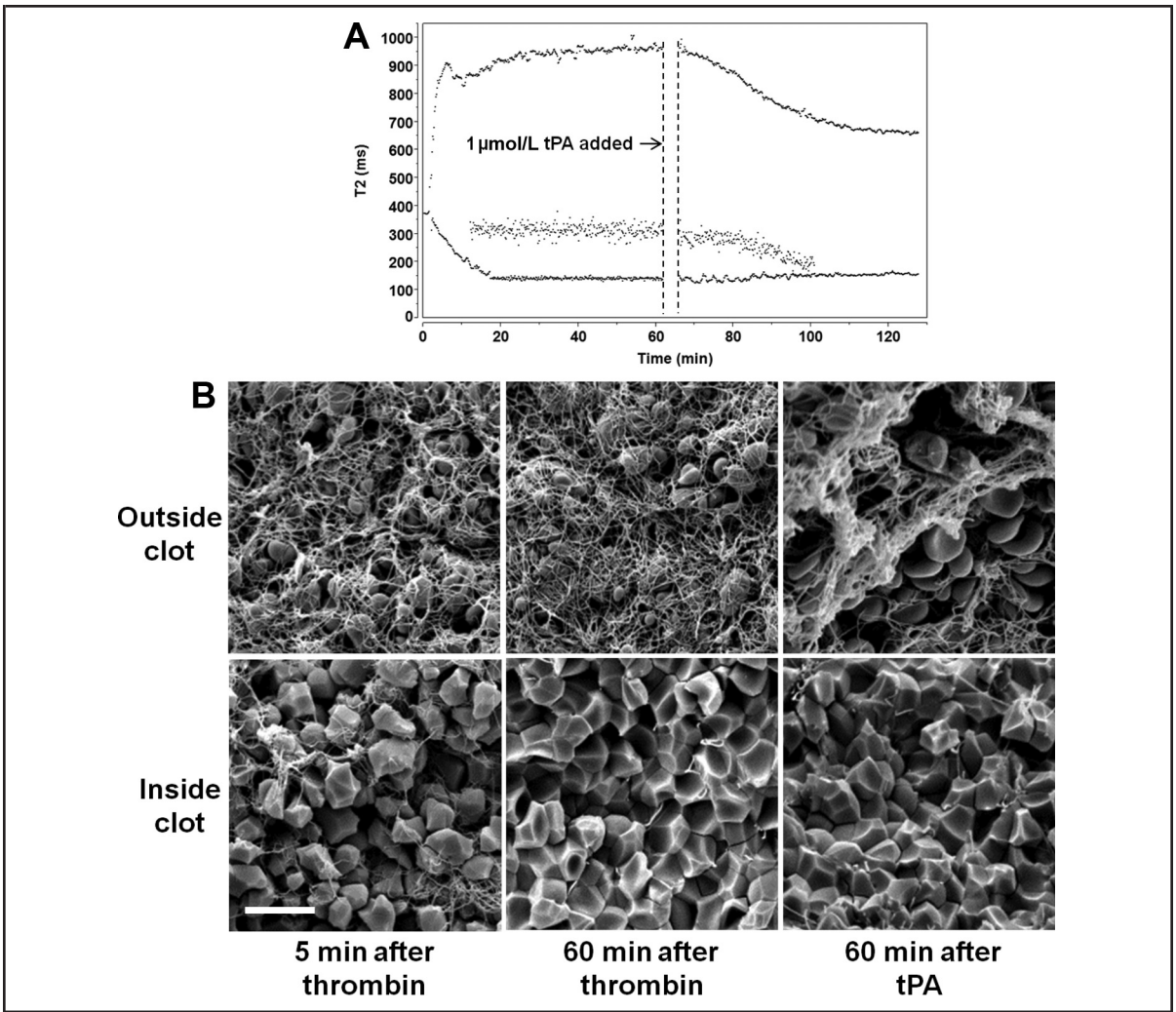

Fig. 4. T2MR signals and corresponding scanning electron microscopy images for samples clotted with 0.2 U/mL thrombin.

(A), T2MR signatures for two re-calcified citrated whole blood samples clotted with thrombin followed by addition of 1 μmol/L tPA. (B), Images collected of both the outside and inside of clots activated by 0.2 U/mL thrombin. The erythrocytes inside this clot were biconcave 5 minutes after the onset of clotting, but were compressed into polyhedral shapes after 60 minutes. Polyhedral erythrocytes remain unchanged after incubation with tPA, as shown in the right hand panels, but the fibrin mesh on the outside of the clot relaxed after partial digestion and released some erythrocytes by 60 min. See the online Supplemental Materials for data collected at 2 U/mL thrombin. Scale bar 10 μm.

consequent release of erythrocytes from the surface of both types of clots (Fig. 4B), corresponding to the drop in the upper T2 peak (Fig. 4A). The persistence of the lower T2MR signal for the more tightly contracted clot is consistent with its relative resistance to fibrinolysis, as previously described *(28)*.

CORRELATION OF T2MR WITH EXISTING DIAGNOSTIC TESTS

Although T2MR can be used to obtain new insights into the physical states of microenvironments within blood samples, it can also be configured to measure standard hemostasis parameters. To demonstrate this, we performed method comparison studies against the Sysmex pocH-100*i* hematology analyzer for hematocrit for which an $R^2 = 0.95$ for 40 donor samples and an mean precision of %CV of 4.8% for n = 10 replicates across 13 donor samples was observed (see the online Supplemental Materials). For prothrombin time against the Stago ST4 system, a correlation of $R^2 = 0.94$ over 68 donor samples from healthy donors and do-

nors treated with anticoagulants and a %CV of 3.5% for n = 10 replicates across 23 donor samples was observed, and for thromboelastography clot strength, a correlation of $R^2 = 0.95$ between T2MR and thromboelastography maximum amplitude values across 10 samples was observed. For platelet function measurements, an overall agreement of 93% was observed between T2MR and light transmission aggregometry for activation by ADP. See the online Supplemental Materials for detailed descriptions of the methods and results for these correlation studies.

Discussion

We have developed and characterized a new diagnostic platform that potentially enables both standard hemostasis measurements as well as measurements that provide novel insights into the dynamics and physical states of blood during clotting and lysis. Assignment of the different T2MR signals to distinct blood and clot constituents permits continuous monitoring of the dynamic states of blood components over a wide range of platelet counts, fibrinogen concentrations, hematocrits, activator concentrations, and other contributors to clotting in whole blood. The T2MR platform allows real-time assessment of the transition of fibrinogen to fibrin polymer, clot contraction by platelets, formation of tightly contracted clots, and fibrinolysis.

We identified a T2MR signal attributable to a recently characterized very tightly contracted blood clot *(27)*. This highly contracted clot was composed of fibrin surrounding tightly packed erythrocytes and was relatively resistant to lysis by tPA *(29)*. The signal attributable to the tightly contracted clot emerged from samples as the hematocrit, platelet count, fibrinogen concentration, and the amount of procoagulant added (e.g., thrombin) increased. This T2MR signal may represent a novel biomarker for a prothrombotic state, with important clinical implications.

Initial correlation and precision studies also demonstrate the potential for clinically relevant measurements of hematocrit, clotting time, platelet reactivity, and clot strength with this platform. T2MR may provide novel insights into overall platelet health because clot contraction requires not only the signaling and membrane receptor functions assessed by platelet aggregometry, but also the interaction between the platelet cytoskeleton and fibrin via integrins in the platelet membrane. Preliminary studies suggest that T2MR may show residual platelet capacity to cause clot contraction in whole blood in the presence of inhibitors that block platelet aggregation and may thus find a place in the monitoring of aspirin and other antiplatelet agents to which biological resistance is encountered in the absence of a laboratory correlate. Additional studies are underway to expand the types of activators and data sets for establishing the reference intervals of ranges for T2MR as correlated with light transmission aggregometry initiated by diverse platelet agonists, thromboelastography, clotting time, and functional fibrinogen. In addition, in-depth studies are needed for validation of the technology with clinical samples.

In common with other currently used clinical coagulation laboratory instruments, the current configuration of T2MR for hemostasis measurements does not provide for measurements under physiologically relevant flow conditions that provide shear force, exposure to endothelium, and subsequent activation of the thrombomodulin/protein C pathway and release of plasminogen activator inhibitor-1. Future modifications of T2MR by integration with flow-through devices *(30–32)* could be used to enable study of these contributions to hemostasis. Additionally, several other magnetic resonance methods, including those utilizing controlled gradients for apparent diffusion coefficient measurements as well as measurement of other relaxation parameters, may provide new insights into the biology of clot formation and lysis. Lastly, to fully understand the clinical impact of this methodology, sample-to-answer automation should be provided to facilitate testing at a wide range of clinical sites.

The T2MR platform combines the flexibility of conforming to standard measurements of hemostasis with analysis of integrated coagulation in whole blood, including the contribution of leukocytes, microparticles, and other factors difficult to assess at present. The relative simplicity of the instrumentation and methodology involving a single transfer of whole blood from a test sample has the potential to permit rapid testing requiring no sample preparation and minimal sample volumes.

Lastly, the data from this proof-of-concept study demonstrate that this platform has the potential to permit rapid and sensitive analysis of whole blood clotting across a spectrum of conditions that cannot be readily assayed using currently available methodology, ranging from impaired hemostasis to hypercoagulable states *(3–5, 7, 33)*. The unique small sample volume requirement is particularly advantageous for pediatric populations, studies of thrombotic and bleeding disorders in small animal models (see the online Supplemental Materials), and point-of-care testing. Future development of this technology holds the promise of providing methods to refine understanding of the interaction between cellular and plasma components in blood, the rapid and sensitive identification of patients at risk for clotting or bleeding, and the ascertainment of relevant endpoints for therapeutic monitoring, such as patients on direct thrombin inhibitors, with a single, simple-to-employ analytic approach that may be suitable for routine use in both research and diverse clinical settings.

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