Journal of Thrombosis and Haemostasis, **16**: 2050–2056 DOI: 10.1111/jth.14244

BRIEF REPORT

Assessment of whole blood coagulation with a microfluidic dielectric sensor

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To cite this article: Maji D, De La Fuente M, Kucukal E, Sekhon UDS, Schmaier AH, Sen Gupta A, Gurkan UA, Nieman MT, Stavrou EX, Mohseni P, Suster MA. Assessment of whole blood coagulation with a microfluidic dielectric sensor. *J Thromb Haemost* 2018; **16**: 2050–6.

Essentials

- ClotChip is a novel microsensor for comprehensive assessment of *ex vivo* hemostasis.
- Clinical samples show high sensitivity to detecting the entire hemostatic process.
- ClotChip readout exhibits distinct information on coagulation factor and platelet abnormalities.
- ClotChip has potential as a point-of-care platform for comprehensive hemostatic analysis.

Summary. *Background:* Rapid point-of-care (POC) assessment of hemostasis is clinically important in patients with a variety of coagulation factor and platelet defects who have bleeding disorders. *Objective:* To evaluate a novel dielectric microsensor, termed ClotChip, which is based on the electrical technique of dielectric spectroscopy for rapid, comprehensive assessment of whole blood coagulation. *Methods:* The ClotChip is a three-dimensional, parallel-plate, capacitive sensor integrated into a single-use microfluidic channel with miniscule sample volume (< 10 μL). The ClotChip readout is defined as the temporal variation in the real part of dielectric permittivity of whole blood at 1 MHz. *Results:* The ClotChip readout exhibits two distinct parameters, namely, the time to reach a permittivity peak (T_{peak}) and the maximum change in permittivity after the peak ($\Delta\varepsilon_{r,max}$), which are, respectively, sensitive towards detecting non-cellular (i.e. coagulation factor) and cellular (i.e. platelet) abnormalities in the hemostatic process. We evaluated the performance of ClotChip using clinical blood samples from 15 healthy volunteers and 12 patients suffering from coagulation defects. The ClotChip T_{peak} parameter exhibited superior sensitivity at distinguishing coagulation disorders as compared with conventional screening coagulation tests. Moreover, the ClotChip $\Delta\varepsilon_{r,max}$ parameter detected platelet function inhibition induced by aspirin and exhibited strong positive correlation with light transmission aggregometry. *Conclusions:* This study demonstrates that ClotChip assesses multiple aspects of the hemostatic process in whole blood on a single disposable cartridge, highlighting its potential as a POC platform for rapid, comprehensive hemostatic analysis.

Keywords: blood coagulation; blood coagulation disorders; blood coagulation factor inhibitors; blood coagulation tests; platelet function tests.

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Received: 26 September 2017
Manuscript handled by: F. R. Rosendaal
Final decision: F. R. Rosendaal, 11 July 2018

Introduction

Early identification of hemostatic dysregulation and bleeding risk is important in the management of patients who are critically ill, severely injured or on antiplatelet/anticoagulation therapies [1]. Conventional laboratory-based coagulation tests are time consuming, labor intensive, and are not reliable indicators of hemostatic risk. Extant handheld point-of-care (POC) devices have uses that are limited to specific patient populations (e.g. CoaguChek for warfarin use), have low thromboplastin and

partial thromboplastin reagent sensitivity (e.g. i-STAT device), resulting in only suboptimal assessment of the coagulation process, and do not provide concurrent information on platelet function. Although thromboelastography (TEG) and rotational thromboelastometry (ROTEM) allow for the analysis of several aspects of clot formation and strength, representing a global measure of the hemostatic process, these viscoelastic tests rely on sensitive mechanical components that are expensive and difficult to miniaturize. Hence, there is an unmet clinical need for a low-cost, easy-to-use and portable platform for comprehensive POC assessment of hemostasis outside of a central laboratory.

To address this need, we adapted the method of dielectric spectroscopy (DS), an electrical, label-free and non-invasive technique, to monitor the hemostatic process *ex vivo* in a disposable microfluidic sensor. DS is the quantitative measurement of permittivity vs. frequency and is a well-established method to extract information on the molecular and cellular components of biological tissues [2,3]. The main response of blood DS measurements in the MHz-frequency range arises from the interfacial polarization of cellular components [4,5]. DS measurements within the resulting dispersion region are used to gain information on the physical properties of blood [6,7]. In particular, DS measurements on blood in the MHz-frequency range are sensitive to aggregation of erythrocytes into a fibrin clot and subsequent erythrocyte deformation as a result of contractile forces from activated platelets [8–10] that characteristically occur during clot formation [11]. DS that assesses the blood coagulation process is termed dielectric coagulometry. We have developed a novel dielectric microsensor, termed ClotChip, which performs dielectric coagulometry on a miniscule volume (<10 μL) of whole blood. Presently, we show that ClotChip readouts are sensitive to several aspects of the hemostatic process, including thrombin formation and platelet activation. These features allow for comprehensive assessment of the hemostatic process *ex vivo* in a potentially portable platform, which is ideal for a POC device.

Methods

ClotChip fabrication and measurements

The ClotChip featured a parallel-plate capacitive sensor to extract the dielectric permittivity of whole blood within a microfluidic channel [12]. Two planar sensing electrodes were separated from a floating electrode through a microfluidic channel to form a three-dimensional capacitive sensing area. With a blood sample passing through this area, the impedance of the sensor would change based upon its dielectric permittivity. ClotChip was fabricated using biocompatible, chemically inert, polymethyl methacrylate (PMMA) plastic substrate and cap (Fig. 1A) [13,14]. The sensor fabrication and assembly process was based on a low-cost (< $1 material cost per chip) batch-fabrication method of screen-printing gold electrodes onto a 1.5 mm-thick PMMA plastic substrate and cap. A double-sided adhesive (DSA) film with thickness of 250 μm was laser micromachined to form the walls of a microfluidic channel with dimensions of 12 mm × 3 mm, and then the ClotChip was assembled by attaching the PMMA cap to the PMMA substrate using the DSA film (Fig. 1B). The fabricated sensor (Fig. 1C) had a size of 26 mm × 9 mm × 3 mm and a total sample volume of 9 μL. The sensor was loaded with whole blood using a micropipette, placed into a thermostatic chamber set at 37 °C, and characterized with an impedance analyzer (Agilent 4294A; Santa Rosa, CA, USA) over a frequency range of 10 kHz to 100 MHz to capture the dispersion region associated with red blood cell (RBC) membrane polarization (Fig. 1D). Measurements were performed at 10-s intervals over a total measurement time of 30 min. The electrical properties of whole blood varied during coagulation, and a measurement frequency of 1 MHz was chosen to maximize the sensitivity to clot formation dynamics, including RBC aggregation and deformation. Therefore, the ClotChip readout was taken as the temporal variation in the real part of blood dielectric permittivity at 1 MHz (Fig. 1E).

Study design

All healthy volunteers and patients who enrolled in this study provided informed consent. The collection and use of blood samples from subjects were approved by the Institutional Review Board of University Hospitals Cleveland Medical Center. Blood samples were drawn by venipuncture into collection tubes containing 3.2% sodium citrate anticoagulant (ratio of blood to anticoagulant = 9 : 1), and were used for both ClotChip measurements and conventional coagulation assays. Healthy volunteers were accrued only if they were off medications and without diagnosis of an illness in the past 4 weeks. Twelve patients with coagulation defects (Table 1) were accrued from a hematology clinic and had been previously characterized by comprehensive medical history, screening coagulation tests and specialized coagulation studies.

ClotChip measurements were performed in a research laboratory by a trained doctoral student within 2 h from the time of blood collection. Prior to measurements and to initiate coagulation, 25.6 μL of 250 mM $CaCl_2$ was pipetted into 300 μL of citrated blood sample that was pre-warmed to 37 °C in a heating chamber, and 10 μL of the mixture was immediately injected into the ClotChip.

Additional ClotChip measurements were performed with whole blood obtained by fingerstick using a 23-gauge lancet and wiping away the first drop of blood. Another two to three drops of blood were collected in a polypropylene tube, after which 10 μL of whole blood was immediately injected into the ClotChip using a micropipette. Duplicate measurements were performed within 1 min following the fingerstick procedure.

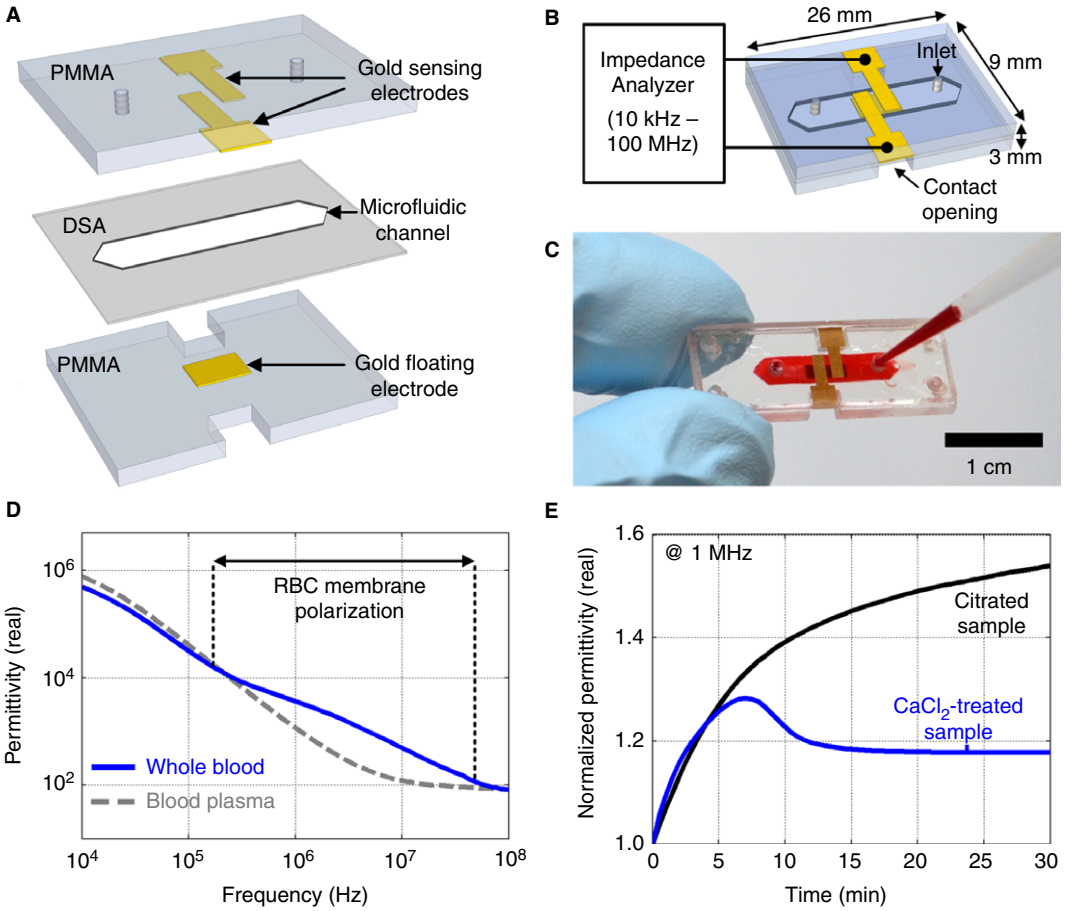

Fig. 1. ClotChip fabrication and testing. (A) ClotChip fabrication and assembly. A double-sided adhesive (DSA) film with thickness of 250 μm was laser micromachined to form the walls of a microfluidic channel. The ClotChip then was assembled by attaching the polymethyl methacrylate (PMMA) cap to the PMMA substrate using the DSA film. (B) Assembled ClotChip sensor and experimental setup. Contact openings in the PMMA substrate allowed electrical connection between the sensing electrodes and an impedance analyzer (Agilent 4294A). Spring-loaded electrical contact pins were used to provide a plug-and-play-type connection and enable rapid user replacement of the disposable sensor. (C) Photograph of prototype ClotChip sensor with sample volume of 9 μL. (D) Sensor measurements showing the real part of dielectric permittivity of human whole blood and blood plasma versus frequency. The permittivity of whole blood versus frequency exhibited a dispersion region (~ 200 kHz to 50 MHz) due to red blood cell (RBC) membrane interfacial polarization (as evident by the absence of this region in plasma measurement). (E) Example of the ClotChip readout for a human whole blood sample (collected in 3.2% sodium citrate anticoagulant) without (black) and with (blue) coagulation initiated by the addition of $CaCl_2$. The ClotChip readout was taken as the time course of variation in the real part of blood dielectric permittivity at 1 MHz. A peak in permittivity was observed for the $CaCl_2$-treated sample, whereas no such peak was observed for the citrated sample, indicating that the permittivity of whole blood at 1 MHz was sensitive to the coagulation process. [Color figure can be viewed at wileyonlinelibrary.com]

Calibration of the measurement set-up was performed daily to implement quality control and ensure accurate electrical measurement of the blood sample. This step was performed using a custom printed circuit board in the form of the ClotChip sensor that contained an equivalent circuit model of whole blood [12]. Additional quality control was implemented by testing a control sample from a pool of known healthy volunteer donors on each day that patient samples were examined.

Platelet inhibition studies

Platelet inhibition studies were performed *ex vivo* with blood samples from 10 healthy volunteers. Samples for ClotChip measurements were prepared by mixing whole blood with 120 mM aspirin in DMSO (final aspirin concentration of 0 to 2 mM), followed by incubation for 30 min at room temperature. Light transmission aggregometry (LTA) experiments were performed using platelet-rich plasma (PRP) within 3 h of blood collection. PRP was diluted to 2.2×10^8 platelets per mL, then treated with aspirin (1 or 2 mM) for 30 min at room temperature. Aggregation was initiated with ADP (5 or 10 μM), arachidonic acid (0.5 mM) or SFLLRN (PAR1 agonist peptide, 10 μM). Aggregation was measured using a lumi-aggregometer (Model 700, Chrono-log Corp). The sample was stirred constantly at 1200 rpm at 37 °C.

Table 1 Baseline characteristics of patients with coagulopathy

Patient	Diagnosis	ClotChip T_{peak} (240–505 s)	PT (9.3–14 s)	APTT (24–35 s)	Specialized coagulation assays
A1	FXII deficiency	3550	13.8	> 120	FXII:C: < 1%
A2	Hemophilia A	730	14	34.4	FVIII:C: 50%
A3	Hemophilia A w/ inhibitor	980	12.2	43.1	FVIII:C: 2–3%
A4	Hemophilia A	735	13.9	39.1	FVIII:C: 2–4%
A5	Hemophilia A	900	13.9	38.3	FVIII:C: 11%
A6	Hemophilia A w/ inhibitor	2420	12.4	41.5	FVIII:C: < 1%
A7	Hemophilia A	1350	13.5	49.4	FVIII:C: < 1%
A8	Hemophilia B	675	13.5	37.7	FIX:C: 10%
A9	Hemophilia B	860	13.9	43.2	FIX:C: 3%
A10	Hemophilia A	990	13	43.3	FVIII:C: 4%
A11	Hemophilia B	1335	13	37.7	FIX:C: 11%
A12	von Willebrand disease type 2B – V1316A polymorphism in A1 region of VWF	680	12.8	29.8	VWF antigen: 44% VWF activity (RCA): 35% VWF multimers: loss of high-molecular-weight multimers FVIII:C: 62%

FVIII:C, factor VIII coagulant activity (%); FIX:C, factor IX coagulant activity (%); FXII:C, factor XII coagulant activity (%); VWF, von Willebrand factor; RCA, ristocetin cofactor assay. Patients with classic hemostatic defects were accrued from a specialty hematology clinic and agreed to participate in this study. ClotChip measurements along with relevant coagulation tests were performed.

Statistical analysis

Data obtained in this study are reported as mean ± standard error of the mean (SEM). The Wilcox matched-pairs signed test was used for comparing outcomes between two paired groups and the Mann–Whitney *U*-test otherwise. All statistical comparisons were two-tailed. The statistical significance threshold was set at the 95% confidence level for all tests ($P < 0.05$). Statistical data analysis was performed using Minitab 17 and GraphPad Prism software suites (GraphPad Software, San Diego, CA, USA).

Results and discussion

ClotChip measurements from 15 healthy volunteers who had normal activated partial thromboplastin time (APTT) and prothrombin time (PT) values are shown in Fig. 2(A). A characteristic rise to a permittivity peak was observed within 240–505 s (T_{peak}). Based on our previous studies, the T_{peak} parameter was taken to be indicative of coagulation time [14,15]. ClotChip measurements then were performed with 12 patient samples obtained from individuals with well-characterized bleeding disorders. Compared with the normal curve, samples from patients with coagulopathy exhibited abnormal curves (Fig. 2B) with a significantly prolonged T_{peak} range of 675–3550 s ($P < 0.0001$; Fig. 2C). The precision of T_{peak} was evaluated by calculating a percentage coefficient of variance (CV) as the ratio of the within-subject standard deviation and the overall mean × 100 [16]. We used duplicate measurements from the 15 normal samples and 12 patient samples to establish a CV for 'normal' and 'high' ranges of T_{peak}, respectively. The normal samples exhibited a mean T_{peak} of 371 s and CV of 7.6%. The patient samples exhibited a mean T_{peak} of 1267 s and CV of 8.6%. The ClotChip CV values are similar to the reported precision (~6%) of a commercially available POC blood coagulation test [17].

Conventional coagulation tests (APTT and PT) were also performed for each sample. It should be noted that the most prolonged APTT coagulation time was observed in the sample with factor XII deficiency, which also had the most prolonged T_{peak}. We also observed prolonged APTT coagulation times as well as prolonged T_{peak} values in all moderate and severe hemophilia samples, accurately capturing this type of coagulopathy. However, for mild hemophilia A and von Willebrand type 2B cases, both the APTT and PT were normal. These patients were diagnosed with specialized coagulation assays following referral to our hematology clinic for work-up of clinical bleeding. In both cases, the ClotChip readout did show a prolonged T_{peak}, indicating its superior sensitivity. Taken together, the data confirmed that ClotChip could capture defects in multiple aspects of the hemostatic pathway, with improved sensitivity as compared with standard screening coagulation tests.

We then investigated the effect of aspirin-induced inhibition of platelet activity on the ClotChip readout. Samples were treated with various concentrations of aspirin *ex vivo*. LTA studies were run on each sample, and we observed that with arachidonic acid-induced platelet aggregation, the percentage aggregation was reduced from > 70% for baseline measurements to < 5% for all aspirin-treated samples, confirming platelet inhibition in response to aspirin treatment. We found that aspirin treatment did not significantly change T_{peak} (Fig. 2D), as we had previously observed with coagulation factor defects. However, aspirin dose-dependently decreased the $\Delta\varepsilon_{r,max}$ parameter, defined as one minus the ratio of final permittivity (i.e.

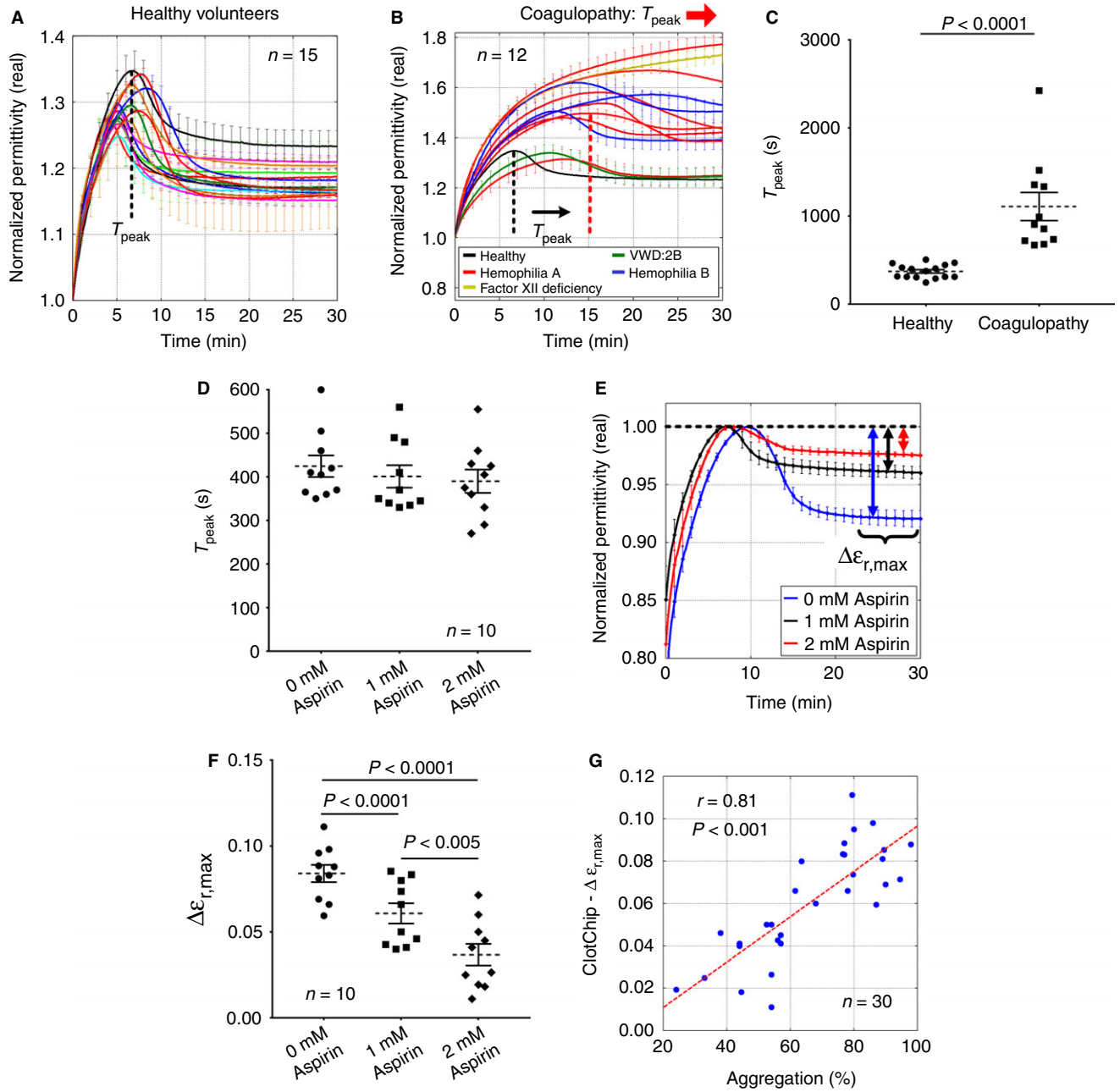

Fig. 2. ClotChip measurements. (A) ClotChip readout from 15 healthy volunteers showed a time-to-permittivity peak (T_{peak}) ranging in 240–505 s. (B) ClotChip readout from 12 patients with coagulation defects showed prolonged T_{peak} compared to the curve from a healthy volunteer. (C) A Mann–Whitney *U*-test comparing T_{peak} in 15 healthy vs. 12 coagulopathic samples, $P < 0.0001$. T_{peak} of 3550 s for the blood sample with FXII deficiency is not shown. (D) Blood samples from 10 healthy volunteers were treated with 1 or 2 mM aspirin, and no significant change in T_{peak} was found when compared to untreated samples. (E) Representative ClotChip readout from a blood sample treated with increasing concentrations of aspirin to inhibit platelet activity. (F) A significant decrease in ClotChip $\Delta\varepsilon_{r,max}$ parameter was observed in blood samples treated with 1 mM aspirin ($n = 10$, $P < 0.0001$) or 2 mM aspirin ($n = 10$, $P < 0.0001$) when compared to untreated samples. (G) For aspirin-treated samples, the ClotChip $\Delta\varepsilon_{r,max}$ parameter showed strong positive correlation to percentage aggregation in light transmission in response to 5 μM ADP ($r = 0.81$, $P < 0.001$, $n = 30$). For all plots of the ClotChip readout, error bars indicate duplicate measurements and are presented as mean ± standard error of the mean (SEM). [Color figure can be viewed at wileyonlinelibrary.com]

permittivity at 30 min) and peak permittivity (i.e. permittivity at T_{peak}; Fig. 2E). Compared with untreated samples, a significant decrease in $\Delta\varepsilon_{r,max}$ was observed for samples that were treated with 1 mM aspirin ($n = 10$, $P < 0.0001$) or 2 mM aspirin ($n = 10$, $P < 0.0001$; Fig. 2F). The ClotChip $\Delta\varepsilon_{r,max}$ parameter exhibited the strongest correlation ($r = 0.81$, $P < 0.001$, $n = 30$) with the percentage aggregation parameter of LTA with 5 μM ADP (Fig. 2G). These data showed that the ClotChip $\Delta\varepsilon_{r,max}$ parameter was sensitive to platelet activity.

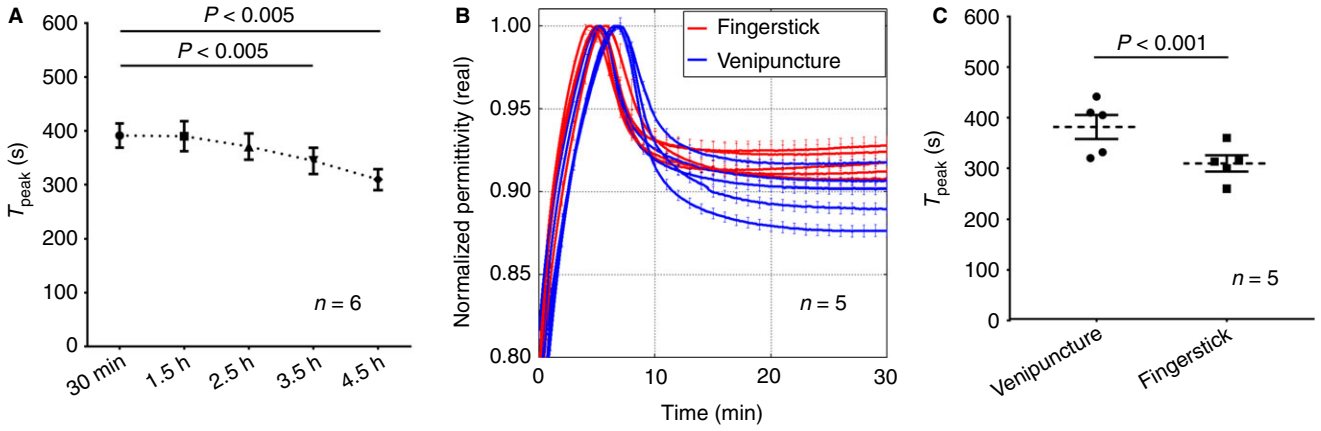

Fig. 3. Effect of pre-analytical conditions on the ClotChip readout. (A) Whole blood stability was analyzed by measuring mean T_{peak} for 6 blood samples stored at room temperature and tested 30 min, 1.5, 2.5, 3.5, and 4.5 h after blood draw. B) ClotChip readout for whole blood obtained from fingerstick and re-calcified whole blood obtained from venipuncture showed nearly identical characteristics. C) A decrease in T_{peak} was observed for blood samples obtained from fingerstick as compared to venipuncture ($n = 5$, $P < 0.001$). [Color figure can be viewed at wileyonlinelibrary.com]

Finally, we investigated the effect of pre-analytical conditions on the ClotChip readout. To test sample stability, we performed repeated measurements at different time-points for blood samples from six healthy volunteers. Samples were stored at room temperature and duplicate ClotChip measurements were performed at 30 min, 1.5, 2.5, 3.5 and 4.5 h after blood collection. The studies showed a downward trend in mean T_{peak} as storage time increased (Fig. 3A). The first of these results (performed 30 min after blood collection) represented the reference T_{peak} for each sample, and the stability at different time-points was analyzed via comparison with this first T_{peak} value [18]. A significant difference was found for groups at 3.5 h ($P < 0.005$) and 4.5 h ($P < 0.005$) compared with the group at 30 min. No significant difference was found for groups at 2.5 h and 1.5 h compared with 30 min. These results demonstrate that ClotChip readout exhibits repeatable characteristics for citrated whole blood tested within 2.5 h from blood draw.

We then investigated the feasibility of using whole blood obtained from a fingerstick blood-collection method. Five healthy volunteers were recruited to donate blood by fingerstick and venipuncture techniques. Figure 3(B) shows nearly identical ClotChip readout for whole blood obtained by fingerstick and re-calcified whole blood obtained from venipuncture. Although we did observe a decrease in T_{peak} values for fingerstick samples (range: 260–360 s) compared with re-calcified venipuncture samples (range: 320–440 s) as shown in Fig. 3(C), the T_{peak} values for all fingerstick samples fell within the range of all 15 healthy volunteers, as reported above (Fig. 2A,C). This study demonstrates the potential of ClotChip to accurately perform with whole blood obtained from a fingerstick.

In conclusion, we show that the ClotChip readout of dielectric permittivity of whole blood at 1 MHz is sensitive to a wide range of hemostatic defects. Specifically, two distinct parameters of the ClotChip readout, T_{peak} and $\Delta\varepsilon_{r,max}$, provide independent information on hemostatic defects arising from non-cellular (i.e. coagulation factor) and cellular (i.e. platelet) components, respectively, thereby allowing a discriminatory assessment of the comprehensive blood coagulation process. Although early work on dielectric coagulometry revealed sensitivity to both clotting time and platelet activity [19–21], this technique was restricted to laboratory-based benchtop equipment and > 100 μL-volume samples [22–25]. The ClotChip sensor used in this study features a simple fabrication process and enables dielectric coagulometry to be performed with < 10 μL of blood sample volume in a disposable sensor. Furthermore, the electrical technique of dielectric coagulometry does not require bulky optical or mechanical components and is thus ideal for a POC platform. Although a small sample size of patients with known coagulation defects was employed in this proof-of-concept investigation, these initial studies pave the way for the development of a portable platform that allows for clinical testing to be performed at the POC. In future investigations we plan to expand the sample size, use the device to screen patients for disease, assess blood coagulation testing phenomena such as lupus anticoagulants, and determine the correlation of test results with bleeding. We believe these studies are needed before translation to clinical practice. Nonetheless, our present work establishes that ClotChip has potential as a portable platform for rapid, comprehensive assessment of hemostasis at the POC using < 10 μL of whole blood.

Addendum

D. Maji, M. De La Fuente, E. Kucukal, U. D. S. Sekhon and E. X. Stavrou performed experiments; M. A. Suster, E. X. Stavrou, P. Mohseni, M. T. Nieman, A. H. Schmaier, U. A. Gurkan and A. Sen Gupta conceptualized and planned experiments; A. H. Schmaier and E. X. Stavrou designed the

study and obtained clinical samples; P. Mohseni, D. Maji, M. A. Suster and E. X. Stavrou prepared the figures; M. A. Suster, D. Maji, E. X. Stavrou and P. Mohseni wrote the manuscript.

Acknowledgments

The authors would like to thank Dr. Lalitha V. Nayak, Case Western Reserve University, for providing additional clinical samples. This work was supported by the American Heart Association Grant-in-Aid 17GRNT33661005 (M. A. Suster and P. Mohseni), American Heart Association Scientist Development Award 15SDG25590000 (E. X. Stavrou), NIH Grants R01 HL126645 and R01 AI130131-01 (A. H. Schmaier), NIH Grant 5R01 HL121212 (A. Sen Gupta), DOD Grant BC150596P1 (A. H. Schmaier), the Case School of Engineering's San Diego-based Wireless Health program, the Case-Coulter Translational Research Partnership, and the Advanced Platform Technology (APT) Center – A Veterans Affairs (VA) Research Center of Excellence at Case Western Reserve University. The contents do not represent the views of the U.S. Department of Veterans Affairs or the United States Government.

Disclosure of Conflict of Interests

D. Maji, U. A. Gurkan, E. X. Stavrou, P. Mohseni and M. A. Suster are inventors of intellectual property that has been licensed by Case Western Reserve University to XaTek, Inc. M. A. Suster and P. Mohseni receive consulting fees from XaTek, Inc.

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