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Blood clot parameters: Thromboelastography and scanning electron microscopy in research and clinical practice

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ABSTRACT

Background: Clotting parameters are informative of overall haematological healthiness of an individual. Particularly, clotting parameters can be used as a measure of the degree of pathology of the coagulation system. Thromboelastography (TEG) is a well-known technique that is an important point-of-care method, as well as research method. Scanning electron microscopy (SEM) is a novel research method, but with possible clinical application. However, there are no clear standardized guidelines for TEG and SEM result interpretation.
Materials and methods: We have an extensive database of results from TEG of hypercoagulable, hypocoagulable and healthy whole blood (WB) and platelet poor plasma (PPP). These results were generated using citrated PPP or WB, followed by the addition of CaCl2, to initiate clot formation. We also have an extensive and comprehensive database of thousands of clot micrographs, prepared for SEM. We reanalysed all our data to compile a user-friendly guideline for TEG and SEM. We also discuss the effects of different storage times on both WB and PPP.
Results: We provide a quick and informative guide that discusses each TEG parameter, in both WB and PPP. Increases or decreases in the various parameters are indicative of either hyper- or hypocoagulability. We also show how hypo- and hypercoagulable clots look like, compared with healthy clots, using SEM analysis of clots created by adding thrombin to PPP.
Conclusion: For optimal and speedy interpretation of a patient's coagulation status, it is essential for the clinician to make an informed and precise decision regarding clotting propensity. We believe this guideline will add to the standardization of TEG parameters, and ultimately contribute to the treatment of patients. These guidelines will also allow researchers to standardize their data interpretations and ultimately allow for the use of a global and inclusive database that might be included in precision medicine approaches.

1. Introduction

Clotting parameters and clot structure are informative measures of overall haematological healthiness of an individual [1–6]. Particularly, clotting parameters can be used as a measure of the degree of pathology of the coagulation status. One of the current point-of-care methods and research techniques that characterizes the viscosity and elasticity of either whole blood (WB) or platelet poor plasma (PPP) is thromboelastography (TEG) [2,7–10]. Scanning electron microscopy (SEM) is an emerging highly sensitive ultrastructural technique that can convey very specific changes to individual cells and fibrin packaging, involved in clotting [2,7,11–15]. However, there are currently no clear guidelines that direct researchers on how to evaluate and interpret TEG results in light of ultrastructural changes visualized with SEM. Only if clear guidelines exist will clinicians be able to use these techniques effectively as a truly point-of-care evaluation of the coagulation status, particularly, but not exclusively of inflammatory conditions.

In this paper we discuss the different TEG parameters of both WB and PPP, and give interpretations of each parameter if increased or decreased. We link these changes to TEG parameters to SEM visualization of fine ultrastructure of clot structure.

2. Materials and methods

2.1. Blood samples

Citrated whole blood was collected from healthy individuals, diabetic patients, thrombo-embolic ischemic stroke, Alzheimer's type dementia and Parkinson's disease individuals from 2014 to 2016. A clinician

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Table 1
TEG clot parameters for whole blood and platelet poor plasma.

Parmeters	Explanation
R value: reaction time measured in minutes	Time of latency from start of test to initial fibrin formation (amplitude of 2 mm); i.e. initiation time
K: kinetics measured in minutes	Time taken to achieve a certain level of clot strength (amplitude of 20 mm); i.e. amplification
A (Alpha): Angle (slope between the traces represented by R and K) Angle is measured in degrees	The angle measures the speed at which fibrin build up and cross linking takes place, hence assesses the rate of clot formation; i.e. thrombin burst
MA: Maximal Amplitude measured in mm	Maximum strength/stiffness of clot. Reflects the ultimate strength of the fibrin clot, i.e. overall stability of the clot
Maximum rate of thrombus generation (MRTG) measured in Dyn cm^{-2} s^{-1}	The maximum velocity of clot growth observed or maximum rate of thrombus generation using G, where G is the elastic modulus strength of the thrombus in dynes per cm^{-2}
Time to maximum rate of thrombus generation (TMRTG) measured in minutes	The time interval observed before the maximum speed of the clot growth
Total thrombus generation (TTG) measured in Dyn.cm^{-2}	The clot strength: the amount of total resistance (to movement of the cup and pin) generated during clot formation. This is the total area under the velocity curve during clot growth, representing the amount of clot strength generated during clot growth

identified the patients, and a qualified phlebotomist did the collection. Samples were collected under very strict aseptically conditions, to prevent contamination of samples. Healthy individuals were not on any chronic medication, did not smoke and if female, were not using hormonal interventions.

2.2. Ethical statement

Collection of blood from the individual diseases, was approved by the Ethical Committee of the University of Pretoria (South Africa). A written form of informed consent was obtained from all donors or family members of donors in the case of Alzheimer's type dementia (available on request). Blood was collected and methods were carried out in accordance with the relevant guidelines of the ethics committee. We adhered strictly to the Declaration of Helsinki.

2.3. Clot property studies

Clot property studies performed with thromboelastography (TEG) were done in the following manner: 340 μL of WB or PPP were placed in a TEG cup and 20 μL of 0.2 M $CaCl_2$ was added. $CaCl_2$ is necessary to reverse the effect of the sodium citrate and consequently initiate coagulation. The samples were then placed in a Thromboelastograph 5000 Hemostasis Analyzer System for analysis. We also looked at the effect of time after collection and storage procedure (30 min, 1 h, 3 h and 24 h at room temperature; and 24 h stored at −4 °C), using both WB and PPP from healthy individuals.

Table 2
TEG parameters of platelet poor plasma and whole blood of healthy individuals (datasets from [16,17]).

	R	K	Angle	MA	MRTGG	TMRTGG	TTG
TEG of a typical healthy PPP citrate profile (n = 71)							
Median	8.0	3.5	61.6	28.3	4.6	10.3	201.6
Mean	8.4	4.5	61.4	28.9	5.1	10.3	216.1
SD	3.0	3.1	10.0	7.4	3.5	3.5	86.7
TEG of a typical healthy whole blood citrate profile (n = 30)							
Median	8.1	4.9	49.8	55.1	2.6	13.9	615.0
Mean	8.0	5.5	51.2	53.7	2.9	13.3	623.1
SD	1.6	2.6	7.3	8.1	1.1	3.5	179.6
TEG ranges (machine specified)	9–27	2–9	22–58	44–64	0–10	5–23	251–1014

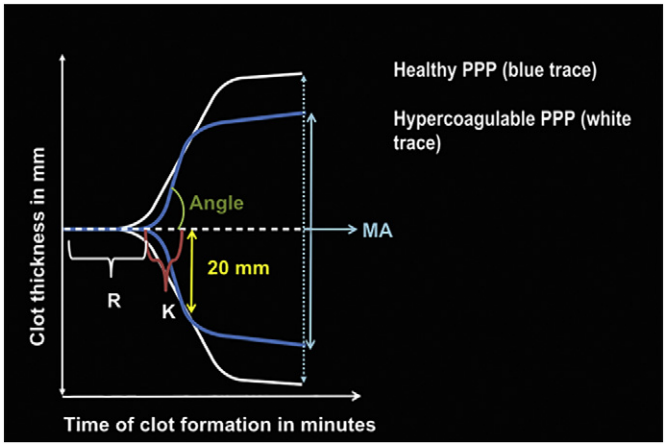

Fig. 1. A representative trace that shows the various TEG parameters.

2.4. Ultrastructural analysis

Scanning electron microscopy (SEM) was used to investigate clot ultrastructure. Thrombin was provided by the South African National Blood Services of SANBS and prepared in biological buffer containing 0.2% serum albumin to obtain a final concentration of 20 U/mL. Thrombin converts fibrinogen to fibrin and thus produces an expansive fibrin fibre network.

Whole blood or PPP (10 μL) were individually mixed with thrombin (5 μL) on glass coverslips. Samples were immediately placed on a dampened filter paper (0.075 M sodium potassium phosphate buffer solution with pH = 7.4) inside an airtight container, thus creating a humid environment for incubation at 37 °C (for 5 min). Samples were subsequently washed for 20 min in 0.075 M sodium potassium phosphate buffer solution (pH = 7.4).

After washing the samples underwent primary fixation with 4% formaldehyde for 30 min and followed by three wash changes in 0.075 M sodium potassium phosphate buffer solution (pH = 7.4) of 5 min each. Sample then underwent a secondary fixation step with osmium tetraoxide (1% OsO_4) for 15 min and again followed with a wash step as mentioned before. Samples were then dehydrated in a series of ethanol concentrations: 30%, 50%, 70%, 90% and three times 100% for 5 min each. Samples were lastly submerged in 100% hexamethyldisilazane (HDMS) for 30 min and air-dried in a flow hood. Samples were mounted on an aluminium platform with carbon tape and coated with carbon before it was examined and micrographed with a Zeiss Ultra plus FEG SEM or Zeiss High Resolution CryoSEM at 1 kV.

3. Results and discussion

Table 1 shows TEG clot parameters of WB and PPP and explanations of each parameter. Table 2 shows averages of TEG parameters for PPP parameters (n = 71) and WB parameters (n = 30) of healthy individuals (datasets previously shown in [16,17]). Fig. 1 shows a representative trace that labels the various parameters. Table 3 clarifies whether PPP or WB is hypo- or hypercoagulable when increases or decreases in the measured TEG parameters are seen. Table 4 shows the time and