A comparative study of tissue factor and kaolin on blood coagulation assays using rotational thromboelastometry and thromboelastography

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Rotational thromboelastometry (ROTEM) and thromboelastography (TEG) have been increasingly used to diagnose acute coagulopathy and guide blood transfusion. The tests are routinely performed using different triggering activators such as tissue factor and kaolin, which activate different pathways yielding different results. To optimize the global blood coagulation assays using ROTEM and TEG, we conducted a comparative study on the activation methods employing tissue factor and kaolin at different concentrations as well as standard reagents as recommended by the manufacturer of each device. Key parameter values were obtained at various assay conditions to evaluate and compare coagulation and fibrinolysis profiles of citrated whole blood collected from healthy volunteers. It was found that tissue factor reduced ROTEM clotting time and TEG R, and increased ROTEM clot formation time and TEG K in a concentration-dependent manner. In addition, tissue factor affected ROTEM alpha angle, and maximum clot firmness, especially in the absence of kaolin activation, whereas both ROTEM and TEG clot lysis (LI30, CL30, and LY30) remained unaffected. Moreover, kaolin reduced ROTEM clotting time and TEG R and K, but to a lesser extent than tissue factor, in-tem and ex-tem. Correlations in all corresponding parameters between ROTEM and TEG were observed, when the same activators were used in the assays compared with lesser correlations between standard kaolin TEG and ROTEM (INTEM/EXTEM). The two types of viscoelastic point-of-care devices provide different results, depending on the triggering reagent used to perform the assay. Optimal assay condition was obtained to reduce assay time and improve assay accuracy. ***Blood Coagul Fibrinolysis*** **26:000–000**

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Introduction

Thromboelastography (TEG; Haemonetics Corporation, Haemoscope Division, Niles, Illinois, USA) and rotational thromboelastometry (ROTEM; Tem Innovations GmbH, Munich, Germany) are two point-of-care viscoelastic tests of hemostasis in the whole blood. In the past few years, both have been increasingly used to diagnose acute coagulopathy and guide blood transfusion in early resuscitation [1–3]. Studies have shown the benefits of ROTEM-guided therapy with fibrinogen concentrate and prothrombin complex concentrate in different perioperative settings, for example, a reduction in transfusion requirements for plasma, red blood cells, and platelets [4]. Early TEG-guided hemostatic resuscitation may have contributed to low hemorrhage-related mortality in trauma patients [5]. A recent study has concluded that TEG variables can replace conventional coagulation tests [e.g., prothrombin time (PT)/international normalized ratio (INR)] for predicting transfusion requirements in trauma [6]. In addition, a rapid turnaround time (time from specimen collection to result availability in <15 min) for PT/INR and fibrinogen level have been utilized at major trauma centers in the North America to facilitate timely transfusion, but the availability of PT/INR information is relatively limited [7].

Although both ROTEM and TEG techniques record the viscoelastic changes that occur during the whole coagulation process, there is a primary hardware difference. ROTEM has an immobile cup, wherein the pin/wire transduction system slowly oscillates an arc of 4°45′. In addition, each ROTEM system has four channels and a built-in computer to operate as opposed to two channels in the TEG system that requires a separate computer to operate. It has been suggested that the ROTEM system uses a ball-bearing system for power transduction, which makes it less susceptible to movement and vibration [8]. In addition, different reagents are used in the two systems based on different hemostasis models. TEG tests are based on a cell-based model of hemostasis [9], whereas ROTEM tests are based on intrinsic and extrinsic coagulation pathways [10].

Studies have shown that the two tests provide different results for diagnosing coagulopathy and guiding transfusion [3]. For example, different transfusion algorithms have been developed for each system [11]. ROTEM-

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guided transfusion tended to recommend fibrinogen concentrate [12] whereas TEG-based algorithms appeared to recommend plasma [13], but both recommendations have resulted in decreased blood transfusion compared with standard laboratory measures of blood coagulation. The difference may be more because of the assays as opposed to the instrument *per se*, as most ROTEM-guided transfusion involved FIBTEM, which is a specific assay for fibrinogen level and function [4]. In contrast, functional fibrinogen assay was less used for TEG-guided transfusion [14]. In addition, there is a lack of studies to compare their utilities for diagnosis of coagulopathy, prediction of mortality, and the requirement for massive transfusion, although both have been reported useful [15,16]. On the contrary, some comparative studies indicated certain correlations between the two systems depending on the measurement parameters [17,18], activation reagents [19], blood samples [18,20] and specific assays [21,22]. The blood components and the type and concentration of activators could influence the interpretation of coagulation profiles measured by the test [23]. Simultaneous assays using various reagents could improve diagnosis and therapeutic outcomes [24].

There is a need to compare the two systems and optimize the assay reagents for better utilization of the technology for guiding blood transfusion. The objective of this study was to investigate the effects of tissue factor and kaolin concentrations on ROTEM and TEG variables in comparison with the standard assay method and ultimately optimize the assays. In addition, a crossover analysis (kaolin on ROTEM, and ex-tem and in-tem on TEG) was also conducted to confirm whether the assay reagents or the device may contribute to the observed effects. We hypothesize that activation reagents are the main contributors to the different assays developed for the two viscoelastic point-of-care systems leading to different diagnostics and transfusion algorithms.

Materials and methods

Reagents

AQ2 Kaolin vials containing kaolin and buffer were purchased from Haemonetics Corporation, Haemoscope Division (Niles, Illinois, USA). Lyophilized reagent consisting of recombinant human tissue factor, synthetic phospholipids, calcium ions, a heparin-neutralizing compound, buffers, and stabilizers (bovine serum albumin) (Dade Innovin; Siemens Canada Limited, Oakville, Ontario, Canada) was dissolved in Milli-Q water and serially diluted to a target concentration before each use [25]. Start-term, in-tem, and ex-tem were purchased from TEM Canada Ltd. (Mississauga, Ontario, Canada) and used as per manufacturer's procedures.

Blood samples

Blood was drawn via phlebotomy from healthy volunteers into BD vacutainers containing 3.2% sodium citrate (Fisher Scientific, Nepean, Ontario, Canada). Blood samples collected from eight individuals were analyzed for the study of the effects of activators concentrations and from another 11 individuals were analyzed by the standard activators as recommended by each manufacturer. The study was approved by the Ethics Committee of the Defence Research and Development Canada, Toronto Research Centre, Toronto, Ontario, Canada. All volunteers provided informed consent.

Rotational thromboelastometry analysis

The citrate whole blood was analyzed using the ROTEM delta system (Tem Innovations GmbH, Munich, Germany). The instrument was periodically checked with ROTROL-N and ROTROL-P for quality controls. Specifically, analyses were performed using 300 μl of whole citrated blood and 20 μl of start-tem (0.2 M calcium chloride) together with 20 μl of standard reagents (i.e., in-tem and ex-tem) or specific activators at various concentrations. In the INTEM test, 20 μl of in-tem reagent composed of ellagic acid, buffer, preservatives, and partial thromboplastin phospholipid from rabbit brain was added. In the EXTEM test, 20 μl of ex-tem reagent composed of recombinant tissue factor, phospholipids, preservatives, heparin inhibitor, and buffer was used. In the tissue factor test, 20 μl of Innovin activator was AQ3 added. For each test, 300 μl of citrated whole blood was then added. The mixture with a total volume of 340 μl was withdrawn and pipetted back to the cup, and the cup was loaded onto the pin immediately to start the test. In addition, some tests were conducted with blood samples that have been first activated with kaolin by adding 1 ml to a kaolin vial (Haemoscope Corporation, Niles, Illinois, USA). The sample was mixed by inversion five times and analyzed as mentioned earlier. The following parameters were recorded for the ROTEM tests: clotting time [CT (s); time from the start of the test until a clot firmness of 2 mm is detected], clot formation time [CFT (s); time from CT until a clot firmness of 20 mm is detected], alpha angle (°), maximum clot firmness (MCF, mm), and clot lysis [LI30 (%)].

Thromboelastography analysis

The citrate whole blood was also analyzed in parallel using a computerized TEG Hemostasis System 5000 (Haemonetics Corporation, Haemoscope Division, Niles, Illinois, USA). The system had to pass the electronics testing and quality control according to manufacturer's protocol prior to sample analysis. To keep the reagent:-blood ratio constant with the ROTEM tests, calcium chloride solution and tissue factor solution at various concentrations, or in-tem or ex-tem were pipetted into a cup at 21 μl each, followed by 318 μl of citrated whole blood. The mixture with a total volume of 360 μl was withdrawn and pipetted back to the cup, and the cup was loaded onto the pin immediately to start the test. The citrated blood was activated with kaolin as mentioned

earlier. In addition, different volumes (0–2 ml) of citrated blood were transferred to a vial with buffered stabilizers and kaolin (Haemoscope Corporation) to alter relative kaolin concentration. The measurement was run until all following interested parameter values were obtained: reaction time, R (s), kinetics time, K (s), alpha angle (°), maximum amplitude (mm), and clot lysis, LY30/CL30 (%).

Comparison of rotational thromboelastometry and thromboelastography

We compared the corresponding parameters between the two instruments performed simultaneously to analyze the same blood sample using the standard activators as recommended by each manufacturer as well as using the same activators. Kaolin is a standard activator for TEG and the test is referred to as kaolin TEG, whereas in-tem and ex-tem were standard activation reagents for ROTEM and the tests are referred to as INTEM and EXTEM. In addition, we compared ROTEM and TEG when the same activator (i.e., kaolin, tissue factor, and ex-tem and in-tem) was used. TEG is normally started manually immediately after samples are mixed, whereas ROTEM can be started automatically as soon as blood is added (before samples are mixed). To eliminate the difference between the devices, therefore, both measurements were manually started, immediately after the samples were mixed once by withdrawing and dispensing 340 or 360 μl in the ROTEM or TEG cup, respectively.

All the TEG and ROTEM parameters were derived in the same way for each assay, except the one for fibrinolysis. The TEG system provided LY30 and CL30. LY30 is computed as the percentage reduction of the area under a TEG tracing from the time maximum amplitude is measured until 30 min after the maximum amplitude. CL30 represents the value of the amplitude of a TEG tracing at 30 min after the maximum amplitude relative to maximum amplitude. The larger the value of LY30, the greater severity of the fibrinolytic process, which is inverse to CL30. The ROTEM system provided LI30 as a measure of fibrinolysis. It is calculated as the ratio between clot firmness (in millimeter amplitude) at CT + 30 min and MCF. Generally, when LY30 is high (i.e., fibrinolytic activity is high), LI30 is low, and vice versa. There are no same fibrinolytic parameters between TEG and ROTEM. CL30 is a fibrinolytic parameter in TEG most similar to LI30 in ROTEM. Therefore, we compared TEG CL30 other than LY30 with ROTEM LI30 for fibrinolysis.

Statistical analysis

Data were represented as mean ± standard deviation. One-way analysis of variance was used to analyze the effects of tissue factor concentration on both ROTEM and TEG parameters, and kaolin concentration on TEG parameters with post-hoc comparison using Tukey's honestly significant difference test. Student's *t* tests were used to compare the corresponding parameters between ROTEM and TEG. Pearson's method was used to evaluate correlations among the parameters. The number of samples we collected allowed 57% and above observed power to detect the effects of tissue factor concentration on ROTEM and TEG parameters except for kaolin TEG LY30 and kaolin ROTEM MCF (Table 1), and above 50% observed power to detect the effects of kaolin concentration on kaolin TEG R, K, and maximum amplitude. Statistical power varied from 0.1 to 1.0 to detect a difference in parameter values between ROTEM and TEG at various tissue factor concentrations. All statistical analyses were performed using IBM SPSS Statistics 21 (IBM Corporation, Armonk, New York, USA) with a significant level of P less than 0.05.

Results

Effects of activators

Tissue factor reduced ROTEM CT and TEG reaction time (R) in a concentration-dependent manner approximating an ultimate time limit, which was even shorter than the values obtained using the ex-tem assay reagent as shown in Fig. 1a. However, ROTEM CFT and TEG kinetics time (K) were increased, both above the control values obtained using the ex-tem and in-tem assay reagents (Fig. 1b). To a lesser extent, tissue factor affected alpha angle, maximum clot strength, and clot lysis in both ROTEM and TEG (Fig. 1c–e). It seems ROTEM was more sensitive to tissue factor concentration and activators than TEG as implied by significant effects on ROTEM alpha and LI30 (Table 1). At the minimal tissue factor concentration (0.08 ng/ml), the coagulation profiles measured by TEG R, K, and ROTEM CT and CFT were still different from those obtained without tissue factor (Table 1). When the same activation reagents (i.e., ex-tem and in-tem) were used, there were no significant differences in corresponding parameter values between ROTEM and TEG, whereas significant differences were seen between TEG R, alpha, and ROTM CT, alpha when kaolin was used (Table 1). However, when activated with kaolin and tissue factor at various concentrations, TEG R and K tended to be larger than ROTEM CT and CFT, TEG alpha and CL30 were smaller than ROTEM alpha and LY30, and TEG maximum amplitude were not different from ROTEM MCF (Table 1). AQ4

The effects of kaolin on ROTEM performed using different activators (tissue factor, ex-tem, and in-tem) are shown in Fig. 2. Together with Table 1, we observed that kaolin reduced the CT at low tissue factor concentrations (<1.0 ng/ml), but had no effects when blood was activated with tissue factor at high concentrations, ex-tem, and in-tem, respectively. On the other hand, greater effects of tissue factor were seen on ROTEM alpha and MCF, in the absence of kaolin activation.

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Table 1 Descriptive data of thromboelastography and rotational thromboelastometry measurements of blood samples activated by various methods

Activation method		Tissue factor (TF) concentration (ng/ml)													
		ex-tem										in-tem	Comparison among activators	Observed power
		0	0.08	0.4	1.0	4.9	9.7	19.5	39.0	77.9	155.8			
Kaolin TEG	R (s)	44.6 ± 7.6	290.6 ± 80.6[a]	116.3 ± 21.3	91.6 ± 20.9[a]	67.5 ± 31.1	56.0 ± 3.5	51.0 ± 3.5	23.0 ± 5.0	22.0 ± 4.9	26.0 ± 9.2	21.0 ± 7.7	157.0 ± 43.3	$F_{11,52} = 23.3$; $P < 0.001$	1.00
	K (s)	74.6 ± 16.2	87.8 ± 25.4	83.3 ± 25.1	85.2 ± 31.9	91.5 ± 34.4	72.0 ± 10.4	61.5 ± 9.0[a]	82.0 ± 28.1	92.4 ± 25.7[a]	186.0 ± 93.7	214.5 ± 101.3	70.0 ± 20.0	$F_{11,52} = 5.5$; $P < 0.0001$	1.00
	Alpha (°)	73.2 ± 3.0	65.4 ± 10.2[a]	71.2 ± 5.3	66.0 ± 10.6	64.9 ± 14.7	74.4 ± 2.8[a]	75.9 ± 2.3[a]	73.9 ± 4.2	72.5 ± 3.9	65.4 ± 4.1	67.2 ± 8.4	69.8 ± 4.6	$F_{11,52} = 1.6$; $P = 0.140$	0.72
	MA (mm)	62.2 ± 4.0	60.8 ± 2.4	55.4 ± 6.8	61.2 ± 7.2	60.7 ± 8.9	59.2 ± 4.9	61.2 ± 3.6	59.6 ± 4.2	57.6 ± 6.7	57.2 ± 5.1	54.5 ± 5.6	60.4 ± 2.4	$F_{11,52} = 1.1$; $P = 0.354$	0.57
	LY30 (%)	1.6 ± 0.4	1.7 ± 1.3	1.4 ± 1.1	1.8 ± 1.3	1.5 ± 2.6	2.4 ± 0.6	2.1 ± 1.3	1.6 ± 1.1	1.6 ± 1.6	1.3 ± 1.5	0.1 ± 0.2	1.9 ± 0.6	$F_{11,52} = 0.687$; $P = 0.744$	0.34
	CL30 (%)	94.8 ± 1.8[a]	95.3 ± 2.9[a]	95.7 ± 2.4[a]	95.0 ± 3.4	95.6 ± 5.5	93.0 ± 1.4[a]	94.3 ± 2.8	95.1 ± 3.2[a]	95.6 ± 3.6[a]	98.9 ± 1.9	99.5 ± 1.0	95.0 ± 1.9[a]	$F_{11,52} = 1.5$; $P = 0.155$	0.70
Kaolin ROTEM	CT (s)	37.5 ± 6.8	189.7 ± 18.0[b]	99.0 ± 12.5[b]	56.7 ± 6.5[b]	50.2 ± 9.2	61.5 ± 3.5	50.5 ± 11.0	31.2 ± 4.9[b]	28.3 ± 9.8	27.7 ± 5.0	24.4 ± 9.9	127.1 ± 39.7	$F_{11,40} = 19.9$; $P < 0.001$	1.00
	CFT (s)	82.6 ± 17.4	83.7 ± 5.5	63.3 ± 10.4	64.7 ± 6.0	61.6 ± 11.9	79.0 ± 8.2	88.3 ± 11.5	123.4 ± 50.5	135.0 ± 36.1	263.0 ± 33.9	371.0 ± 180.1	79.4 ± 22.5	$F_{11,40} = 15.3$; $P < 0.001$	1.00
	Alpha (°)	72.9 ± 3.5	73.7 ± 0.6	74.0 ± 5.0	77.7 ± 0.6	75.5 ± 5.4	74.3 ± 1.5	72.3 ± 1.5	67.0 ± 7.4	65.5 ± 6.4	62.5 ± 9.3	62.5 ± 7.3	74.6 ± 4.0	$F_{11,40} = 2.4$; $P = 0.026$	0.87
	MCF (mm)	60.0 ± 5.2	57.0 ± 4.4	60.8 ± 7.4	62.7 ± 5.7	57.8 ± 6.3	57.3 ± 4.9	58.3 ± 4.2	56.5 ± 7.4	55.5 ± 7.0	55.8 ± 5.8	53.0 ± 3.4	57.3 ± 5.6	$F_{11,40} = 0.9$; $P = 0.522$	0.40
	LI30 (%)	99.3 ± 0.9	98.3 ± 0.6	99.2 ± 0.8	98.5 ± 0.7	98.0 ± 2.1	97.3 ± 1.5	98.0 ± 1.0	99.2 ± 0.8	99.5 ± 0.6	100.0 ± 0.0	100.0 ± 0.0	98.1 ± 1.4	$F_{11,40} = 1.9$; $P = 0.068$	0.74
ROTEM	CT (s)	36.3 ± 10.1	250 ± 15.1	130.6 ± 7.2	85.3 ± 11.6	61.0 ± 7.1	60.0 ± 19.4	52.0 ± 13.5	34.5 ± 4.2	28.8 ± 6.2	22.3 ± 9.7	23.4 ± 8.1	141.6 ± 29.2	$F_{11,47} = 86.0$; $P < 0.001$	1.00
	CFT (s)	81.1 ± 12.2	108.3 ± 17.1	59.2 ± 8.2	76.3 ± 28.0	86.5 ± 34.4	80.6 ± 7.5	75.3 ± 9.2	94.7 ± 14.2	114.7 ± 23.0	181.0 ± 46.8	426.8 ± 195.8	69.3 ± 10.1	$F_{11,47} = 14.3$; $P < 0.001$	1.00
	Alpha (°)	73.7 ± 2.8	76.0 ± 4.6	78.0 ± 1.8	73.0 ± 6.3	75.0 ± 6.8	73.3 ± 0.5	74.8 ± 1.7	70.2 ± 1.3	67.8 ± 4.6	65.3 ± 9.0	53.0 ± 12.5	74.6 ± 4.0	$F_{11,47} = 8.2$; $P < 0.001$	1.00
	MCF (mm)	62.9 ± 4.6	63.0 ± 6.2	67.0 ± 1.8	62.0 ± 7.5	64.3 ± 6.9	59.8 ± 2.9	60.8 ± 2.9	59.0 ± 3.7	58.7 ± 3.9	59.0 ± 5.2	53.6 ± 3.0	60.0 ± 3.7	$F_{11,47} = 2.9$; $P = 0.005$	1.00
	LI30 (%)	99.6 ± 0.8	99.0 ± 1.7	98.8 ± 1.5	98.5 ± 1.0	99.3 ± 1.5	98.0 ± 0.8	99.0 ± 0.8	99.4 ± 0.5	99.7 ± 0.5	100 ± 0.0	100 ± 0.0	98.7 ± 1.2	$F_{11,47} = 0.4$; $P = 0.96$	0.71

~~CT, clotting time; ROTEM, rotational thromboelastometry; TEG, thromboelastography.~~ Data represent mean ± standard deviation. [a] Significant difference between kaolin TEG and kaolin ROTEM ($P < 0.05$). [b] Significant difference between kaolin ROTEM and ROTEM ($P < 0.05$).

The effects of kaolin at relatively different concentrations (amount of blood added to kaolin vial) on TEG are shown in Fig. 3. Kaolin activation reduced TEG R and K, but had no significant effects on TEG alpha, maximum amplitude, LY30, and CL30. There were no further effects of kaolin among various concentrations on the TEG variables.

Correlations between rotational thromboelastometry and thromboelastography

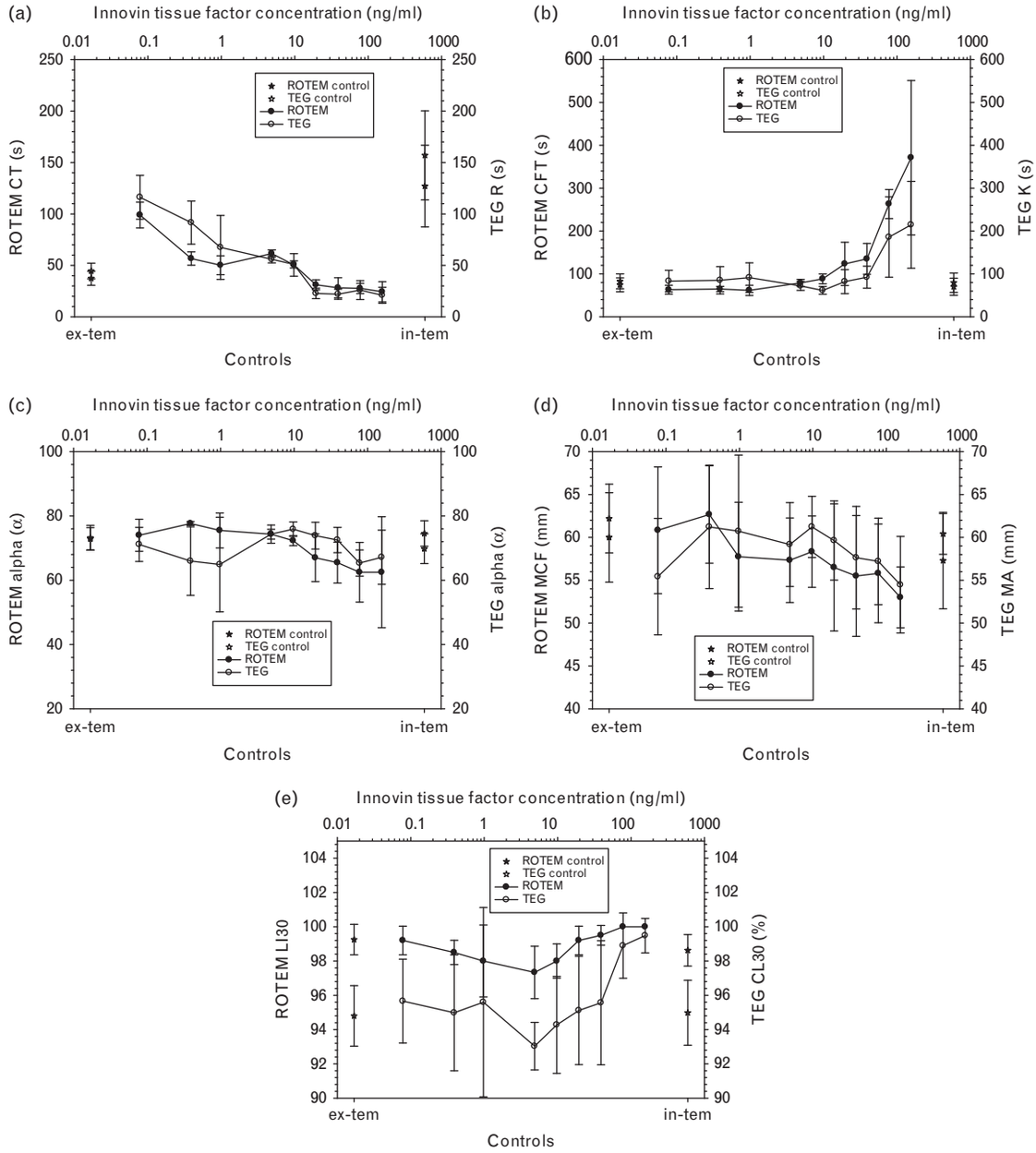
Significant correlations were seen between kaolin TEG R and INTEM CT ($r = 0.54$, $P = 0.005$) (Fig. 4a), kaolin TEG K and EXTEM CFT ($r = 0.412$, $P = 0.041$) (Fig. 4b), maximum amplitude and INTEM MCF ($r = 0.679$, $P < 0.001$), and EXTEM ($r = 0.641$, $P = 0.001$) (Fig. 4d). The kaolin TEG had a larger range of R (168–456 s) than INTEM CT (106–197 s) and EXTEM CT (23–63 s), comparable range of K (48–138 s) with INTEM CFT (52–107 s) and EXTEM CFT (65–123 s), a wider distribution of alpha angle (40–79°) than INTEM alpha (69–79°) and EXTEM alpha (66–77°), a range of maximum amplitude (51–68 mm) similar to INTEM MCF (48–64 mm) and EXTEM MCF (51–66 mm), and a larger range of CL30 (72–100%) than INTEM LI30 (95–100%) and EXTEM LI30 (81–100%).

In contrast, significant correlations were shown for all corresponding parameters between TEG and ROTEM performed with the same activators (Fig. 5). Specifically, Fig. 5a shows a high correlation between TEG R and ROTEM CT ($r = 0.919$, $P < 0.001$), and K and CFT ($r = 0.610$, $P < 0.001$). Figure 5b shows a moderate correlation between TEG alpha and ROTEM alpha ($r = 0.466$, $P = 0.005$) and a high correlation between maximum amplitude and MCF ($r = 0.652$, $P < 0.0001$). Figure 5c depicts a high correlation between CL30 and LI30 ($r = 0.654$, $P < 0.0001$) and between CL30 and LY30 ($r = 0.928$, $P < 0.0001$). The correlation coefficients were higher compared with those observed for the standard TEG and ROTEM tests. Moreover, when the same activators (tissue factor at various concentrations, kaolin, in-tem, and ex-tem) were used, TEG provided a range of R (12–198 s) close to ROTEM CT (11–174 s), a range of K (48–384 s) smaller than ROTEM CFT (49–579 s), a very similar range of TEG alpha angle (41–81°) to ROTEM alpha angle (43–80°), comparable maximum amplitude (44–73 mm) with MCF (47–69 mm), and slightly larger range of CL30 (89–100%) than LI30 (95–100%).

Discussion

In this study, we optimized tissue factor and kaolin concentrations for minimal assay time. Tissue factor is a cell surface glycoprotein that binds coagulation factor VII to trigger extrinsic coagulation pathway. Kaolin is mineral clay that activates coagulation factor XII

Fig. 1

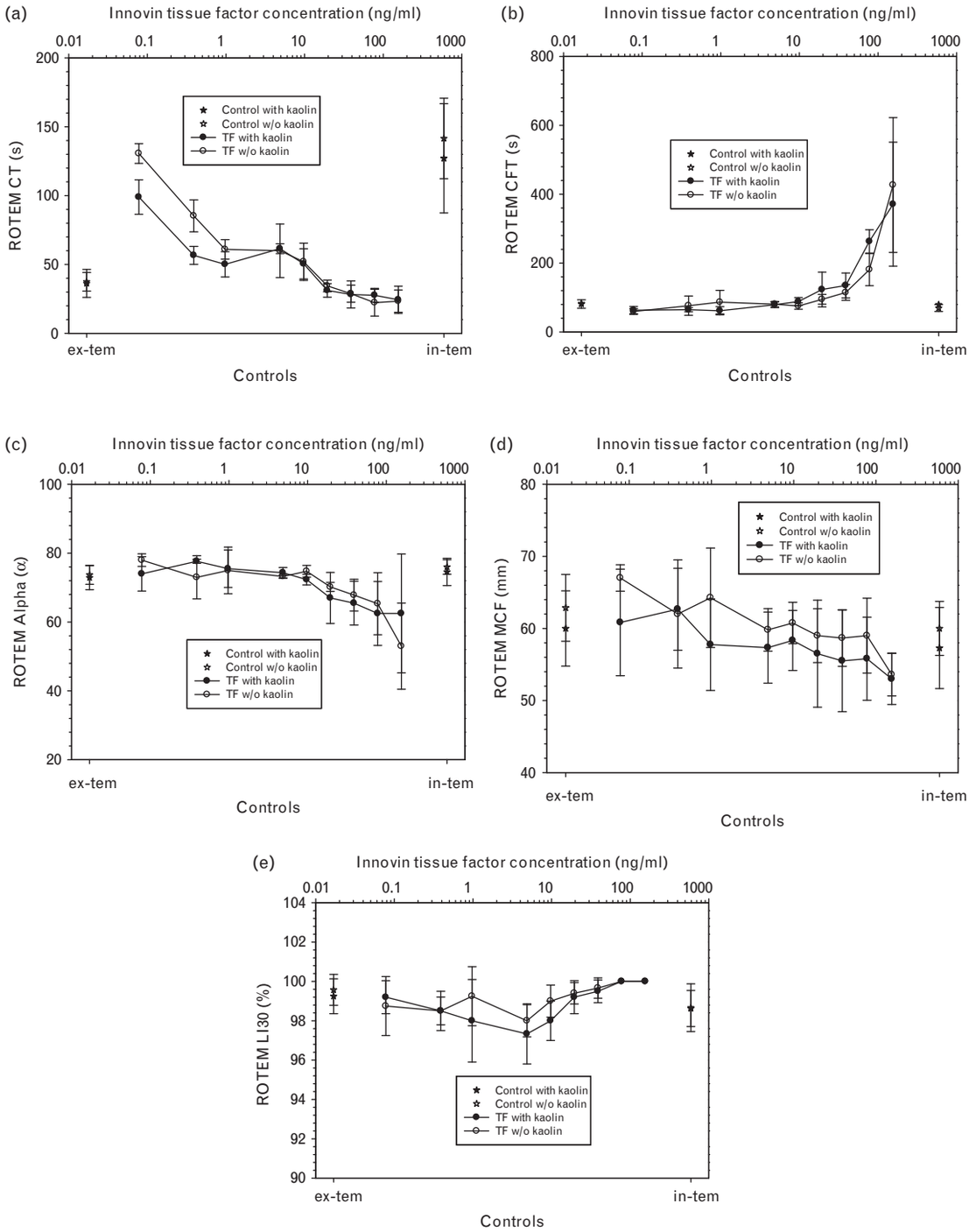


Effects of tissue factor concentration on coagulation and fibrinolysis of kaolin-activated citrated blood in comparison with standard controls as measured by rotational thromboelastometry and thromboelastography. Data represent mean ± standard deviation ($n = 3–8$). (a) Clotting time (CT) and reaction time (R). (b) Clot formation time (CFT) and clotting time (K). (c) Alpha angle. (d) Maximum clot firmness (MCF) and maximum amplitude (MA). (e) Clot lysis (LI30 and CL30). ROTEM, rotational thromboelastometry; TEG, thromboelastography.

through its surface charges to trigger intrinsic coagulation pathway.

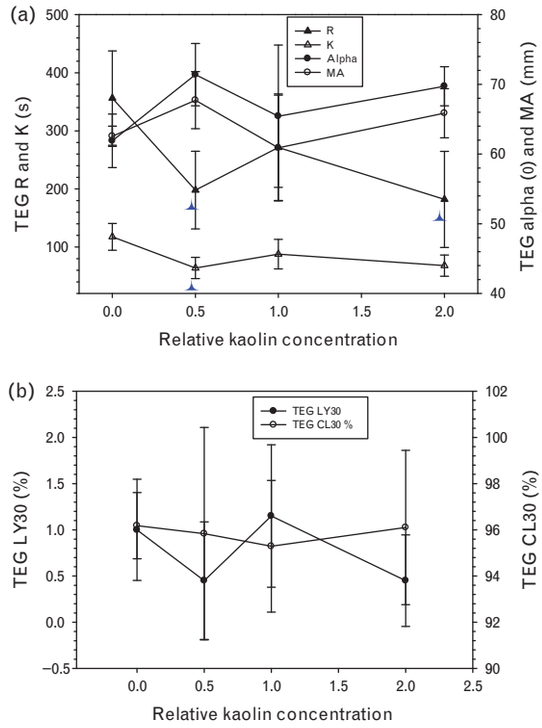
The tissue factor concentration in our study ranged from 0.08 to 155.8 ng/ml. At a high concentration range (≥19.5 ng/ml), tissue factor led to a shorter CT and R, but a longer CFT and K compared with ex-tem. Therefore, to reduce the time for TEG and ROTEM assays, the optimal concentration should be around 19.5 ng/ml, corresponding to that of the stock solution prepared according to the manufacturer's instructions [25]. At this concentration, TEG and ROTEM variables showed lower or comparable variations than those obtained with ex-tem and in-tem (Table 1).

Fig. 2



Effects of tissue factor concentration on rotational thromboelastometry of citrated blood with and without (w/o) kaolin activation in comparison with standard controls. Data represent mean ± standard deviation ($n = 3–8$). (a) CT. (b) CFT. (c) Alpha angle. (d) MCF. (e) Clot lysis (LI30). CFT, clot formation time; CT, clotting time; MCF, maximum clot firmness; ROTEM, rotational thromboelastometry; TEG, thromboelastography; TF, tissue factor.

Fig. 3



Effects of kaolin concentration on (a) TEG reaction time (R), clotting time (K), alpha angle, and maximum amplitude (MA); and (b) TEG clot lysis (LY30 and CL30). Different relative kaolin concentrations were obtained by adding different volumes (0–2 ml) of citrated blood into a kaolin vial (Haemonetics Corporation, Haemoscope Division, Niles, Illinois, USA). Data represent mean ± standard deviation ($n = 3–8$). One-way analysis of variance indicated a significant effect of kaolin activation on TEG R ($F_{3,18} = 3.51$, $P = 0.042$) and K ($F_{3,18} = 4.56$, $P = 0.018$). *Significant difference from no kaolin activation ($P < 0.05$). TEG, thromboelastography.

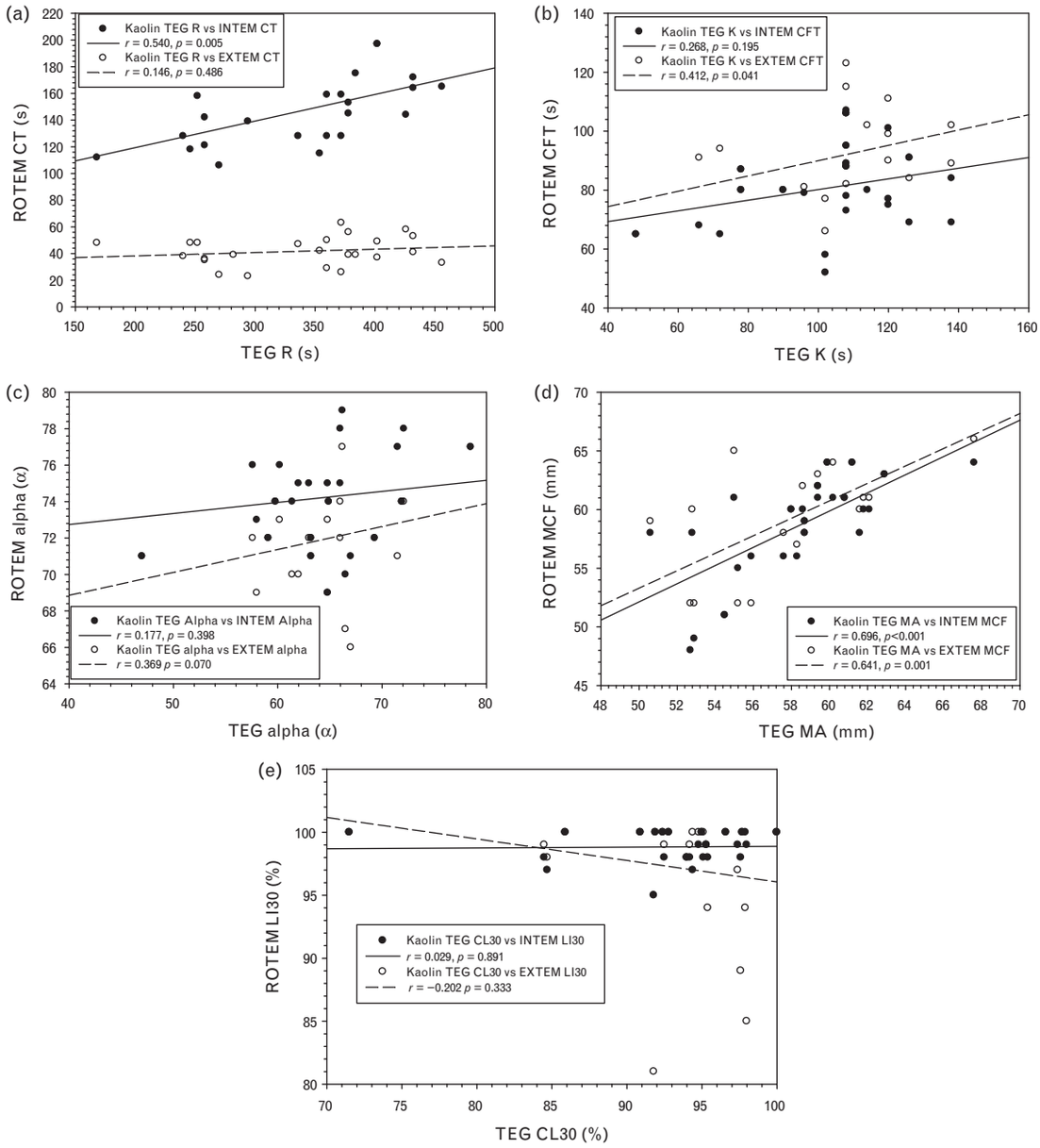
Previous research used a range of dilutions of the tissue factor preparation from 1 : 17 (~350 pM) up to 1 : 170 000 (~0.035 pM) [23,26], or specific dilutions of 1 : 3333 [27], 1 : 17 000 (corresponding to a theoretical level of ~0.35 pM) [26], 1 : 42 000 [28], and 1 : 50 000 [29] in either TEG or ROTEM. However, the 'optimal' tissue factor amount to use is not yet determined. This is the first study optimizing and comparing the effects of tissue factors on all key parameters between the two systems.

Our findings of the effects of tissue factor and kaolin on whole-blood coagulation profiles are consistent with other results reported by Sørensen *et al.* [23,26] and Johansson *et al.* [30]. Specifically, the concentration-dependent decrease in TEG R and ROTEM CT, and minimal effects on TEG maximum amplitude and ROTEM MCF by tissue factor is consistent with the results reported by Sørensen *et al.* [23], who studied the effects of tissue factor on ROTEM parameters in a dilution from 1 : 17 to 1 : 170 000, but did not report the effects on ROTEM CFT, alpha, and LI30. The concentration-dependent increase in TEG K and ROTEM CFT differs from a previous study, wherein tissue factor was found to lead to a trend of decreased ROTEM CFT of citrated canine blood [31]. The difference is likely due to differences in blood (human vs. canine) and dilutions of tissue factor (45–85 000 vs. 3400–85 000). Together with alpha, TEG K and ROTEM CFT describe the kinetics of the formation of a stable clot (amplification and propagation phase of coagulation) through both activated fibrin and platelets. It may be speculated that at very high concentrations, tissue factor binds factor VII and predominantly activates factor X excluding the involvement of factor IX and VIII complex in intrinsic pathway. The factor IX and VIII complex is more potent for activating factor X [10]. As a result, this slows down further thrombin generation and rate of clot formation at high tissue factor concentrations, prolonging K or CFT and impairing alpha. Another plausible explanation could be that factor VII was saturated with high tissue factor and could not bind to tissue factor containing phospholipid vesicles. Subsequently, factor X or IX could bind to these particles, reducing tissue factor and factor VII complex [23].

TEG R and ROTEM CT were most profoundly impacted by the type and strength of activators, which were expected as the two parameters reflecting the time to initial fibrin polymerization, primarily a function of the rate of initial thrombin formation. The more significant effects of tissue factor on TEG R/K compared with angle and maximum amplitude are consistent with other reports investigating the effects of tissue factor in healthy [30] and hemophiliac individuals [28], and dogs [31]. As reported, both tissue factor and kaolin merely accelerated the initiation phase of the coagulation process, but had a less marked impact on the other phases of coagulation (e.g., propagation and lysis) and clot strength [30,31].

Consistent with the previous observations, our results demonstrate that tissue factor and kaolin exert different effects on both TEG and ROTEM variables [28]. On the contrary, there were less effects of kaolin concentration on both ROTEM and TEG variables compared with the tissue factor concentration, but there was an influence on the tissue factor effects. In addition, in-tem contained a stronger contact activator (i.e., ellagic acid) than kaolin as indicated by shorter TEG R and INTEM CT obtained by using in-tem and there were no effects of kaolin on TEG and ROTEM performed with in-tem. These results suggest that as long as a strong enough initial thrombin burst occurs, the addition of activator does not influence the parameters. Taken all the results together, assay time can be shortened with an optimal tissue factor concentration, without compromising alpha angle and clot strength, by using citrated blood without kaolin activation.

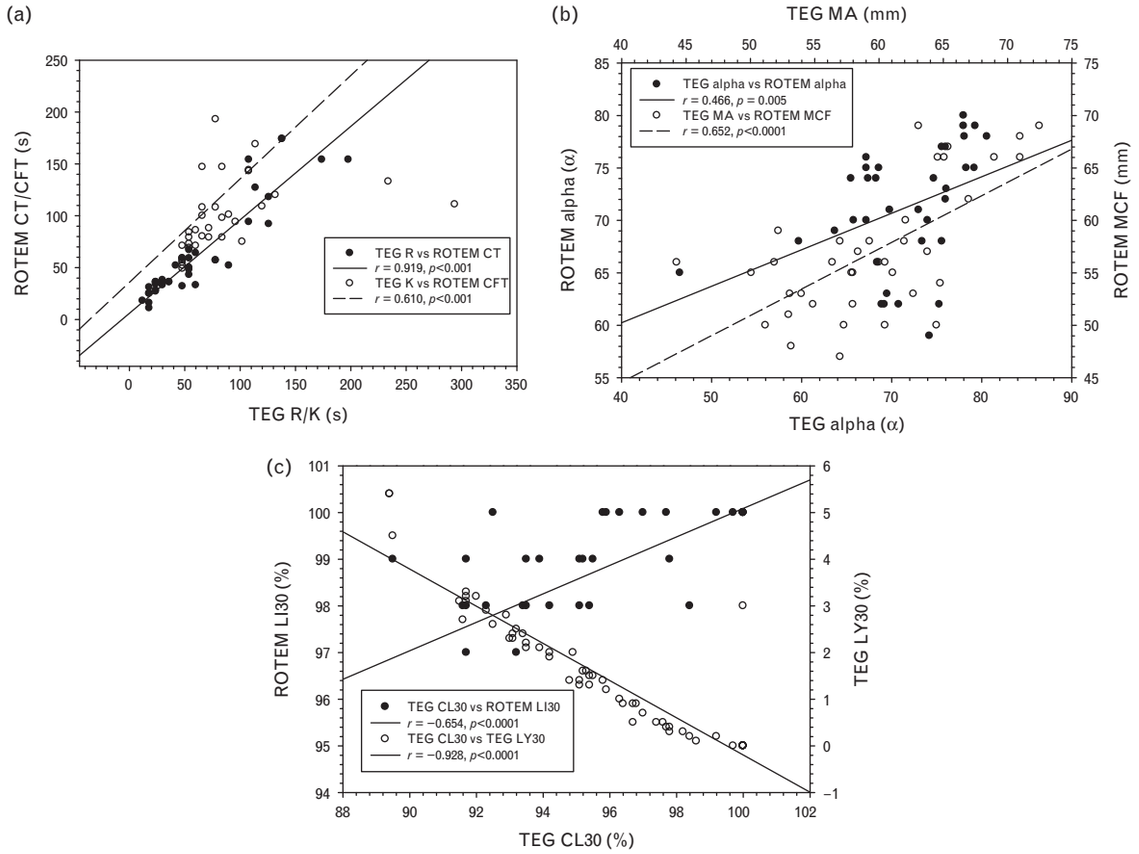
Fig. 4



Correlations between thromboelastography activated by kaolin and rotational thromboelastometry activated by in-tem and ex-tem in (a) coagulation time (R vs. CT); (b) clot formation time (K vs. CFT); (c) alpha angle; (d) maximum clot strength (MA vs. MCF); and (e) clot lysis (CL30 vs. LI30). Significant correlations ($P < 0.05$) were found between TEG R and INTEM CT, TEG K and EXTEM CFT, and TEG MA and INTEM/EXTEM MCF. CFT, clot formation time; CT, clotting time; MA, maximum amplitude; MCF, maximum clot firmness; ROTEM, rotational thromboelastometry; TEG, thromboelastography.

The differences in the parameter values between TEG and ROTEM performed with their standard reagents were generally consistent with others [17,18] and could be ascribed to the differences in both devices and assay reagents used [22]. The differences related to the equipment functions include the mechanisms for cup/pin rotation, detection of the rotation, cup materials, and interior surface properties [18,19]. In terms of reagents, kaolin is a weaker contact activator than ellagic acid in INTEM and is different from tissue factor activation in EXTEM [24]. The longer TEG R, K than both INTEM and EXTEM CT, CFT are consistent with the findings reported by Venema *et al.* [17], who used the same activation methods, but different types of blood (native blood for TEG and citrated blood for ROTEM). The smaller TEG alpha compared with INTEM and

Fig. 5



Correlations between thromboelastography and rotational thromboelastometry activated by the same activators (tissue factor, kaolin, in-tem, and ex-tem) in (a) coagulation time and clot formation time (R/K vs. CT/CFT); (b) alpha angle and maximum clot strength (alpha/MA vs. alpha/MCF); and (c) fibrinolysis (LY30/CL30 vs. LI30). Significant correlations ($P < 0.05$) were found for all the parameters. CT, clotting time; ROTEM, rotational thromboelastometry.

EXTEM alpha for some samples is also in agreement with their report. Tomori *et al.* [18] reported the same differences as ours when comparing TEG and ROTEM for evaluation of coagulation stages of hemorrhaged swine using the same volume ratio of $CaCl_2$ and citrated whole blood and no additional activators. The longer R and smaller alpha were also found in kaolin activation TEG of normal plasma compared with INTEM CT and alpha, but there was no difference between TEG maximum amplitude and INTEM MCF [19]. However, the TEG maximum amplitude is within the same range as INTEM and EXTEM MCF with high correlations, which is consistent with the literature reporting most association between TEG maximum amplitude and ROTEM MCF [17,18,20]. Minimal bias in the maximum clot strength and high correlations between TEG and ROTEM implies that this parameter was not profoundly affected by activation methods. As TEG CL30 was calculated from the amplitude 30 min after maximum amplitude, it should be generally smaller than INTEM LI30, which was calculated from the amplitude 30 min after CT as elucidated in our study. The most differences in clot initiation (i.e., R and CT) and propagation (K, CFT, and alpha) between TEG and ROTEM in our results were consistent with the different activator types to which these parameters are particularly sensitive [32].

In addition to the comparative studies of TEG and ROTEM performed with standard but different activators, we also compared the two systems activated by the same reagents.

The closer relationship and comparable range of parameter values between TEG and ROTEM performed with the same activators are consistent with the findings by Nielsen [19], who compared TEG and ROTEM for normal, hypocoagulable, and hypercoagulable plasma samples activated with celite, and by Scharbert *et al.* [21], who compared TEG and ROTEM for platelet mapping assay using the same reagents. A larger TEG maximum amplitude than ROTEM MCF was reported

in another study comparing TEG and ROTEM for whole blood fibrin-based clot tests using the same functional fibrinogen reagent [22]. A very recent study reported no disparity between TEG and ROTEM for any parameters (R vs. CT, alpha vs. alpha, and maximum amplitude vs. MCF) when in-tem and rapid TEG reagents were used on both instruments. Significant differences were found in the angle and maximum firmness of the clot for ex-tem and kaolin reagents as well as in the clot time and maximum firmness of the clot for recombinant tissue factor-phospholipid reagent [33]. However, no correlations between the two systems were investigated. The clinical significance of the difference between TEG and ROTEM may be more associated with their correlations than their absolute parameter values.

In summary, our study confirmed that TEG R and ROTEM CT were sensitive parameters to tissue factor concentrations as observed in other studies [23,26,31]. We also found effects of tissue factor on TEG K and ROTEM CFT, alpha. The differences in the standard tests between the two systems may be mostly because of the activation methods (kaolin/ellagic acid vs. tissue factor) leading to different coagulation pathways (intrinsic vs. extrinsic). Larsen *et al.* [24] reported that multiple analyses by INTEM, EXTEM, and FIBTEM could provide more detailed and accurate diagnosis and transfusion guidance with a shorter runtime than a single analysis by ROTEM with kaolin activation alone. Both compatibility and incompatibility were indicated by various degrees of the correlations between TEG and ROTEM, which were dependent on the activators used.

A potential limitation of our study is the small sample size. However, the main objective of the study was to elucidate and compare the characteristic changes of TEG and ROTEM measurements as a function of tissue factor and kaolin concentrations and not to evaluate interindividual variability or reference ranges.

Conclusion

Tissue factor is a strong activator altering clot initiation and propagation. Its optimal concentration could be obtained to reduce assay time and improve assay accuracy. The two systems showed limited interchangeability with the best correlation between TEG maximum amplitude and ROTEM MCF, when standard reagents were used as per manufacturer's instruction for each instrument. The two systems appeared more interchangeable with the highest correlation between TEG R and ROTEM CT, when the same activators were used. The clinical significance of this study for diagnostic and therapeutic applications requires further investigation.

Acknowledgements

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Conflicts of interest

There are no conflicts of interest.

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