Point-of-Care Tests in for Blood Coagulation in the Perioperative Period

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Thromboelastography utilizing the TEG® analyzer systems (*Haemonetics Corporation, Boston, MA*) and thromboelastometry utilizing the Rotem® analyzer systems (*Instrumentation Laboratory, Bedford, MA*) are presently benchmark tests for goal-directed blood component transfusions, in scenarios of bleeding and hemorrhage in cardiac, obstetrical, blunt, and penetrating trauma, brain trauma, and solid organ transplantation. It isis essential to know that there are no studies to compare Rotem and TEG. The choice between the two lies in which product was deployed by a department or hospital administration. Both tests rely on the concept of viscoelasticity. A substance is thought of as just a solid or just a liquid. One that is viscoelastic may have both properties. Elastic substances may undergo strain when stretched but will return to its normal state once that stressing factor is removed. Viscosity gives the material more stability and resists stretching [1]. Blood is a viscoelastic substance. Plasma demonstrates pure viscous behavior, while other components of blood are elastic. The viscoelasticity of blood is under the influence of four factors: (1) the environment (temperature), (2) interplay (RBC orientation and aggregation), (3) plasma factors (osmotic pressure, pH, concentration of fibrinogen and other plasma proteins), and (4) RBC factors (viscoelasticity and deformability of erythrocytes and their membranes).

There is little difference in blood viscoelasticity among normal cases but becomes significant with certain pathological states or surgical interventions. The formation of a clot results from the polymerization of factors that generate fibrin. The ongoing reactions generate a three-dimensional polymer network. This network changes the viscoelasticity prior and through the phase of fibrin generation. The changes in viscoelasticity during the clotting phase can be measured and presented to the anesthesiologist as a computerized tracing) [2].

The thromboelastogram (TEG®) tracing is nonlinear, and while valuable, it lacks a mathematical way to determine accuracy of the curves. Viscoelastic testing (TEG® and Rotem) has more than stood the test of time and remains an essential part of goal-directed blood product administration. Their utility is now in question as new tests based on different sciences are making their way to the market and appear to be more accurate due to the measurements that are linear.

Figure 21.1 is a TEG 5000 (Haemonetics Corporation®). To generate test results utilizing a TEG® 5000 analyzer, a small sample of whole blood (native, citrated or heparinized depending on tests run) is placed in a 37-degree cuvette (Fig. 21.2). A disposable pin is suspended from a torsion wire into the cup. The cup rotates through an angle of 4° for 45 min with each cycle lasting approximately10 s (Fig. 21.2). As the blood begins to clot and it adheres to both the pin and the cuvette, the clot will begin to transmit the rotation of the cup to the pin. The pin is suspended by a torsional spring which adds an elastic element to the system. The extent of resulting pin rotation is directly proportional to the strength of the clot. The corresponding numeric results and tracing represent all of the phases of clot formation and lysis [2].

Figures 21.3 and 21.4 illustrate all of the components of a TEG tracing to understand rate, strength, and stability or sustainability of a clot. The R reflects the "Reaction Time" and represents the coagulation pathways resulting in the initial thrombin burst and generation of fibrin or factor IIa.

A long "R" time may denote that factors are deficient and goal-directed fresh frozen plasma, or newly developed synthetic factors (10,9,7,2-KCENTRA®) [3]. Utilization of a

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Fig. 21.1 TEG 5000. (With permission from Haemonetics Corporation)

cuvette impregnated with heparinase (identified with a blue colorant in the disposable plastic cup) can help determine if systemic heparin is responsible for the observed delay in clot formation.

Depending on the coagulation status of the patient and on the type of test run, it takes minutes or longer for the entire body of the TEG results to be displayed (Figs. 21.3 and 21.4).

The alpha (α), angle parameter is the slope of the line beginning at the point that the tracing diverges from the baseline line and is tangential to the TEG tracing. This represents the acceleration of fibrin buildup and cross-linking.

Critical information of the TEG analysis lies in the MA or maximum amplitude. It is the widest component of the TEG and represents the platelet fibrinogen interaction and overall clot strength. A general rule of thumb is that 80% of clot strength is contributed by platelets and 20% to fibrinogen [4, 6–8]. The clinician might surmise that if the MA is narrow, platelets are most likely deficient or not functioning. However, a TEG Functional Fibrinogen Assay measures the fibrinogen contribution to clot strength and provide greater specificity to guide therapy. The TEG Platelet Mapping® [5, 6] is an adjunctive test run on the same TEG analyzer that can assess platelet function of the MA if it is suboptimal.

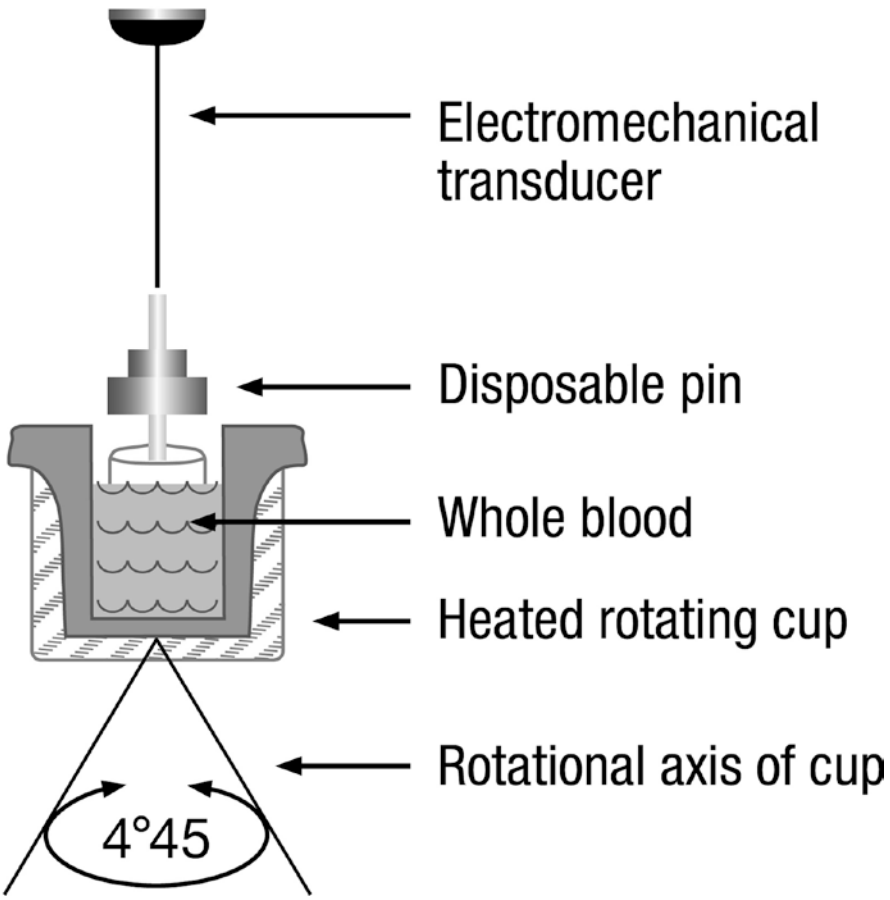

Fig. 21.2 The technology of TEG where the cup rotates. (With permission from Haemonetics Corporation)

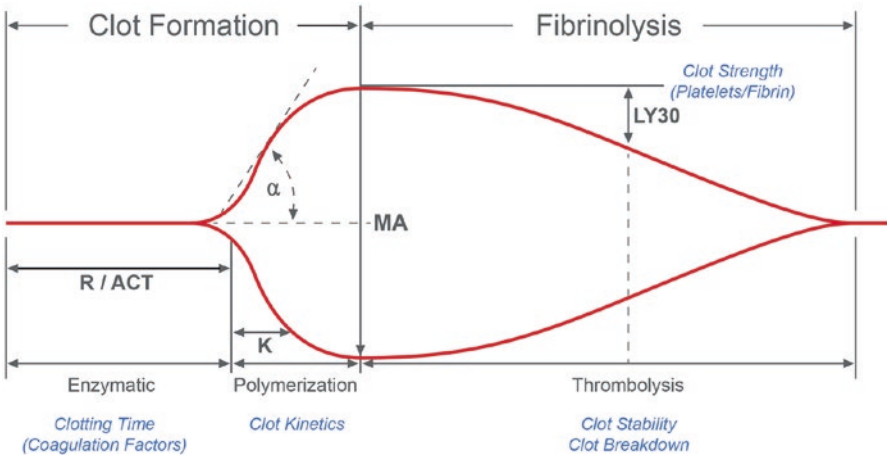

Fig. 21.3 TEG components. (With permission from Haemonetics Corporation)

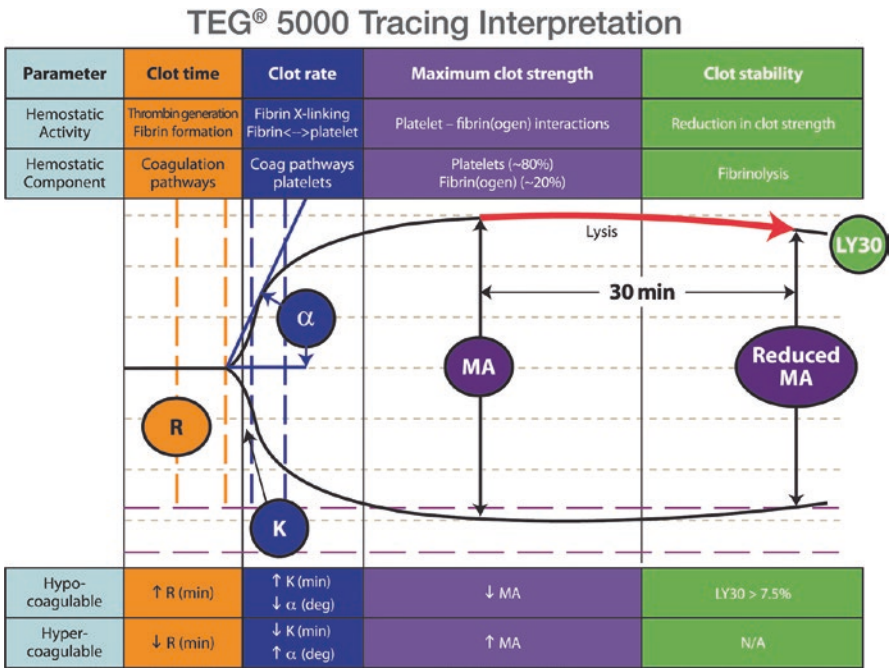

Fig. 21.4 Detailed figure demonstrating each component of a TEG using the TEG 5000. (With permission from Haemonetics Corporation)

The platelet function analyzer measures the speed of platelet adhesion.

Clot instability (Figs. 21.3 and 21.4) appears in this illustration as the MA declines near the end of the TEG tracing. The clot loses its stability as the amplitude of the tracing declines. This reflects fibrinolysis in the blood sample. Fibrinolytic inhibitors may be used prophylactically in some settings, such as CV surgery and some trauma setting. In settings where the hazard of thrombosis is much greater, and prophylactic anti-fibrinolytics are not desired, those drugs can quickly correct hyperfibrinolysis when it is detected. Fibrinolytic inhibitors, to have maximal effect, should be administered before hemorrhage begins for maximal prophylaxes. Once fibrinolysis begins, fibrinolytic inhibitors like aminocaproic acid and tranxamic acid are not helpful.

D-dimers are more useful as a tool of exclusion for VTE but can be elevated by a number of inflammatory states related to fibrinolysis. Even if a clot is confirmed, the D-dimer shows what has already happened in terms of clot breakdown. The TEG tracing shows the presence of active fibrinolysis by elevated clot lysis (LY30) or the lack of fibrinolysis by a stable MA.

Assays Available on the TEG 5000 Analyzer System Include

Kaolin (+/− heparinase) - An intrinsic pathway activated assay. This thrombin-generated tracing identifies underlying hemostatic characteristics and risk of bleeding or thrombosis.

RapidTEG (+/− heparinase) - An intrinsic and extrinsic pathway activated assay increases the coagulation process rapidly assess coagulation properties.

Functional Fibrinogen - An extrinsic pathway activated assay uses a potent GPIIb/IIIa platelet inhibitor to isolate fibrin contribution to clot strength. Used in conjunction with Kaolin, TEG can assess relative contribution of platelets and fibrin to overall clot strength.

Platelet Mapping (Haemonetics Corporation©) includes a thrombin-generated tracing (kaolin) and platelet receptor-specific tracing(s) (ADP/AA). Identifies the level of platelet function and inhibition using the patient's underlying hemostatic potential from the Kaolin TEG as the reference point.

The TEG® 6s Analyzer System

TEG 6s (Haemonetics Corp, Boston MA) (Fig. 21.5) is the newest platform in the thrombelastography portfolio. It is a cartridge-based system which dramatically increases the ease of operation and the reproducibility of results [5, 6, 8]. All reagents are already in the cartridge and are mixed with the blood when a sample is added to the cartridge by means of a simple transfer pipette. Another benefit is a reduction in the amount of blood required to obtain the results. A cartridge with four assays can be run with 340 mcl of blood. These changes make the device well suited to use in a variety of care settings [1]. Each cartridge provides results from multiple tracings to provide data using a variety of assays to provide the quickest, most specific results to guide treatment decisions.

Fig. 21.5 The cartridge-based TEG® 6s analyzer. (With permission from Haemonetics Corporation)

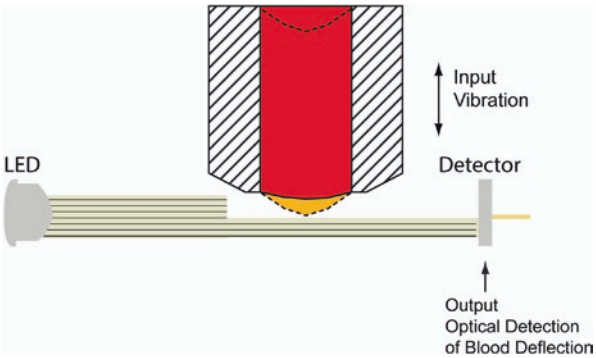

Fig. 21.6 The new technology of TEG 6000. (With permission from Haemonetics Corporation)

Because it is a cartridge design, it no longer uses a cup and pin methodology, instead uses resonant frequency to assess the clot (Figs. 21.6 and 21.7). Each assay occupies a separate channel of the cartridge, to a total of four channels. Blood is added to the cartridge, where it is mixed with the reagents, then channeled into a capillary tube with a meniscus of blood at the testing chamber end of the capillary tube. A range of radiofrequencies are applied to the tube. The frequency which causes the blood to distend the furthest into the test chamber, blocking light from a photodetector, is the resonant frequency. Each resonant frequency is associated with a specific clot strength. As the clot moves through the process of clot initiation, reaches maximum clot strength, and potentially begins to break down, those changing resonant frequencies are plotted to produce the familiar TEG tracing (Figs. 21.6 and 21.7).

There are currently three cartridges available for the TEG 6s system. A Global Hemostasis cartridge provides four assays: citrated kaolin, citrated kaolin with heparinase, citrated rapid TEG, and citrated functional fibrinogen. This cartridge is FDA cleared for use in CV surgery and cardiology procedures. The Platelet Mapping® cartridge uses the same reagents as the Platelet Mapping (Haemonetic Corporation) assays in TEG 5000 to provide information on percent inhibition/aggregation and residual platelet reactivity, using ADP and arachidonic acid as the agonists (Figs. 21.6 and 21.7). This is used to assess the patient's response to antiplatelet therapies. The most recent cartridge is approved for use in trauma. It includes assays for citrated kaolin, citrated

RapidTEG™, and citrated functional fibrinogen. Using these assays in combination, it is possible to more specifically address hemostatic defects between platelets, fibrinogen, factors, heparin effect, and fibrinolysis (Figs. 21.8 and 21.9).

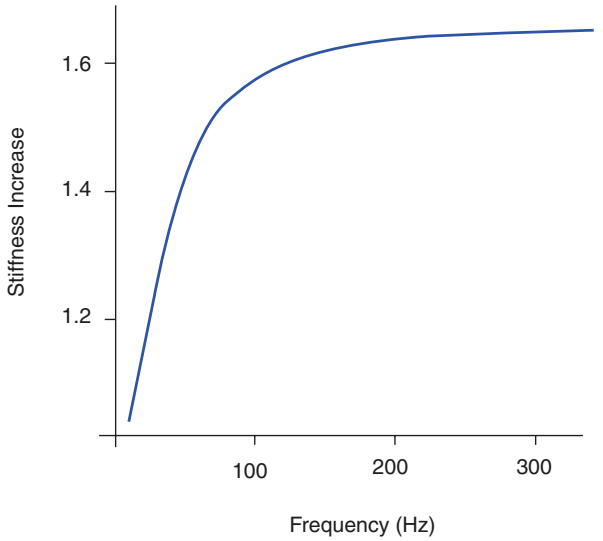

Fig. 21.7 New to the TEG system technology is the measurement of clot viscoelasticity using a resonance method. To measure the clot strength with the resonance method, the sample is exposed to a fixed vibration frequency. With LED illumination, a detector measures up/down motion of the blood meniscus. The frequency leading to resonance is identified and then converted to the TEG system readout. Stronger clots have higher resonant frequencies and higher TEG readouts. (With permission from Haemonetics Corporation)

Educational Tools

Rotational Thromboelastometry

ROTEM® or rotational thromboelastometry **(TEM ®)** is an alternative method of viscoelastic testing (Fig. 21.10). The cups in TEM determine the interaction of normal coagulation factors of blood with inhibitors, anticoagulant drugs, platelets, red blood cells, and fibrinolytics. ROTEM as in TEG utilizes a whole blood to assess clotting. Only the heparinase cups which are available for TEG have reactive agent.

Blood (300 µl) anticoagulated with citrate is placed into a cuvette using an electronic pipette. A disposable pin is attached to a shaft which is connected with a thin spring (the equivalent to Hartert's torsion wire in thrombelastography) and slowly oscillates back and forth. The signal of the pin suspended in the blood sample is transmitted via an optical detector system. The developing clot slows down the pin as the clot forms.

The test starts by adding appropriate reagents. The instrument measures and graphically displays the changes in elasticity at all stages of the developing clot. It is essential to know that the clot formed may become unstable by activating those factors that generate fibrinolysis. The typical test temperature is 37°C, but different temperatures can be selected, as in patients with hypothermia (Fig. 21.11).

By adding specific reagents, TEM like TEG can find specific points in the coagulation cascade where a problem exists.

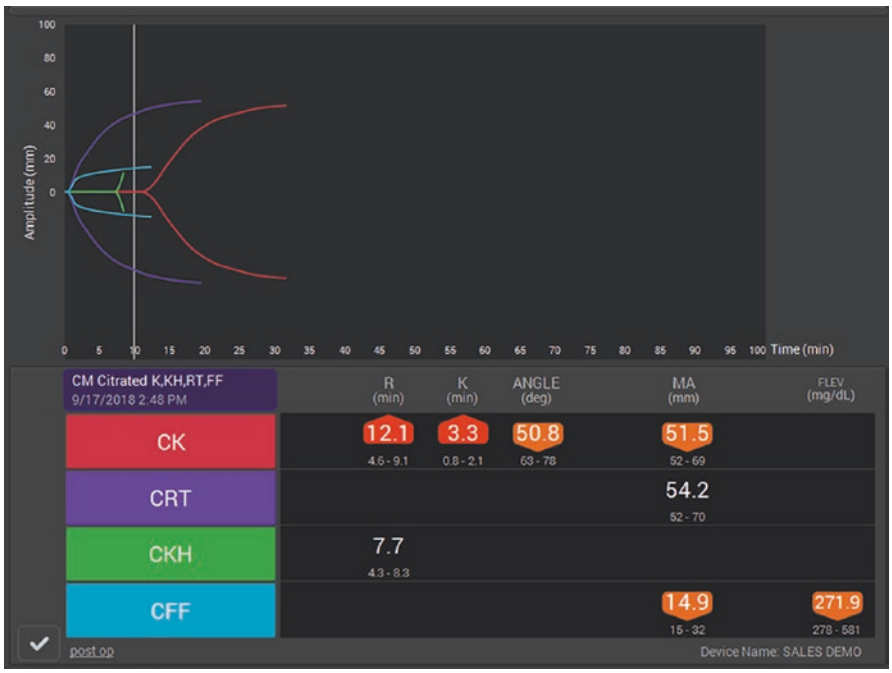

Fig. 21.8 Tracings on the left from the Global Hemostasis cartridge. CK citrated kaolin, CKH citrated kaolin with heparinase, CRT citrated RapidTEG, CFF citrated functional fibrinogen. The *white line* is a 10 min marker, by which time several parameters are already available to guide decision-making

Fig. 21.9 Tracings above are from the Platelet Mapping cartridge. *HKH* heparinized kaolin with heparinase, represents maximum platelet activation, or no inhibition. *ActF* represents clot without platelet contribution, or 100% inhibition. ADP and AA tracings reflect strength of clot when those agonists are added to the activator. These last two are the patient's residual platelet reactivity after inhibition is assessed

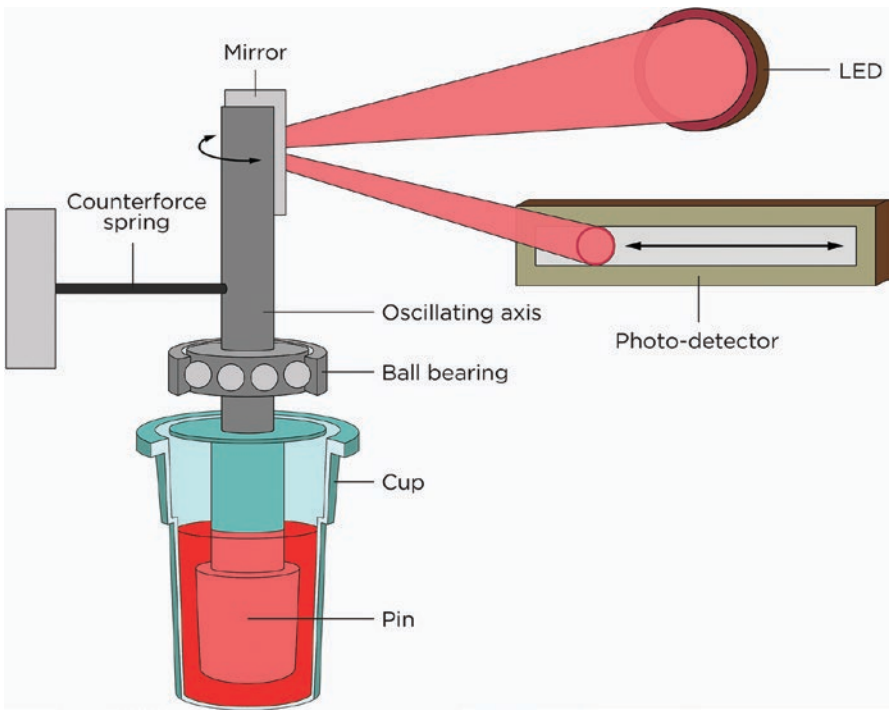

Fig. 21.10 The technology of ROTEM. Opposite from TEG, the torsion wire moves due to viscoelastic forces of clotting. In TEG the cup moves

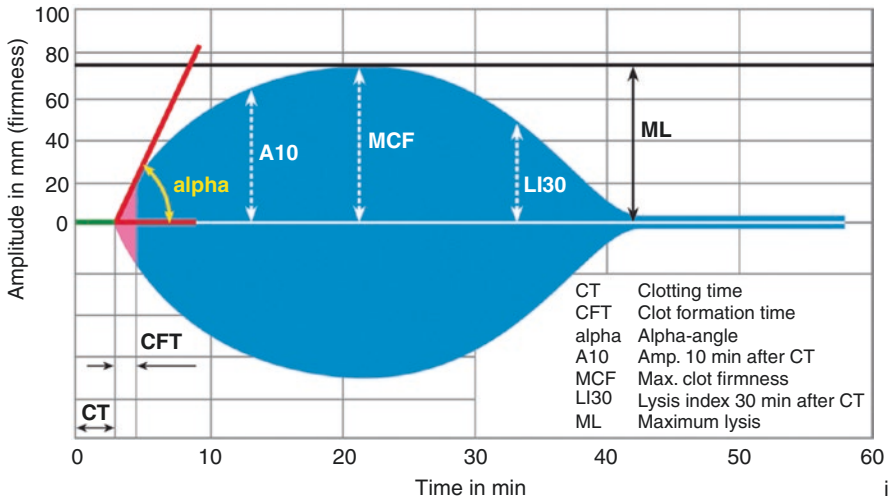

Fig. 21.11 This figure is the final result of ROTEM. While similar to TEG, the parameters measured for clot formation are different. Compare Fig. 21.3 to this Figure

As explained by Instrumentation Laboratories Worldwide (Fig. 21.12):

1. INTEM – Contains phospholipid and elagic acid which are activators and provides information similar to that of the aPTT
2. EXTEM – Contains tissue factor as an activator and provides information similar to that of the PT
3. HEPEM – Contains lyophilized heparinase for neutralizing heparin
4. APTEM – Contains aprotinin for inhibiting fibrinolysis
5. IBTEM – Utilizes cytochalasin D, **a** platelet inhibitor which blocks the platelet contribution to clot formation, allowing qualitative analysis of the functional fibrinogen component

TEG and TEM measurements, for the most part, are not interchangeable. The time of initial fibrin formation is the R time in TEG or clotting time in TEM. Clotting time or CT is the time from the test beginning until the amplitude of 2 mm is reached (Fig. 21.5). The CT time is increased or prolonged by hereditary or acquired inhibitors, (hemophilia or warfarin), factor deficiencies, or when factor function becomes impaired as with the direct thrombin inhibitors. Simply stated, when the CT line increases, coagulation factors are needed. In hemophilia, TEG/TEM monitoring is helpful when factor 10a activity is blocked as seen with apixaban with resumed activity when this drug was discontinued [7, 8].

Application of the TEG® System in Trauma

Trauma is the second leading cause of death worldwide with 40% mortality associated with massive hemorrhage. The balance between hemostasis and fibrinolysis is disrupted by acidosis, hypothermia and hemodilution, tissue damage, exposure, and fluid/blood product administration tip scales away from hemostasis. Acute traumatic coagulopathy, mediated by activation of the thrombomodulin-protein C system, further promotes fibrinolysis.

The Prospective, Observational, Multicenter, Major Trauma Transfusion Trial (PROMMTT) investigated the "Comparative Effectiveness of a Time-varying Treatment with Competing Risks [9], which recognized coagulopathy in 42% of trauma patients. Management of DIC typically involved conventional coagulation analysis to guide transfusion protocol. Since the utilization of TEG in Germany, it has become increasingly popular in the acute monitoring of coagulation for cardiac and liver transplantation cases and has expanded into other medical specialties, other than trauma.

One of the earliest prospective studies [10] investigating the utility of TEG in the assessment of trauma patients demonstrated that of the parameters measured, (demographics, medical history of coagulopathy, medications, TEG indices, platelet count, PT/PTT, revised trauma score, and injury severity score), only TEG and injury severity score were accurate predictors of early transfusion [10]. The Injury Severity Score assesses trauma severity. It correlates with mortality, morbidity, and hospitalization time after trauma.

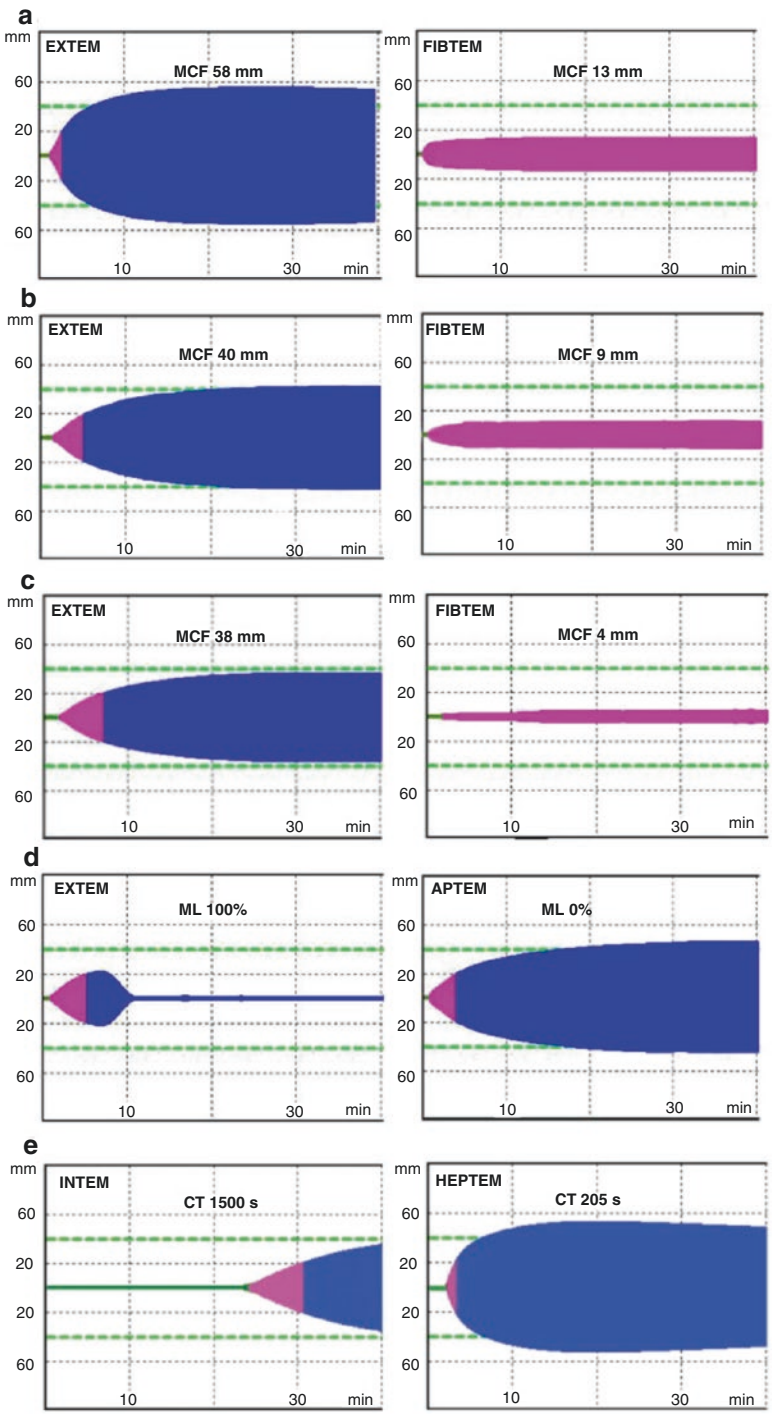

Fig. 21.12 INTEM This test activates the contact phase of hemostasis. The result is influenced by coagulation factors, platelets, fibrinogen, and heparin. In the absence of heparin, INTEM is a screening test for the hemostasis system. It is used for therapeutic decisions regarding the administration of fresh frozen plasma, coagulation factors, fibrinogen, or platelets HEPTEM. This assay represents an INTEM assay performed in the presence of heparinase, a heparin (or LMWH)-degrading enzyme. The difference between HEPTEM and INTEM CT-value comparison confirms the presence of heparin.
EXTEM test activates hemostasis via the physiological activator tissue factor. The result is influenced by extrinsic coagulation factors, platelets, and fibrinogen. EXTEM represents the extrinsic pathway. This assay is not influenced by heparin (heparin inhibitor included in the EXTEM reagent). It guides goal-directed therapy with the deployment of coagulation factors, fibrinogen, or platelets.
FIBTEM test is an EXTEM-based assay for the fibrin part of the clot. FIBTEM eliminates the platelet contribution of clot formation by inhibiting the platelets irreversibly with cytochalasin D, a potent inhibitor of actin polymerization which disrupts actin microfilaments, an essential part of a cytoskeleton-mediated contractibility apparatus of the platelet. The use of cytochalasin is more favorable than using glycoprotein IIb/ IIIa inhibitors which block platelet incompletely.
FIBTEM allows for the detection of fibrinogen deficiency or fibrin polymerization disorders, e.g., induced by certain plasma expanders, and may identify rapidly the need to substitute fibrinogen.
APTEM test is an EXTEM-based assay for fibrinolysis. APTEM compared to EXTEM allows to detect fulminant hyperfibrinolysis.

It is used to define the term major trauma. A major trauma is defined as the Injury Severity Score being greater than 15.

Consistent with the PROMMTT trial, 75% of the trauma patients were diagnosed with coagulopathy. Of those, 87% were hypercoagulable and the remaining hypocoagulable. By design, this study did not allow for TEG coagulation analysis to alter clinical decision-making. The authors noted that TEG data can predict the magnitude of hemostasis derangement and may be implemented into the approach for transfusion.

A subsequent porcine study [11] compared PT, PTT, and activated clotting time against TEG indices in hypothermia versus hemorrhagic shock. Hypothermia directly results in inhibition of platelet function and reduction of clotting factor activity. This caused a hindrance to initial clot formation. In contrast, hemorrhage elicits massive bleeding, disseminated intravascular coagulation, and thrombotic/embolic complications; all impairing clot strength and stability. In this investigation, pigs were subjected to sham, hypothermic, hemorrhagic, or hypothermia and hemorrhagic conditions.

PT and PTT lacked sensitivity and activated clotting time lacked specificity in identifying the condition associated with the coagulopathy. TEG accurately differentiated between the conditions due its comprehensive assessment of the coagulation process. The authors recommended implementing TEG data into treatment of hypothermia- and hemorrhagic shock-related coagulopathy [11].

Adding to the pre-existing literature, a study published in 2012 [12] evaluated nearly 2000 trauma patients with a median injury severity score of 17. Twenty-five percent of these patients presented with overt shock, and of these, 28% were transfused. After controlling for age, mechanism of injury, weighted-revised trauma score, base excess, and hemoglobin, the investigators deduced that the TEG r-time predicted RBC transfusion with greater superiority than PT/PTT/INR. The TEG α angle predicted plasma transfusion with greater superiority than fibrinogen; and, the TEG maximum amplitude predicted platelet transfusion with greater superiority than platelet count. These correlations improved in transfusion, shock, and head injury cases. Additionally, the cost of r-TEG was only marginally greater than that of the five conventional tests (PT, PTT, INR, platelet count, fibrinogen) [12].

In a prospective study of 272 trauma patients [13], TEG r and k time were available within 5 min and maximum amplitude and α angle within 15 min as compared to conventional coagulation tests that were not available until 48 min. r-TEG values, ACT k-time, and r values predicted red blood cell, plasma, and platelet transfusion within 2 h of arrival; specifically, ACT > 128 predicted massive transfusion, whereas ACT < 105 predicted no transfusion.

Investigators at Ben Taub General in Houston [14] published a study following the hospital's transition from massive transfusion protocol guided by TEG to a 1:1:1 ratio of blood, plasma, and platelets [14]. Their data demonstrated no difference in resuscitation strategy in patients receiving greater than 6 units of red blood cells or in patients with blunt trauma receiving greater than 10 units of red blood cells. The 1:1:1 protocol had greater mortality rates than the TEG protocol in cases of penetrating trauma with transfusion of 10 or more units of red blood cells. This suggests that the 1:1:1 protocol may not be extrapolated and hemostatic to all patients.

Following this investigation, the Denver Health Medical Center [15] with a level 1 trauma center published a randomized clinical trial to test the hypothesis that massive transfusion protocol guided by TEG improves clinical outcomes compared with massive transfusion protocol goal directed by conventional coagulation assays (CCA, PT, PTT, fibrinogen, platelet count, and d-dimers). A total of 111 patients with a median injury severity score of 30 were enrolled and of whom 27% presented with penetrating trauma. As compared to 36% mortality in CCA-guided transfusion group, the TEG-guided transfusion group had a 19% mortality rate. This difference was believed to be secondary to decreased early hemorrhagic death in the TEG group. There were significantly more plasma and platelet transfusions within 2 h of arrival and overall more cryoprecipitate transfusions in the CCA guided transfusion arm. There were no differences in the volume of crystalloid or red blood cell units transfused. The TEG-guided transfusion group was also associated with decreased ventilator dependence and ICU hospitalization.

In summary, acute traumatic coagulopathy is associated with greater transfusion requirements, prolonged ICU hospitalization, and increased incidence of multi-organ complications. As compared to patients without coagulopathy, those diagnosed with coagulopathy have 3–4 times greater mortality rate overall and 8 times greater mortality rate within the first 24 h of injury.

Given these statistics, rapid identification and treatment, with modalities such as TEG, improved and ongoing coagulopathy.

Application of Thromboelastography/Thromboelastometry in Obstetric Hemorrhage

The 2017 American College of Obstetricians and Gynecologist guidelines define postpartum hemorrhage (PPH) as bleeding with signs and symptoms of hypovolemia or cumulative blood loss of >1000 mL within the first 24 h of delivery. The national incidence of PPH varies between 1% and 10% of all deliveries, and PPH remains one of the top 5 causes of obstetric morbidity and mortality globally.

While pregnancy induces a hypercoagulable state, the postpartum period elicits fibrinolysis. Hyperfibrinolysis is associated with the consumption and depletion of coagulation factors (particularly fibrinogen) and inhibition of clot formation. This phenomenon is critical to the development of acquired coagulopathy of PPH and is targeted in the management of PPH [16]. Conventional laboratory tests for hyperfibrinolysis (D-dimer, fibrinogen) reflect indirect measures of coagulopathy through report of historical events and may take 60–90 min to result [17]. This either delays goal-directed transfusion therapy or, in emergent cases, unnecessarily promotes empiric treatment of suspected hyperfibrinogenemia. Point-of-care TEG analyses allow for early identification of the hemostatic derangements during pregnancy. A recent prospective longitudinal study comparing TEG parameters demonstrated a hypercoagulable profile with increased clot strength and decreased fibrinolysis during pregnancy as compared to 8 weeks postpartum [18]. A subsequent study, investigating TEG parameters in massive obstetric hemorrhage, (MOH; defined as >2 L estimated blood loss) reported rapid initiation of clotting, decreased clot strength, and decreased

fibrinolysis in MOH as compared to normal delivery. The same study also compared TEG to conventional coagulopathy analyses (PT, PTT, fibrinogen, antithrombin, D-dimer) and suggested integration of viscoelastic assays into transfusion protocol can rapidly diagnose etiology of bleeding and thereby improve response time [19].

Rotational thromboelastometry (ROTEM) provides the FIBTEM assay as a measure of clot strength; this analysis is available within 10 min and accurately differentiates among hypofibrinogenemia, hypofibrinogenesis, and hyperfibrinolysis, thereby theoretically informing fibrinogen replacement therapy [20]. A prospective, observational study compared the utility of FIBTEM (TEM surrogate for plasma fibrinogen level) and conventional fibrinogen in 356 women with 1–1.5 L PPH. The investigators recognized FIBTEM, but not fibrinogen/PTT/PT, as an independent predictor of progression of bleeds >2.5 L, ≥4 U packed red blood cells (PRBCs), and 8 U allogeneic products [20]. It was noted that FIBTEM was an early biomarker for PPH severity/progression. Fibrinogen <2 g/L and FIBTEM <10 mm were associated with prolonged bleeding, increased frequency of invasive procedures, and earlier transfusion. Based on these findings, the authors deduced ROTEM has the potential to markedly improve clinical outcomes via early administration of fibrinogen concentrate and decreased transfusion of high-volume blood products, including RBCs, FFP, and platelets. These results were replicated with similar findings in subsequent investigations with one observational study showing a 1.8-fold reduction in total MOH transfusions with integration of a ROTEM-guided transfusion protocol [21].

A recent multicenter, double-blinded, randomized controlled trial studied the efficacy of early fibrinogen replacement guided by visco-elastometric measures. The trial enrolled 55 females with PPH of 1–1.5 L. Participants with FIBTEM <15 mm were randomized to fibrinogen concentrate or placebo transfusion with the primary outcome comparing total number of blood products transfused. The results indicated no statistically significant reduction in transfusion requirements between the two groups. These findings also suggested FIBTEM of 15 mm as too high as an interventional trigger. The authors further concluded that at FIBTEM A5 > 12 mm or fibrinogen >2 g/L, normal physiologic hemostasis still occurs and does not warrant fibrinogen replacement [21].

The utility of TEG [22] expands beyond PPH to include recognition and management of catastrophic amniotic fluid embolism, gray platelet syndrome, Glanzmann's thrombasthenia, hemophilia, platelet storage pool disorder, placental abruption, disseminated intravascular coagulation, and HELLP syndrome. Despite these findings, the primary limitation to widespread integration of TEG/TEM-guided transfusion protocols remains the lack of large randomized controlled trials. Further investigations with high-power and multicenter involvement are needed.

New Technologies for Clot Assessment and Clot Stability

- Sonorheometry
- Microfluidic devices
- Quartz crystal microbalance
- Laser speckle rheology

Sonorheometry

Sonic Estimation of Elasticity via Resonance (SEER) or sonorheometry is a technology that employs ultrasound waves to measure changes in viscoelastic properties during the process of coagulation. This identifies and qualifies clot formation [23].

Repetitive high-frequency ultrasound signals propagate through the blood sample in an air-sealed cartridge generating gentle nudging on the blood clot early in the process of its formation. Clot displacement (i.e., shear modulus) will occur which will generate series of returning frequency echoes (Fig. 21.13 *left panel*). Tracking and analyzing the returning echoes can estimate the sample motion over time and creating a displacement curve. The shape of the displacement curve is directly related to the shear modulus of the sample at any point time [24].

Changes over time in shear modulus is a direct physical measure of clot stiffness. Repeating signals over time creates a signature time-displacement curve. Figure 21.13 reflects the dynamic changes in shear modulus of the sample during coagulation at any given point in time.

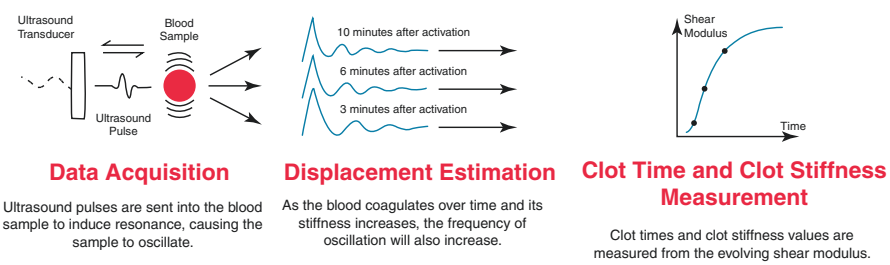

Fig. 21.13 Sonorheometry technology. (Copy permitted by Quantra HemoSonics)

The shear modulus is the elastic properties of materials [25]. It is a parameter of materials to resist displacement when exhibiting external forces, and it is measured by Pascal (Pa). For example, bone tissue has a shear force of 3.3 GPa shear, while natural rubber has 600 Pa and liquids has 0 Pa. The "Quantra Hemostasis Analyzer," designed by HemoSonics, is a point-of-care (POC) test that provides quick results in a critical care environment. It generates complete test results within 15 min of test initiation [24].

The analysis cartridge allows a small sample to be collected, provides no physical contacts with the blood sample, and will permit detection of early soft clots identification. It has been demonstrated that a large shear stress applied by instruments during measurements like ROTAM [26] did disrupt clot formation. The lack of disruption to the sample during the processing provides high sensitivity to detect soft/weak clots which are often associated with clinical bleeding [27]. The "Quantra" analyzer cartridge is a multi-channeled and a single-use disposable plastic component (Fig. 21.14). It has four independent channels, each containing pre-filled lyophilized reagents that enable simultaneous differential testing without the need for any reagent preparation or pipetting. Lyophilization of the reagents provides stability at room temperature [28].

The cartridge is the only component of the device that is in direct contact with blood which prevents potential biohazard spills. The cartridge protects the sample from environmental factors interference, such as temperature, vibration, or evaporation.

Cartridges

The QPlus Cartridge was designed to evaluate a patient's functional coagulation status in major surgeries. The cartridge can be stored at room temperature and immediately available for acute bleeding situations without warming or special preparation. This cartridge provides all the parameters of Quantra analyzer (Fig. 21.15) except for clot stability to lysis (CSL) which can be measured by the QStat® Cartridge [28]. CSL measures changes to clot stiffness change in the presence of tranexamic acid which is the function of fibrinogen. It is useful in level 1 traumas, liver transplantation, complicated obstetrics, cardiac surgery, and critical care units.

Fig. 21.14 Measured parameters on hemosonics analyzer (QPlus Cartridge)

QPlus Cartidge Measurements

Parameter	units	Description	Measurement
Clot Time (CT)	Seconds	Clot time citrated whole blood.	Clot time measured in Channel #1 with activator of the intrinsic pathway (kaolin).
Heparinase Clot Time (CTH)	Seconds	Clot time in citrated whole blood with heparin neutralization.	Clot time measured in Channel #2 with activator of the intrinsic pathway (kaolin) and heparinase.
Clot Time Ratio (CTR)	No units	The CTR parameter may indicate the prolongation of the intrinsic pathway clotting time that is likely due to the influence of unfractionated heparin. CTR values are not directly correlated with heparin levels in the sample; if CTR is > 1.4, it is only indicative of heparin in the sample.	Calculated as the ratio of clot time values of Channel #1 over Channel #2 (CT/CTH).
Clot Stiffness (CS)	hecto Pascals (hPa)	Stiffness of the whole blood clot.	Clot stiffness measured in Channel #3 with an activator of the extrinsic pathway (thromboplastin) and heparin inhibitor (polybrene).
Fibrinotgen Contribution to Clot Stiffness (FCS)	hecto Pascals (hPa)	Contribution of functional fibrinogen to overall clot stiffness.	Clot stiffness measured in Channel #4 with an activator of the extrinsic pathway (thromboplastin) and heparin inhibitor (polybrene) and platelet inhibitor (abciximab).
Platelet Contribution to Clot Stiffness (PCS)	hecto Pascals (hPa)	Contribution of platelet activity to overall clot stiffness.	Calculated by subtracting clot stiffness value of Channel #4 from Channel #3.

Fig. 21.15 Test results as displayed on hemosonics analyzer

Microfluidic Devices

The Wyss Institute team led by Wyss Institute Founding Director Donald Ingber, M.D., Ph.D., has developed a novel microfluidic device in which blood flows through a life like network of small "vessels," where it is subjected to true-to-life shear stresses and force gradients of the human vascular network. Using automated pressure sensors and a proprietary algorithm developed by the Wyss team, data acquired from the device is analyzed in real time, precisely predicting the time at which a certain blood sample will obstruct. The hemostasis monitoring microdevice mimics rapid changes in blood flow dynamics associated with stenosis or narrowing of small blood vessels by pumping pressurized blood flow through the device's microfluidic channels [29].

In this schematic and series of magnified insets (Fig. 21.16) from left to right, the microfluidic channels progress from pre-stenosis, to stenosis, to post-stenosis, simulating the narrowing of blood vessels that can often occur in patients as a result of medical conditions or treatments. The effects of stenosis on blood clotting tendency are visible: blood clotting protein fibrin (green) and blood platelets (red) are seen coagulating as they progress through the device, and most notably in the post-stenotic region.

By combining Wyss's fabricated microfluidic device that mimics blood flow dynamics of small arterioles with their novel data analysis software, a quantitate hemostasis in real-time and predict if blood clots will develop in an individual or in a blood sample. The integrated low-cost miniaturized equipment requires less than 1.0 ml of blood sample and have made it ideal as a bedside monitor to identify and quantify clot formation and platelets function precisely and in real time.

In the past 10 years, several microfluidic devices were produced to study different biological reactions like enzymatic reaction kinetics and fluid viscosity [30]. Schoeman et al. designed a microfluidic chip to measure clot formation evoked by blood flow. Blood samples flow from a high-pressure flow channel into a lower-pressure receiver channel until a hemostatic clot formed (Fig. 21.17). The channels were coated with procoagulants: collagen and tissue factor. This allowed him to measure normal physiological blood clot to form within a clotting time of 7.5 min.

Schoeman designed another microfluidic chip with defects in reagents to mimic hemophilia A with anti-Factor VIII antibody, and he identifies an unstable clot formation as would be expected in vivo. His team also demonstrated that treatment of blood with antiplatelet P2Y12 receptor inhibitor substantially delayed the clotting time [31].

Microfluidics cell can be designed in different complex geometries that mimic stenosed arteries to study various pathological events and aim to evaluate an individual patient's coagulopathy or compliance with treatment.

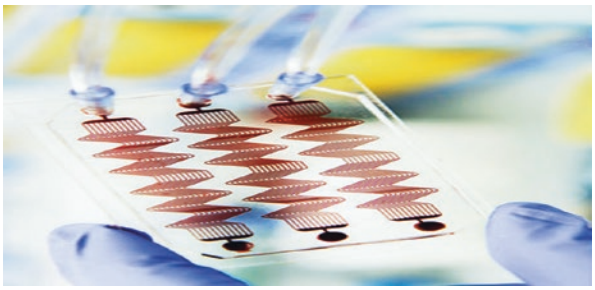

Fig. 21.17 Microfluidic 3 channels cartridge. (Courtesy of Wyss Institute at Harvard University)

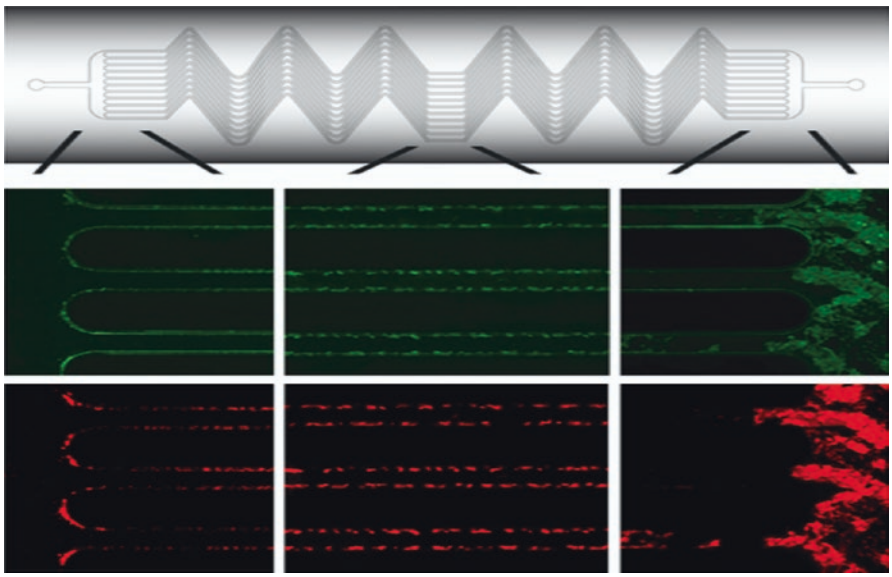

Fig. 21.16 Schematic microfluidic channels. (Courtesy of Wyss Institute at Harvard University)

Recently, the incorporation of endothelium into the operation of these devices remails a future possibility to be readily available at bedside in order to assist in clinical decision making [32–34].

In summary, microfluidic device measures patient clotting abilities under any specific physical flow pattern and, as a result, can be an invaluable tool for clinical diagnostics.

Quartz Crystal Microbalance

The basis of QCM operation relates to quartz's physical property of piezoelectricity, and as the alternating electric current passes on to the crystal, a mechanical energy generates oscillating waves with a recordable frequencies [35].

Crystal's thickness and cut is detrimental as a trade-off the generated frequency, the thinner is the crystal the higher is the resonant frequency [36].

The QCM sensor consists of a thin circular quartz that is sandwiched between a pair of electrodes and a blood sample will face the electrodes (Fig. 21.18). When sufficient AC voltage is applied to the electrodes, the crystal gets excited and generates oscillation, and a resonance will be generated with a specific frequency and will be detected and measured by a sensor. During the process of clot formation, a mass will be adsorbed on the electrodes causing change in frequency and resonance [37]. When a mass is attached to the sensor, the frequency decreases. If the mass is rigid, the decrease in frequency is proportional to the size of the mass. This linear relationship between changing in mass adsorbed on the surface of quartz crystal electrodes and proportional reduction in frequency was discovered by Sauerbrey in 1959 and established CQM algorithm foundation [33].

However, Sauerbrey equation is linear for rigid mass formation due to its even distribution with sufficiently thin adsorbed layers [34]. Therefore implementing a dissipation parameter will allow analysis of soft films that do not obey the linear relation between change in frequency and change in mass. The soft or viscoelastic will be underestimated as it is not fully coupled to the oscillating crystal. By adding and measuring the dissipation to the QCM, one can determine the adsorbed viscoelastic (soft) mass on the plate [38, 39].

Dissipation done by shutting off the driving voltage to the crystal and the energy from the oscillating crystal dissipates from the system [40]. This procedure can be repeated over 200 times per second, which gives QCM-D great sensitivity and high resolution. QCM-D measures both frequency and dissipation of the quartz crystal [41].

Laser Speckle Rheology

Laser speckle rheology (LSR) is a novel approach to evaluate the frequency-dependent process of viscoelastic blood clot formation using non-contact optical approach. A laser beam will illuminate the blood sample during the process of clot formation; light rays will be scattered by the multiple particles in the blood sample. The scattered speckles created will be captured on a high-speed camera (CMOS) (Fig. 21.19). The scale of time-varying speckle images correlated with a time-real viscoelastic clot formation and will be captured and recorded by the CMOS and analyzed by a computer software.

The Brownian motion phenomenon is one of the elements in the process of LSR evaluation, and it is defined as a random motion of particles suspended in a fluid resulting from their collision with the fast-moving particle in the

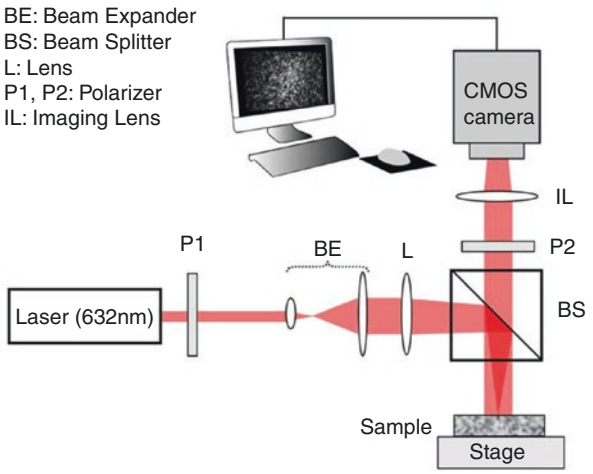

Fig. 21.19 Technology of laser speckle

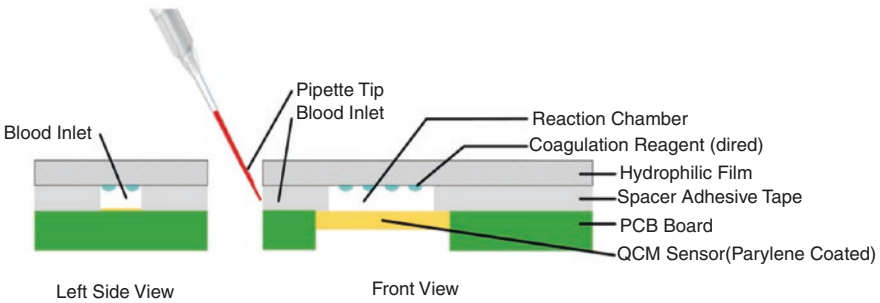

Fig. 21.18 QCM-D transducer consisting of a piezoelectric quartz sensor with two differently sized gold electrodes

fluid. The Brownian motion of blood clot has less restrictions on the scale of scattered particles reflecting wider range of speckle [42]. During the progress of clot formation with more fibrin-formation and platelets aggregation, the clot gets stiffer and restricts the scatter displacements. The camera will register lesser area of speckle fluctuations during the course of clot stiffness.

Blood coagulation status has been measured by relating the time scale of speckle intensity fluctuations with the clinically relevant coagulations parameters, including clotting time and fibrinogen content.

Markandey et al. demonstrated a close correlation between coagulation metrics measured using LSR and conventional coagulation results of activated partial thromboplastin time, prothrombin time, and functional fibrinogen levels [43].

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