Review: Study of the Blood Coagulation by Ultrasound

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Abstract

This paper provides a detailed review of the in vitro, in vivo, and clinical applications of ultrasound to understand blood coagulation. The paper focuses on the effect of blood rheology on clotting mechanisms, especially on the use of ultrasound to detect the viscoelastic properties of blood clots. After a short introduction, the paper describes how quantitative ultrasound parameters can be used to study the process of blood coagulation. Ultrasound parameters can be used for tissue characterization, and so the process of blood coagulation can be monitored continuously by measuring the ultrasound backscatter, attenuation coefficient, and velocity. Furthermore, several ultrasound elastography methods including quasi-static strain imaging, shear wave, and acoustic-radiation-force imaging are introduced for measuring the viscoelastic properties of blood clots. The advantages and drawbacks of these ultrasound-based approaches for detecting blood coagulation are discussed. Finally, new areas of research, the clinical application of ultrasound, and areas of potential future developments are presented.

Keywords: Ultrasound, Blood coagulation, Clot formation, Viscoelastic properties, Ultrasound elastography

1. Introduction

Blood coagulation forms part of hemostasis, which is a major host defense mechanism that aims to prevent blood loss from an injured vessel. Clinical laboratory examination of blood coagulation analysis allows observation of the amount of vitamin K in the blood indirectly and diagnosis of liver disorder. The treatment of drugs used in some hereditary and hemorrhagic diseases depends on the process of blood coagulation by measuring the clotting time or prothrombin time [1]. The coagulation of blood is a complex process during which solid clots are formed in the blood. The conversion of fibrinogen into fibrin and the subsequent covalent cross-linking of fibrin play important roles in this process, and can be induced by an imbalance between coagulant and anticoagulant factors. Although coagulating blood is vital to the preservation of life, blood clots can impede blood flow in the vessels. Thrombus formation is responsible for most heart attacks and strokes and complicates other pathological conditions such as coronary thrombosis, peripheral deep venous thrombosis, pulmonary embolus, and can eventually cause death unless brought under control [2]. In addition, paralytic patients confined to bed usually suffer from intravascular clots due to coagulation that tends to occur when the flowing blood is obstructed for a few hours in any vessel of the body. Therefore, a clear understanding of blood coagulation properties is crucial for clinical diagnosis, and techniques for detecting blood coagulation need to be developed.

A common method to assess the process of blood coagulation involves adding blood to three or four test tubes and then tilting the tubes at 30° intervals until the blood can no longer flow [3]. The current assays frequently used to assess blood coagulation properties are based on mechanical impedance, electromagnetism, rheometer, Sonoclot analyzer, and photometry [4,5]. Moreover, ultrasonic modality can be performed in real time and noninvasively, and it was proposed as a potential method for studying blood coagulation and detecting thrombus in vivo. However, many aspects of studying blood coagulation by ultrasound remain to be investigated since new discoveries often raise more questions than they answer.

This literature review focuses on introducing the ultrasonic modality for studying blood coagulation both in vitro and in vivo. Firstly, the acoustic properties of blood and clot tissues are described in terms of characterizing coagulating blood. Several ultrasonic methods for detecting blood coagulation are then reviewed, and their advantages and drawbacks are discussed. Finally, future developments of
ultrasonic modality in the blood coagulation field are summarized.

2. Assessments of blood coagulation based on ultrasound parameters

2.1 Ultrasound backscatter

When an ultrasound wave propagates through biological tissues, its energy may be diverted by reflection and or refraction or absorbed by the medium and converted into heat [6]. Scattering will occur within the tissue if the interfering particles are much smaller than the acoustic wavelength. The scattering of ultrasound from blood is mainly attributed to red blood cells (RBCs), because they are larger and much more numerous than other types of blood cells. Ultrasound backscattering has been extensively utilized to characterize certain blood properties, such as the hematocrit, fibrinogen concentration, and the size of RBCs [7-10]. The effects of turbulence, shear rate, shear stress, and flow disturbance in flowing whole blood and RBC suspensions have also been investigated by Doppler ultrasound [11-15]. In addition, the rheological impacts of RBC aggregation on flowing blood have been explored using ultrasound backscattering and Doppler ultrasound [16,17]. Since the ultrasound backscatter depends on the particle structure in tissues, rheological studies have involved liquid blood [7,18] or coagulating blood by measuring the backscatter in vitro [19,20]. The power backscattered from coagulating human whole blood was measured for 24 hours using a 7.5-MHz transducer by Shung et al. [21], who found that the backscatter increased by 18.5 ± 1.2 dB (mean ± SD) during the clotting period. Coagulating porcine blood was continuously monitored using a 10-MHz transducer by Huang et al. [22], as shown in Fig. 1. Blood coagulation was induced by adding CaCl₂ solution to 45%-hematocrit whole-blood sample; results indicated that the formation of a clot with the blood turning into a solid gel caused the fluctuations in the ultrasound scattering to cease. A quantitative analysis of the backscattered signal showed that the formation of fibrin fibers caused the average integrated backscatter to increase by 5.8 dB. Blood coagulation properties at different hematocrits ranging from 25% to 55% were studied by the same group [23]. That study demonstrated that blood hematocrit substantially affects the viscosity and rheological properties of blood during coagulation, which can be detected by monitoring the ultrasound backscatter. Since high-frequency ultrasound allows higher-resolution detection of the tissue microstructure [24-27], high-frequency ultrasound backscatter has been used to measure the blood properties [28] and blood coagulation [29]. Furthermore, high-frequency ultrasound backscatter from human blood was measured at frequencies ranging from 20 to 40 MHz during the process of coagulation [30]. The results showed that it is possible to temporally divide the blood coagulation process into several stages. The effects of heparin treatment on blood coagulation were also determined in a murine model using the same high-frequency ultrasound device [31].

Figure 1. A typical ultrasonic image during the blood coagulation. The figure indicates that the formation of a clot with the blood becoming a solid gel results in the cessation of fluctuations in the ultrasonic scattering after approximately 2200 s [22].

2.2 Ultrasound attenuation

As mentioned above, some of the ultrasound energy propagating in biological tissue is lost due to the dispersion of energy by reflection and scattering, and by tissue absorption [32]. Since the ultrasound attenuation of tissue depends on the viscoelastic properties and the relaxation phenomenon of the medium, the blood properties of pH and oxygen tension have been detected by measuring blood’s ultrasound attenuation [33-35]. Furthermore, Shung et al. studied the process of blood coagulation by measuring the ultrasound attenuation by clotting blood [21]. They measured the 7.5-MHz ultrasound-attenuation coefficient for 44%-hematocrit human blood for 24 hours, during which time the average attenuation increased by 2.30 ± 0.22 dB/cm [21]. A similar result was obtained using 10-MHz ultrasound for continuous monitoring of 45%-hematocrit coagulating porcine whole blood [22]. The effects of the hematocrit value on blood coagulation were also studied by the same group [23]. The normalized broadband ultrasound attenuation coefficients (nBUA) were measured from porcine whole blood at hematocrits ranging from 25% to 55%, as shown in Fig. 2. The results showed that increases in viscosity and biochemical interactions in blood during clotting caused the variation of ultrasound attenuation to increase with the hematocrit. Also, the ultrasound attenuation seems to be a more suitable parameter for detecting the early stage of clot formation when the hematocrit is higher than 40%. The high-frequency ultrasound-attenuation coefficient for human blood plasma during clotting was measured for frequencies ranging from 8 to 22 MHz [36]. In order to explore the effect of heparin treatment on blood coagulation, the 10- to 30-MHz high-frequency attenuation coefficient was measured for both human and rat whole blood during coagulation [31].

2.3 Sound velocity

The characteristics of an acoustic longitudinal wave as an elastic wave vary with the mechanical properties of the tissue [32]. Since the ultrasound velocity is inversely proportional to
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Figure 2. nBUA as a function of time obtained from coagulating blood of (a) 25%, (b) 35%, (c) 45%, and (d) 55% hematocrit. The reaction time is indicated as an arrow symbol, which is defined as the nBUA to be dramatically increased. The coagulation time was increased with the increase of hematocrit [23].

The product of the density and compressibility of the tissue, the hematocrit, density, and compressibility properties of blood have been studied by measuring the ultrasound velocity [37-39]. The speed of sound in human coagulating blood was measured [21], and the sound velocity had increased by approximately 3.36 cm/s at 24 hours after clotting. Voleišis et al. [40] and Huang et al. [22] also measured the sound velocity in human and porcine whole blood during clotting, respectively. The velocity of high-frequency sound has also been measured for human and rat blood [30,31]. General speaking, the sound velocity increases gradually during blood coagulation, as shown in Fig. 3. Their study concluded that the sound velocity varies during particular stages of blood coagulation. Therefore, measurements of sound velocity are useful to characterizing the clotting process, such as fibrin network formation and the kinetics of clot retraction that is linked to platelet function.

2.4 Ultrasonic characterization of blood coagulation under flowing conditions

All of the above ultrasound-based studies investigated coagulating blood under static conditions, whereas blood coagulation is a dynamic process that generally depends on the shear rate and shear stress of blood flow in vessels [41-44]. Thus, other feasibility studies have measured the variation of viscosity of coagulating blood at different shear rates [45]. Ultrasound backscattering has been frequently used to characterize properties of blood under various flow conditions [11,12,46]. Specifically, the strength of ultrasound backscatter by blood has been found to depend on the shear rate of the flow and RBC aggregation [47-49]. Furthermore, the properties of coagulating blood have been assessed based on ultrasound signals backscattered by blood in an excised section of the porcine aorta [50]. The progress of thrombus development in an ex vivo shunt model was monitored using intravascular ultrasound [51]. In addition, the Doppler velocity and Doppler power were found to allow variations of stirred blood properties during coagulation to be sensitively detected [52]. Color Doppler imaging was used to detect the flow velocity during blood coagulation when the RBCs were displaced by induced acoustic streaming [53]. The effect of shear rate on blood clotting was explored by measuring ultrasound backscattering in coagulating blood at different flow velocities [54]. The results consistently demonstrate that a higher shear rate tends to prolong the coagulation time of flowing blood. In addition to traditional backscatter measurements, statistical models based on backscattering signals have also been used to

Figure 3. Sound velocity as a function of time during blood coagulation and clot formation. The measurements were performed at 10 MHz ultrasound from porcine whole blood [22].
characterize the blood [55,56] and blood coagulation properties [57] under flowing conditions. The two major components of the thrombotic process are aggregation and blood coagulation. Both aggregation and blood coagulation in vessel increase the size of scatterers in blood. However, the process of blood coagulation is quite a different physiological phenomenon from RBC aggregation. The lower value of Nakagami parameter for flowing blood may be due to a stronger envelope near the center of the tube that changed the probability density function (PDF) from a Rayleigh to a pre-Rayleigh distribution. The value of Nakagami parameter consequently approached 1 during the clotting phase, in which the resolution cell of the ultrasonic transducer contains a large number of distributed scatterers, the envelope statistics of the ultrasonic backscattered signals are similar to a Rayleigh distribution. As the clot formed and the blood became for more than 20 gel, the Nakagami parameter increased rapidly to its highest level of higher than 1. The PDF in this condition probably has a post-Rayleigh distribution corresponding to the structure of blood containing large scatterers such as a fibrin meshwork and blood clots. All of these studies demonstrate that ultrasonic techniques can be applied to feasibly and sensitively detect and assess blood coagulation under dynamic conditions.

3. Measurements of the viscoelastic properties of clots

3.1 Ultrasound elastography

While the analysis of ultrasound parameters of coagulating blood can be used to indirectly determine the viscoelastic properties of clots, direct measurements of clot mechanical properties are also needed. Ultrasound elastography has been used to measure the mechanical properties of soft tissues for more than 20 years [58]. This method is based on comparisons of ultrasound images obtained when the tissue is subjected to axial forces. The ages of blood clots in deep veins have been evaluated by using quasi-static elastography to assess the mechanical properties of blood clots both in vivo and in vitro [59,60]. An animal model was also subjected to ultrasound elasticity imaging [61,62]. The viscoelastic properties of deep vein thrombosis were measured directly in order to assess the stiffness of blood clots. Imaging clot elasticity may make it possible to both monitor and differentiate clots, and thereby provide an urgently needed noninvasive method for thrombosis staging.

3.2 Shear wave

Since the shear wave velocity is directly related to elasticity in an elastic medium, measurements of this velocity in biological tissue can be used to infer or diagnose pathological conditions [32]. This prompted the use of an ultrasonic shear-wave method to detect the process of blood coagulation in vitro [63]. The prothrombin time and activated partial thromboplastin time of coagulating blood were determined in that study, one limitation of which was that it only made measurements on formed blood clots, and did not assess the dynamics of blood clotting. This prompted the continuous variation of the blood coagulation process to be monitored using the shear wave velocity and shear wave attenuation, which are linked to the viscoelastic properties of blood clot [64]. However, in vivo measurement of shear wave would be needed before applying this method to assess the viscoelastic properties of thrombosis.

3.3 Acoustic radiation force

In addition to quasi-static elastography, dynamic elastography based on an acoustic-radiation-force technique was developed to evaluate the viscoelastic properties of biological tissue [32]. It is hypothesized that an acoustic radiation force can be applied to create localized tissue displacements, which would be directly correlated with localized variations of the tissue viscoelastic properties [65,66]. A radiation-force method to detect the backscattered signals from spherical glass particles in the blood has been described, in which particle motions are strongly influenced by the rheological changes that occur during the process of coagulation [67]. In addition, Doppler ultrasound was used to evaluate the process of blood coagulation by measuring the acoustic streaming induced by applying an acoustic radiation force [68]. Furthermore, the relative elasticity and viscosity of blood clot were assessed by an acoustic-radiation-force technique [69]. This method was also used to investigate the dynamic process of coagulation of human blood [70]. However, the applied force is unknown due to the absorption coefficient and acoustic impedance differing between the tissues. Therefore, no absolute values of clot elasticity and viscosity were given in these acoustic-radiation-force technique reports, except where the applied force was known. This problem was overcome using a method based on measuring the displacement of a solid sphere in response to a time-dependent acoustic radiation force [71,72]. The blood clot viscoelastic properties were subsequently measured in porcine whole blood at hematocrits from 0% to 40% [73], revealing that the shear modulus of blood clots decreased from 585 ± 127 Pa in plasma to 168 ± 26 Pa at a hematocrit of 40%. These data suggest that the concentration of fibrinogen plays the main role in determining clot elastic properties.

4. Discussion

Blood coagulation is a complex physiological mechanism. It is well known that over 50 factors may affect the blood coagulation mechanism. The normal process of blood coagulation involves three essential steps. First, in response to rupture of the vessel or damage to the blood itself, a complex cascade of chemical reactions occurs in the blood involving more than a dozen blood coagulation factors, the net result of which is the formation of a complex of activated substances collectively called prothrombin activator. Second, the prothrombin activator catalyzes the conversion of prothrombin into thrombin. Third, the thrombin acts as an enzyme to convert fibrinogen into fibrin fibers that enmesh platelets, blood cell, and plasma. Finally, the blood becomes a solid gel, and a clot forms that is composed of a meshwork of fibrin
fibers running in all directions that entraps blood cells, platelets, and plasma. All the essential steps require activated platelets, plasma cofactors, and Ca$^{2+}$ [3].

As described above, the ultrasound-based methods used to study blood coagulation properties have mainly employed measurements of acoustic parameters of clotting blood and ultrasound elastography. The backscattered ultrasound signals have been shown to vary with the size, shape, concentration, density, and elastic properties of the scatterers in a biological tissue [6,7,11,74,75]. The formation of fibrin fibers increases the size and changes the shape of the scatterers, which increases the echogenicity during the clotting process [21-24,76]. The fluctuation in backscattered signals during clotting may be due to the turbulent motion of the fluid caused by fibrinogen converting into fibrin fibers [21,22,29,77], as shown in Fig. 1. During the last stage of blood coagulation, many erythrocytes are trapped in the fibrin meshwork, the blood increases in viscosity and tends to become a solid gel, and therefore the ultrasound attenuation increases, as shown in Fig. 2. There is experimental evidence that ultrasound absorption by biological materials is affected by molecular interactions [78]. Therefore, the increase in attenuation could be due to an increase in viscosity and other frictional factors that consume energy, which is lost as heat [79].

The formation of fibrin fibers changes the sound velocity, which is inversely proportional to the square root of compressibility, and the compressibility of blood clots reduces due to the blood becoming stiffer [21]. Therefore, the increased sound velocity during clotting might be attributable to the variation of blood clot compressibility, as shown in Fig. 3. A method based on the analysis of the ultrasound parameters of blood clots could achieve the goals of real-time and continuous observation, since most of the acoustic properties associated with changes in tissue characteristics can be preserved without any loss of the original physiological information. Measurements of quantitative ultrasound parameters for assessing the blood coagulation process are suitable for laboratory examinations because detailed variations of clotting can be detected by continuously monitoring the acoustic parameters. Even though it is very difficult to obtain accurate values of these parameters in vivo, the obtained data could still have clinical relevance to human care because real-time ultrasound imaging has been shown to be a valuable tool for diagnosing intracranial hemorrhage and thrombi.

In contrast, ultrasound elastography could make it feasible to measure the viscoelastic properties of blood clots in vivo. Because the clot stiffness increases monotonically with the fibrin content [80], it is reasonable to use ultrasound elastography to estimate the clot maturity. Leg veins are typically located near the skin surface, which makes ultrasound elastography useful for detecting thrombosis [59]. However, such measurements may contain errors since the imaging systems are operated manually and have poor signal-to-noise ratios. In addition, only the relative viscoelastic properties of clot can be obtained by quasi-static strain imaging because the compression force is unknown. Another problem is erroneous diagnosis when the clots and surrounding tissues (e.g., vessel wall or muscle) have similar stiffnesses. Therefore, a combination of elastography and color flow imaging may make it possible to simultaneously detect and stage blood clot in vivo. Similar problems also occur in dynamic elastography based on the acoustic radiation force, since the magnitude of this force applied to the clot is unknown due to differences in the absorption coefficient and acoustic impedance in the surrounding tissues [66]. Even though the viscoelastic properties of clot have been assessed by an acoustic-radiation-force method in vitro [69], it remains difficult to determine the stiffness of clots using commercial acoustic-radiation-force impulse imaging in vivo because of the applied impulse being too short to induce a sufficient displacement of the clot [69]. Moreover, measuring acoustic streaming by Doppler ultrasound is not suitable for the in vivo assessment of clot properties [68]. However, the detailed process of blood coagulation can be monitored continuously by measuring acoustic streaming in vitro [70].

Since the blood flow velocity in microcirculation is slower than it is in other blood vessels, the local concentration of clotting factors may be increased in plasma, particularly in patients who have a blood coagulation problem. Most ultrasound studies have not explored clotting blood under microcirculation level. The reason may be that the resolution of ultrasound was not good enough. However, this problem may be resolved by using the ultrasonic contrast agents. Fibrin-targeted perfluorocarbon nanoparticles were used for marking the thrombus with ultrasound in canine femoral artery in vivo [81]. Therefore, it is possible to detect the blood coagulation in microcirculation by high-frequency ultrasound imaging via ultrasonic contrast agents.

5. Summary and future developments

Several ultrasonic methods used to study blood coagulation have been briefly reviewed in this report. Since quantitative ultrasound parameters can be used for tissue characterization, the process of blood coagulation has been monitored continuously by measuring the ultrasound backscatter, attenuation coefficient, and sound velocity in vivo. All of the obtained results indicate that the acoustic properties of blood are affected by the hematological and hemodynamic properties of coagulating blood. However, the ability to accurately measure these ultrasound parameters in vivo is needed before applying this method for clinical diagnosis. It might be possible to use ultrasound elastography to both detect and differentiate clots and, therefore, provide an urgently needed noninvasive means of clot staging. However, quasi-static and dynamic elastography methods cannot provide absolute values of clot elasticity unless the applied force is known. A feasible method might be based on measuring the displacement of a solid sphere or gas bubble in a blood clot in response to a time-dependent acoustic radiation force, as shown in Fig. 4 [73]. Future studies could focus on developing laser-based diagnosis combined with acoustic-radiation-force elastography. For example, a gas bubble could be created in a
blood clot by stimulation with a nano- or femtosecond laser pulse by a laser attached to an intravascular ultrasound probe. The subsequent application of an acoustic radiation force to the laser-induced gas bubble could allow the viscoelastic properties of the clot to be estimated, thereby providing an improved diagnostic strategy. Additional work should also be oriented toward using ultrasound to obtain a better understanding of the blood coagulation process under a dynamic or flowing condition.

Figure 4. (a) Block diagram of the experimental setup for assessing the viscoelastic properties of blood clot using a solid-sphere-based acoustic-radiation-force approach. (b) The results of sphere displacement in response to a time-dependent acoustic radiation force. The viscoelastic properties of blood clot can be reconstructed by fitting the sphere displacement curve in response to applied force [73].

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References


