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A Study on Ratio of Loss to Storage Modulus for the Blood Clot

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ABSTRACT

In this study, the rheology of blood clot is measured with the help of rotational rheometer. Several shear strains (0.5, 1 and 2%) are applied with two frequencies (5 and 10 Hz) from the incipient time of clot formation and the response of the sample is measured in the form of shear stress and the phase lag which is interpreted with storage and loss moduli. In this study the ratio of loss to storage modulus is studied and the blood clot gel-point as the transition from viscoelastic fluid to viscoelastic solid is investigated. By increasing the frequency, "tan delta" decreases before the gel-point and increases after the gel-point which indicates the viscoelastic fluid and viscoelastic solid behaviour,respectively. Moreover, by increasing the shear strain, "tan delta" varies with lower rate at fluidic stage (Ra1 and Ra3) and with higher rate at solid stage (Ra2 and Ra4). It is also shown that increasing the shear strain causes a delay on gel-point formation.

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1. INTRODUCTION

1. 1. Rheology of Blood Clot The viscoelasticity of blood clot is a mechanical indicator in order to predict the probability of clot rupture and/or strength when exposed to various forces inside the blood current. This may lead to further embolization and so fatal event [1]; thus the behavior of blood clot stuck inside the coronary vessel is seriously important. Moreover regarding the treatment of such disease (coronary vessel blockage due to the blood clot) the mechanical characteristic of the blood clot determines how to choose the appropriate medical care; for e.g., the percutaneous coronary intervention (PCI) can only be applied for aspiration of clot with specific value of mechanical characteristic[2].

The rheology of blood clot is defined with the help of dynamic modulus: storage and loss. The blood clot mechanical characteristic (dynamic modulus) is a crucial parameter which indicates whether a blood clot easily ruptures and leads to further embolization or not [3].

Moreover, the study on blood clot rheology provides useful information for the recognition of different diseases including myocardial infarction, vessel peripheral disease and cancer [4].

The clotting time (the time needed to convert an incipient clot to a solid clot) is a very important factor which could be determined by the gel-point factor.

It is also worth noting that three types of blood clot exist: fibrin clot, plasma clot and whole blood clot. Most of the researches are focused on the characteristic of fibrin and plasma [5]. However, the study on whole blood clot viscoelasticity is of great demand particularly to predict acute myocardial infarction (AMI) fatal level as well as treatment of AMI with PCI method. The purpose of this study is to investigate the mechanical characteristic of whole blood clot which was barely addressed by the other authors. The rheology of a material could be measured by several techniques including: dynamic ultrasound elastography, thromboelastograph and rotational rheometer. Thromboelastograph is an old fashion measurement

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approach which is not able to measure the blood clot in its incipient stage. In dynamic ultrasound elastography only a high frequency could be applied to measure the blood clot rheology. This method either cannot measure the blood clot rheology in its incipient stage [6]. To measure the blood clot rheology through rotational rheometer, a low frequency is applied so that the blood clot rheology could be measured since the incipient stage of clot coagulation [4, 5, 7]. In this study, the rotational rheometer is used for clot rheoleogy measurement with shear strain of 0.5, 1 and 2%. The shear strain less than 3% will guarantee a linear regime. Rotational rheometer is able to measure the rheology of the material through applying steady [5, 7-9] and oscillatory (dynamic) measurement [4, 5, 7]. An oscillatory (dynamic) measurement is performed within this study for the measurement of rheology of porcine whole blood clot. Oscillatory measurement provides a much more reliable data compared to steady measurement.

The availability of human blood is not easy in terms of risk of infection, donor limitations and frequent access. On the other hand, the human blood is similar to porcine blood in terms of structure and hemostatic. According to Verbiest [10] and based on some published works [11-14], the porcine coagulation cascade and coagulation factors are analogous to those of humans, also the platelets of humans and pigs have similar morphology and size. Furthermore, similar to human platelets, porcine platelet agonists are thrombin, collagen and ADP. There are still differences, e.g. epinephrine does not activate porcine platelets, as it does in human platelets and the arachidonic acid pathway in pigs does not develop to the same level as it does in human blood. These differences are insignificant in the complicated system of thrombus formation and porcine blood and hemostatic system are comparable to those of humans and can be used for experiments in thrombus formation.

2. MATERIALS AND METHOD

Immediately after the blood sample collection, it is first mixed with the anticoagulant in order to prevent the start of coagulation. The coagulation is then initiated in the laboratory by adding the coagulant to neutralize the anticoagulant immediately before the start of the measurement. Some droplets of the blood sample is inserted on the plate of the rheometer which is further spread all over it by approaching the upper plate of the rheometer to the lower one in order to wholly fill the gap and create a meniscus. In our study the Ares (Rheometrics, Inc., Piscataway, NJ, USA) is used. Such device is a strained control rheometer i.e. the specific amount of strain is applied to the sample and the response is measured as the stress. The frequently shear strain is applied based on the following correlation:

$$g^* = g_m e^{iwt} \tag{1}$$

where w is the frequency of applied shear strain and g_m is the maximum value of shear strain. In a linear regime the stress follows the same frequency with a phase lag as follows:

$$t^* = t_m e^{i(wt+d)} \tag{2}$$

where t_m is the maximum value of shear stress and d is the phase lag between the induced shear strain and the measured shear stress. The correlation between shear strain and shear stress is as follows:

$$t^* = G^* g^* \tag{3}$$

The G^* is the dynamic moduli. It is obtained through the following calculation:

$$G^* = \frac{t_m}{g_m}\cos d + i\frac{t_m}{g_m}\sin d \tag{4}$$

The "real" and the "imaginary" terms are written as follow:

$$G^* = G' + iG'' \tag{5}$$

where:

$$G' = \frac{t_m}{g_m}\cos d \tag{6}$$

and

$$G'' = \frac{t_m}{g_m}\sin d \tag{7}$$

G' called as "storage modulus" is a measure of the stored energy in every cycle which will be recovered at the end of the cycle while G'' called as "loss modulus" is the dissipated energy per cycle which is out of phase with respect to the shear strain. The parameter "tan delta" is defined as the ratio $\frac{G''}{G'}$ which will be exploited to depict the clotting time and the gel-point.

3. EXPERIMENTAL TESTS ON CLOT RHEOLOGY

The rheology test can be done by applying the shear strain and measuring the shear stress. The shear strain can be imposed either with a frequency or without a frequency i.e. with a constant rotation. The former test will provide us with dynamic moduli (storage and loss) while the latter will end up to the viscosity of the material which is not a complete data to describe a viscoelastic fluid similar to blood clot. The test of

rheology with frequently shear strain is called dynamic test while with an imposed constant shear strain is called steady test. For several reasons a dynamic test approach is exploited within this study: the blood flow material has both viscosity and elasticity behavior and only can be described by dynamic moduli obtained through dynamic test. Moreover, through the steady test the risk of not forming coagulation or rupturing the network of fibrin increases. Conversely, the dynamic measurement has the least influence on the coagulation process and does not rupture the clot fibrin network as the maximum shear strain does not exceed 3%. Additionally, in order to prevent the sample exposure to air and dehydration, a thin layer of low viscose oil is added around the meniscus of the blood sample. The dehydration issue is crucial and inevitable if the oil is not used for the meniscus coverage. In steady measurement, the use of oil is not feasible; due to the complete rotation of rheometer plate the oil will disturb the sample meniscus and lead to an incorrect rheology measurement. It should be also noted that the complete rotation of the upper plate in steady test will loose the contact between the sample and the upper plate which may lead to totally wrong measurement.

Some studies indicate that the silicon plates may delay the clotting time significantly. Accordingly a stainless steel material is chosen for the rheometer plates in this study which is reported to have the least influence on clotting process [4, 15].

4. RESULTS AND DISCUSSION

Three shear strain: 0.5, 1 and 2% are applied with the frequency of 5 and 10 Hz. The diagram of "tan delta" vs. time indicates the ratio of $\frac{G''}{G'}$ (loss modulus over storage modulus). The transition from viscoelastic fluid to viscoelastic solid (the gel-point) could be indicated through the cross point of "tan delta"for various frequencies. This is because at the gel-point, both G' and G'' exhibit a power law behavior having a correlation with w^a and thus "tan delta" becomes independent of w. It is shown that the gel-point increases by increasing the shear strain rate.

The blood clot formation has three stages, at the first stage polymerization takes place between the monomers. This stage is followed by the formation of fibrin network and their cross linking. At this stage a sudden increase in both storage and loss moduli takes place, however the storage modulus increases with a higher rate. At the third stage, both dynamic moduli decrease due to the retraction which takes place because of the protein activity trapped inside the fibrin network and so causing a slight shrinkage [6, 16].

While the first stage is related to viscoelastic fluid behaviour of clot the second and third stage are related to the viscoelastic solid behaviour of clot. The increase on shear strain causesa larger displacement imposed to the coagulating blood clot through the rheometer upper plate. This phenomenon postpones the second and third stage of blood clot coagulation; thus by increasing the shear strain, the gel-point shifts to the right so that at shear strain 0.5, 1 and 2%, the gel point occurs at 267, 524 and 597 s. More precisely, the gel point of 1% shear strain is 1.96 times higher than that of 0.5% shear strain, and the gel point of 2% shear strain is 2.23 times that of 0.5% shear strain. Increasing the shear strain with 1% from 1 to 2 leads to an increase in gel point to 1.14 times.As shown in Figures 1,2 and 3, by increasing the frequency "tan delta" decreases before the gel- point and increases after the gel-point. This phenomenon is also seen in the fibrin blood clot as well as plasma blood clot measured in the literature [17] and presented in Figures 4 and 5. It could be seen that there are several similarities and differences between plasma clot, fibrin clot and whole blood clot: in all cases (fibrin clot, plasma clot and whole blood clot) there is a transition from fluidic behaviour to the solid behaviour which occurs at the gel-point. Moreover at fluidic stage, the increase in frequency leads to decrease of "tan delta" and at the solid stage increasing the frequency leads to the increase of "tan delta", however they occur with different slopes. The general behaviour of "tan delta" vs. time is characterized through two main slopes; Ra1 and Ra2 for 5 Hz and Ra3 and Ra4 for 10 Hz. For each frequency,the first slopes (Ra1 and Ra3) is higher than the second slopes (Ra2 and Ra4). This means right before and after the gel point, $\frac{G''}{G'}$changes with higher rate while after this period when the solid clot is perfectly formed, "tan delta" and so the ratio of $\frac{G''}{G'}$ does not change with high rate. The slope of the diagram of "tan delta" vs. time is also presented on Table 1. The highest Ra1 and Ra3 are related to the shear strain 0.5% while the highest Ra2 and Ra4 are related to the shear strain 1% and 2%, respectively. Thus, at the fluid stage, the slope of the diagram and so the rate of the change of $\frac{G''}{G'}$ modulus is the highest for the lowest shear strain rate (0.5%), while at the solid stage, the slope of the diagram is higher for the higher shear rate. More precisely, the first slope Ra1 is higher for 0.5% shear strain than the other two cases, 3.58 time compared to 1% and 2.6 time compared to 2% shear strain, respectively.The second slope Ra2, which is one order smaller than the previousone, generally increases with increasing shear strain, from 3.5e-5 for 0.5% to 5.36e-5 for 2%. From 0.5 to 1% shear strain, the slope increases 1.68 times, while it 10% decreases from 2% to

1% shear strain.The first slope Ra3 decreases with one order from 0.5 to 1% shear strain, but increases back with further shear strain increase from 1% to 2%.Comparing values of 0.5% shear strain to 2% shear strain, Ra3 decreases 3.64 times.The second slope Ra4 increases one order with increasing shear strain from 0.5 to 1%; further increase in shear strain with 1 % (from 1 to 2%) causes an increase in slope Ra4 to 1.72 times.

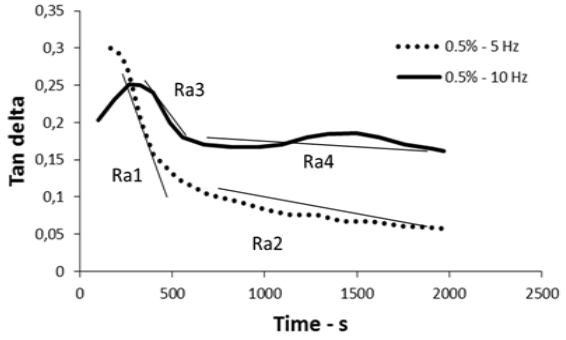

Figure 1. Tan delta, representative of $\frac{G''}{G'}$, for shear strain 0.5% and frequencies 5 and 10 Hz.

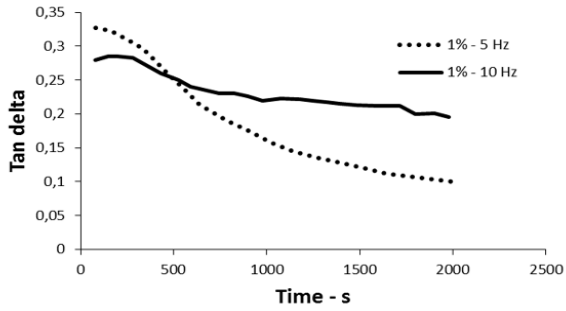

Figure 2. Tan delta, representative of $\frac{G''}{G'}$, for shear strain 1% and frequencies 5 and 10 Hz.

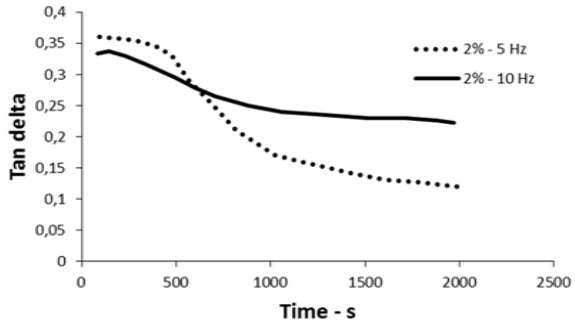

Figure 3. Tan delta, representative of $\frac{G''}{G'}$, for shear strain 2% and frequencies 5 and 10 Hz.

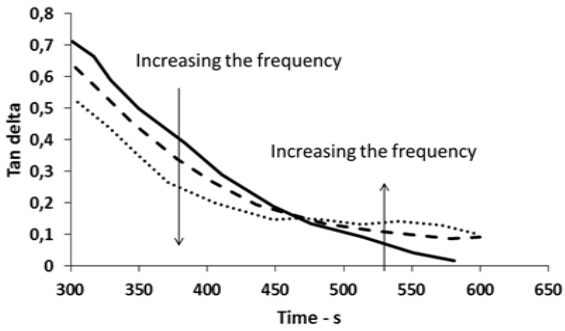

Figure 4. Tan delta vs. time for "plasma" blood clot.

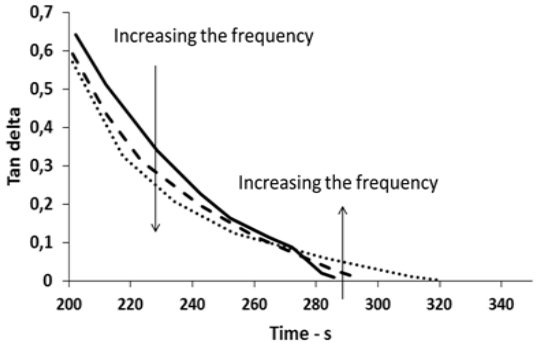

Figure 5. Tan delta vs. time for "fibrin" blood clot.

TABLE 1. Values of Ra1, Ra2, Ra3, Ra4 and the time of gel-point for shear strain 0.5, 1 and 2% and frequencies of 5 and 10 Hz.

0.5% Shear strain	1% Shear strain	2% Shear strain
Ra1 7.7e-4 1/s	Ra1 2.15 e-4 1/s	Ra1 2.96 e-4 1/s
Ra2 3.5 e-5 1/s	Ra2 5.9 e-5 1/s	Ra2 5.36 e-5 1/s
Ra3 3.9 e-4 1/s	Ra3 7.1 e-5 1/s	Ra3 1.07 e-4 1/s
Ra4 1.4 e-6 1/s	Ra4 1.1 e-5 1/s	Ra4 1.9 e-5 1/s
gel-point 267 s	gel-point 524 s	gel-point 597 s

5. CONCLUSION

The dynamic moduli of sample of blood clot are measured with the help of rotational rheometer. Three shear strains of 0.5, 1 and 2% are applied with the frequency of 5 and 10 Hz and the ratio of dynamic moduli are measured during the clotting process. During the course of coagulation, blood sample experience three stages;a low rate growth of dynamic moduli, high rate growth of dynamic moduli and decline of dynamic moduli linked to polymerization and cross linking of the fibrin network as well as clot retraction, respectively. The lowest shear strain rate (0.5%) has the highest rate

of change (Ra) except for Ra2. Moreover the gel-point delays and the level of “tan delta” increases at the gel-point by increasing the shear strain. It is also worth noting that the dynamic test done in this study not only let us to capture the dynamic moduli to quantify the blood clot rheology but also provides the privilege of the usage of the oil to cover the sample meniscus against any dehydration.

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چکیده

در این مطالعه، رئولوژی لخته خون با استفاده از دستگاه رئومتر چرخشی اندازه گرفته می شود. کرنشهای برشی متعددی (0/5، 1 و 2 درصد) در دو فرکانس (5 و 10 هرتز) از زمان اولیه تشکیل لخته به کار گرفته می شود و پاسخ نمونه به شکل تنش برشی و تاخیر فاز اندازه گیری می گردد که با مدول ذخیره سازی وکاهش تفسیر می شود. در این تحقیق، نسبت مدول کاهش به ذخیره سازی مورد مطالعه قرار گرفته و نقطه-ژل لخته خون به عنوان گذار ازسیال ویسکوالاستیک به جامد ویسکوالاستیک بررسی شده است. با افزایش فرکانس، " تن دلتا " قبل ازنقطه-ژل کاهش و بعد از نقطه-ژل افزایش می یابد که به ترتیب رفتار سیال ویسکوالاستیک و جامد ویسکوالاستیک را نشان می دهد. علاوه بر این،با افزایش کرنش برشی، " تن دلتا " با نرخ پایین تری در فاز سیال (Ra1 و Ra3) وبا نرخ بالاتری در فاز جامد (Ra2 وRa4) تغییر می کند. همچنین نشان داده می شود که افزایش تنش برشی باعث تاخیر در تشکیل نقطه-ژل می گردد.

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