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Review

Rheometry and associated techniques for blood coagulation studies

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Abstract

This review considers various rheometrical approaches that have been adopted to study blood coagulation, with special reference to the rheological assessment of clotting time and studies of the evolution of viscoelasticity during the course of fibrin polymerization and cross-linking. The significance of the Gel Point in blood coagulation studies is discussed as a common feature of many of these studies in that they attempt to detect a liquid-to-solid transition during coagulation. Coagulation studies based on various forms of complex shear modulus measurements are considered, the latter being based principally on controlled stress and controlled strain rheometers. Also considered are the long established technique of thromboelastography and several emerging techniques such as wave propagation measurements, free oscillation rheometry, quartz crystal microbalance measurements and surface plasmon resonance.

Keywords: Blood coagulation; Clot viscoelasticity; Incipient clot; Gel Point; Clot formation time; Blood clot detection

Contents

1. Introduction

Blood coagulation involves fibrinogen–fibrin transformation due to the catalytic action of thrombin and the subsequent establishment of a three-dimensional gel network of fibrin fibres [1,2]. Fibrin gels are the main structural scaffolds of

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the haemostatic plug or 'clot' formed in vertebrate blood coagulation, a process involving significant changes in viscoelasticity. Some of these changes have been associated with pathologies such as myocardial infarction (MI), peripheral vascular disease, cancer and diabetes, and have stimulated studies of the rheological properties of coagulating blood, particularly their viscoelastic properties [3–5]. Viscoelastic properties are among the most sensitive measures of fibrin polymerization and clot structure and there are numerous examples of their physiological importance [2]. The connection between disease and clot properties has been reported in terms of an increased incidence of MI in patients with elevated levels of plasma fibrinogen, which results in less deformable clots [5]. In a partially occluded blood vessel the viscoelastic properties of a clot will determine whether the flowing blood causes it to deform reversibly or irreversibly, rupture, or embolize. The viscoelasticity of a blood clot also determines how it responds to treatments, such as coronary artery angioplasty.

The goal of relating the viscoelastic properties of fibrin gels to their microstructure using complementary rheological and morphological studies has been an area of significant activity [6–8]. The three-dimensional network of fibrin fibers formed in vivo constitutes the primary microstructural basis of a blood clot [9] and the time required for conversion of fibrinogen into a clot network is an important clinical parameter called the 'clotting time' (CT). This term is often used synonymously with the term 'Gel Point' in studies of fibrin gels [10] and it is clearly important to consider the rheological significance of the Gel Point insofar as it defines the transition between a (pre-gel) viscoelastic fluid and a (post-gel) viscoelastic solid. The key point in the context of blood coagulation is that the blood clot is required to perform a haemo*static* function and the properties of a viscoelastic *solid* are necessary in order to perform this function. It follows that rheometrical detection of the Gel Point provides a basis for assessing the clotting time of blood in terms of the establishment of an *incipient* clot.

Many rheological studies have attempted to use the rheometrical detection of a Gel Point (GP) as a basis for assessing the clotting time (CT) of blood. However, it is important to distinguish between those techniques which provide absolute values of viscoelastic properties, under conditions where the lengthscales and timescales of deformation are controlled, and other (usually less sophisticated) techniques which provide more qualitative assessments of rheological changes during coagulation. In particular it is necessary to consider how the choice of rheological technique can have a significant impact on the assessment of clotting time. This point is addressed in the present paper which considers techniques including small amplitude oscillatory shear, free oscillation rheometry and various wave propagation techniques. Also considered is the long established technique of thromboelastography and several emerging techniques are discussed including quartz crystal microbalance (QCM) measurements and surface plasmon resonance (SPR)-based assessments of coagulation parameters. Various other tests, both optically and mechanically based, have been used to characterise blood coagulation. They include spectrophotometry and photometric techniques such as nephelometry and turbidimetry, which are commonly used to study coagulation of blood plasma in the bulk phase, while ellipsometry is used to study plasma/surface interactions. These techniques are not considered in detail herein as the focus of this review is mainly the direct assessment of viscoelasticity during blood clotting. References to other techniques may be found elsewhere [11–13].

2. Techniques for blood coagulation monitoring

2.1. Thromboelastography

The thromboelastograph (TEG) [14,15] was introduced in the late 1940s to provide a global coagulation profile and has found use in assessing the consequences of major blood loss and the effect of fibrinolysis during various surgical procedures. It is claimed to be capable of identifying numerous coagulopathies, and to be sensitive to platelet and fibrinogen levels in whole blood coagulation.

The TEG consists of an inner cylinder (the 'pin') suspended on a torsion wire and an outer cylindrical cuvette (the 'cup') which performs unsteady oscillation. A sample (ca. 0.36 ml) of blood is placed within the cup which rotates back and forth, typically every 10 s, through an angle of 4° 45 min. The pin movement is plotted as a displacement (in mm) on a chart record or 'thromboelastogram', the time between the start of TEG data collection (at the 'SP time') to a pin movement greater than 2 mm being defined as the reaction time (the 'R time')—that deemed necessary to initiate fibrin network formation. Other TEG parameters are defined in Fig. 1.

In TEG measurements clot 'rigidity' is assessed in terms of pin displacement. Such measurements involve both elastic and viscous contributions from a coagulating blood sample and it is important to recognise that even Newtonian liquids, with a sufficiently high-shear viscosity, will generate substantial thromboelastograph readings [16]. Moreover, TEG measurements have not been related in a rigorous way to parameters such as the complex shear modulus, $G^*(\omega)$, which is obtained in oscillatory shear experiments involving the application of a harmonically varying shear strain γ of the form $\gamma = \gamma_0 \cos \omega t$. The components of G^* are the storage modulus (G') and the loss modulus (G'') which characterise elastic and viscous properties, respectively.

The TEGs principal drawback is that the strain amplitude associated with its operation is uncontrolled and decreases progressively during coagulation. Shear applied during clotting substantially weakens clots in blood, plasma [17] and fibrinogen solutions [18] and the linear viscoelastic behaviour of clots is restricted to a shear strain range $\gamma < 2\%$ [19]. Larger strains produce strain-hardening behaviour [20]. Typical TEG measurements will involve shear strain amplitudes