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Magnetoelastic transducers for monitoring coagulation, clot inhibition, and fibrinolysis

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Abstract

Magnetoelastic transduction has been used to detect and monitor the viscosity changes that occur during the biological reactions of coagulation and fibrinolysis. Magnetoelastic sensors can be used, because the characteristic resonance frequency of the magnetoelastic strip shifts in response to the changes in fluid viscosity. At a set frequency, the output signal can be obtained over time to develop a coagulation and/or dissolution profile, which display the change in viscosity of a plasma sample that has undergone either coagulation or fibrinolysis. For coagulation screening, an exogenous tissue factor is added to an anticoagulated plasma sample to initiate coagulation. Further studies were performed to investigate fibrinolysis through the addition of plasmin. Plasmin is used in two different ways—as a competitive inhibitor before the initiation of clotting and also as a protease to dissolve the previously formed clot. This method is a viable option for the monitoring of processes that are paramount to maintaining hemostasis.

Keywords: Magnetoelastic transduction; Blood coagulation; Fibrinolysis; Thromboplastin; Plasmin

1. Introduction

The blood coagulation cascade is a complex biological system that is an essential component of hemostasis (Brown, 1984), the ability of the body to maintain normal blood flow. There are four major steps to the hemostatic process. Upon injury, the initial reaction is vasoconstriction to decrease the blood flow at the site of the wound. Platelets become activated and then begin to aggregate, forming a temporary plug to reduce blood flow. During this step, the blood coagulation cascade becomes activated, which sets off a series of enzymatic reactions that ultimately form a fibrin clot to completely stop the bleeding. Once the injured tissue has been repaired, the final stage in this process is fibrinolysis, i.e., the dissolution of previously formed clots (Brown, 1984; Sultan et al., 1984). The hemostatic process is a delicate balance between activators, proteases, and inhibitors that maintains normal function of the vascular system.

The blood coagulation cascade is activated upon injury to a vessel wall. Depending on whether the wound results in exposure of external tissue factors, the blood clot formation will proceed through one of two pathways. The intrinsic pathway is initiated by injury to a vessel wall and requires clotting factors VIII–XII, while the extrinsic pathway requires a vascular injury that exposes tissue factor and requires clotting factors VII and X. Both pathways converge at a common point and proceed to the production of a fibrin network or clot (Brown, 1984; Sultan et al., 1984).

Once a fibrin clot is formed in the body, it must be removed to enable the body to return to normal vascular function. The reversal of the coagulation process is done through the fibrinolytic system, which keeps the body free of deposited clots. The primary enzyme responsible for this action is plasmin,

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a proteolytic enzyme that is found in the body in its inactive form, plasminogen (Brown, 1984; Booth, 1999). Plasminogen is a β-globulin that complexes with both fibrinogen and fibrin and gets adsorbed by the fibrin clot (Rijken and Sakharov, 2000). Plasminogen activators are required to catalyze the conversion of plasminogen to plasmin. Once activated, plasmin digests fibrin to form fragments called fibrin degradation products. These products inhibit platelet aggregation and the interaction of fibrinogen with thrombin; the latter catalyzes the conversion of fibrinogen to fibrin to form a clot. In this way, plasmin acts as a feedback inhibitor to the coagulation process. Free plasmin is quickly neutralized by α_2-antiplasmin or other inhibitors to regulate the system. However, if plasmin is found in its active form during the coagulation process, a competition between the coagulation and fibrinolysis will occur, and the time required for the formation of a fibrin clot will be prolonged, or clotting will not occur (Brown, 1984; Booth, 1999).

Magnetoelasticity is of considerable interest as a transduction method for the development of sensors (Grimes and Kouzoudis, 2000; Grimes et al., 2002), and can be an efficient and inexpensive technique for biomedical monitoring (Puckett et al., 2003). When exposed to a time-varying AC magnetic field, magnetoelastic sensors oscillate at a fundamental frequency that is dependent on the chemical composition and the physical dimensions of the strip. A DC field is also used to offset the magnetic anisotropy of the strip by aligning the magnetic domains of the film to maximize its vibrational amplitude. The oscillation of the sensor creates a magnetic flux, which can then be detected remotely by a pick up coil. The signal can also be detected acoustically by the use of a microphone or by the modulation of backscattered light from a laser beam (Jain and Grimes, 2001). Magnetoelastic sensors behave similarly to surface acoustic wave (SAW) devices in that they respond to changes in physical parameters, such as mass loading, viscosity (Grimes and Ong, 2000; Loiselle and Grimes, 2000), pH (Cai and Grimes, 2000), and pressure (Grimes and Kouzoudis, 2000). Magnetoelastic sensors are commonly found in anti-theft devices on retail products (Ryan, 1997).

Herein, magnetoelastic transduction is used as a means to monitor the viscosity changes that occur during both the coagulation and the fibrinolytic processes. The reagents used in a prothrombin time (PT) assay (Carville and Guyer, 2000) were used to initiate the extrinsic coagulation pathway and consequently form a fibrin clot. PT is a screening test for determining deficiencies within the extrinsic blood coagulation pathway. Specifically, a thromboplastin reagent containing calcium chloride was used to induce clotting in a platelet-poor plasma sample. Plasmin was evaluated as both a competitive inhibitor to the coagulation process and as a proteolytic enzyme that dissolves clots. Plasmin was added either before the initiation of the coagulation process or during the formation of the clot in order to visualize how the clotting profiles change over time. By using magnetoelastic transduction, we were able to obtain profiles of: (1) the extrinsic coagulation process; (2) the competitive inhibition of the coagulation process; and (3) clot dissolution. The ability to detect viscosity changes that accompany biological events, such as coagulation and fibrinolysis, allow magnetoelastic transduction to be a viable method for monitoring these processes.

2. Methods and materials

2.1. Chemicals and other materials

Viscosity measurements of glycerol solutions were performed using a falling ball viscometer (Gilmont Instruments, Barrington, IL). Glycerol was obtained from Mallinckrodt (Phillipsburg, NJ). ThromboMax with calcium, a lyophilized rabbit brain thromboplastin reagent containing calcium, was acquired from Trinity Biotech (Wicklow, Ireland). Plasmin (9.9 mg/mL) was procured from Haematologic Technologies (Essex Junction, VT). Citrated plasma samples (400–450 mL/bag), either fresh or frozen, were obtained from the Central Kentucky Blood Center (CKBC). Frozen plasma samples were thawed in a 37 °C water bath for 10 min. The plasma was separated to platelet-poor (supernatant) fraction after centrifugation at 2500 × *g* for 10 min.

2.2. Magnetoelastic setup

The magnetoelastic measurements were performed on a custom-made Helmholtz coil configuration, using an 80 mOe sinusoidal AC magnetic field for interrogation of the magnetoelastic films and a 3.9 Oe DC magnetic field to offset the anisotropy of the sensor material. Ribbons of the 2826MB Metglas™ magnetoelastic alloy (Honeywell, Morristown, NJ) were cut into strips with the dimensions of 6.35 mm × 38.1 mm with a thickness of 30 μm.

Data collection was achieved via two separate Visual Basic programs specifically written for this purpose. The first operates in a frequency sweep mode. The computer controls the signal generator that creates the applied magnetic field and scans a specific frequency range. The resulting magnetic flux is then recorded as a voltage. This program can also be utilized to perform multiple frequency scans with a designated delay between each scan. The second program records signal output over time at a set frequency. This program was used to obtain the coagulation profiles. For more details on the magnetoelastic setup, see Puckett et al. (2003).

2.3. Measurement procedure

Frequency sweeps were performed on both a bare magnetoelastic strip and a strip with a 5-μL droplet of glycerol solution placed onto the sensor's surface. Glycerol solutions of varying viscosities were used to identify the signal change that accompanies changes in solution viscosity. A falling ball viscometer was used to determine the viscosities of the glycerol stock solutions according to the manufacturer's