Ex vivo Time Evolution of Thrombus Growth through Optical and Electrical Impedance data fusion

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Ex vivo Time Evolution of Thrombus Growth through Optical and Electrical Impedance data fusion

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Abstract. We designed a novel sensor specifically aimed at *ex vivo* measurements of white thrombus volume growth; a white thrombus is induced within an artificial micro-channel where hemostasis takes place starting from whole blood under flow conditions. The advantage of the proposed methodology is to identify the time evolution of the thrombus volume by means of an original data fusion methodology based on 2D optical and electrical impedance data simultaneously processed. On the contrary, the present state of the art optical imaging methodologies allow the thrombus volume estimation only at the end of the hemostatic process.

1. Introduction

Cardiovascular diseases and thrombotic complications are among the major causes of morbidity and mortality of the western world. Herein, we propose a novel methodology for the concurrent evaluation of platelet aggregate formation and activation of the coagulation cascade (fibrin formation) under *ex vivo* conditions that closely mimic the *in vivo* ones.

Current instruments estimate the thrombus growth through image processing of data acquired by inverted or confocal microscopes. For the imaging of white thrombus formation a fluorescent marker for platelets is used (e. g. quinacrine). The inverted microscope estimates the volume of white thrombi growth from a digital bi-dimensional (based on pixel luminance) data image of a whole blood sample and matematically extrapolates the 3D size, therefore the result may not reflect the actual volume of thrombi. On the other hand, the confocal microscope is a very expensive instrument and its use cannot be extended to a clinical screening; moreover it does not analyze the kinetics of thrombus formation and/or the thrombus stability since the volume estimation is performed at the end of the process.

In the paper, we present a novel methodology to measure the volume of white thrombus during its formation, by simultaneously monitoring electrical impedance and 2D optical image of pixel luminance of an aggregating blood sample.

Electrical Impedance Spectroscopy (EIS) on blood [1 – 2] has received a considerable attention because of non–invasive and real–time monitoring of the clotting process. The measurement bench is composed by an impedance meter, an optical microscope with a CCD camera, a control panel implemented on a Personal Computer and a developed sensor containing the microfluidic connections and electrodes to perform EIS. The control panel communicates via USB interface with the impedance meter and the CCD camera and acquires simultaneously the image and impedance information. At a time instant and at a specific frequency, the acquired impedance data are post processed and compared to a corresponding computed impedance obtained from an electromagnetic numerical model of the

overall system formed by the microchannel, containing the thrombus, and the gold electrodes. The geometry of the thrombus is represented by a solid model deduced from the 2D optical image of pixel luminance. For each pixel, the height of the thrombus with respect to surface of the slide, is assumed proportional to the luminance according to a scale factor. In order to determine such a scale factor, a number of 3D electrical simulations are performed by modifying the scale factor in order to match the computed impedance with the measured one; the white thrombus volume is then evaluated by the thrombus solid model. The 3D electrical analysis tool is based on the Discrete Geometric Approach developed by the authors [3].

2. System Description

The developed microfluidic chip is connected to an LCR meter and posed under an optical microscope with a CCD camera; both LCR meter and CCD camera communicate with a calculator through USB interface and a software control panel simultaneously acquires optical and impedance data. Blood flows inside the sensor at a controlled temperature of 37 °C and at a controlled flow rate in order to simulate the *in vivo* conditions.

The sensor is composed by a Poly-Dimethyl-Siloxane (PDMS) substrate where the microchannel with microfluidic connections is molded and a quartz slide forming the upper wall of the channel where gold electrodes and electrical connections are sputtered. At every experiment a collagen is spread on the quartz slide to induce the thrombus formation and the microchannel is then assembled as shown in Figure 1 (a) and (b).

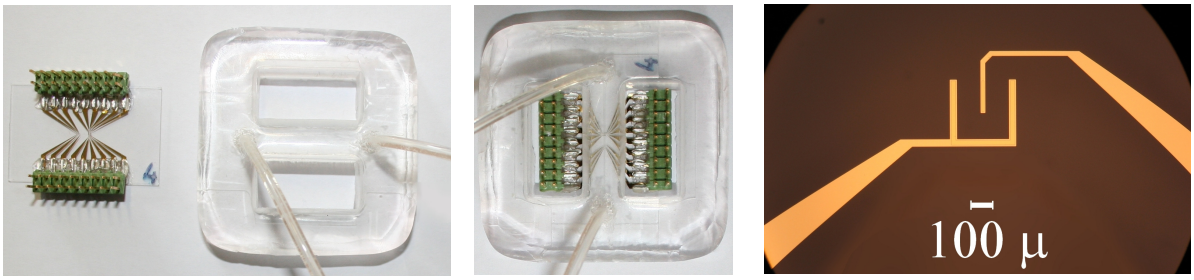


Figure 1: (a) view of PDMS substrate with fluidic connections and glass with sputtered gold electrodes; (b) assembly of substrate and slide; (c) microscope view of the electrodes disposition

Whole blood flows for a specific time interval (3 min) and thrombi grow from the surface of the slide where collagen has been spread. In order to characterize the accuracy of the estimated volumes with the actual volumes of the white thrombus, at the end of the experiment a 3D scan of the investigation area is performed with a confocal microscope.

2.1. Sensor Description and Measurement Setup

The developed microfluidic chip is composed by a PDMS substrate with fluidic connections and a quartz slide with 200 nm thick sputtered gold electrodes.

On the PDMS substrate it is molded an open microchannel without the upper wall whose dimensions are 400 µm in width, 250 µm in depth and 25 mm in length; also the fluidic connections have been molded on this substrate. The upper wall of the microchannel is formed by a quartz slide with 200 nm thick sputtered gold electrodes, arranged as shown in Figure 3(c).

The impedance spectroscopy measurements have been performed using the high precision LCR meter Agilent E4980A in the frequency range 1-300 kHz with eight logarithmic spaced steps, a two-wire configuration and a drive voltage of 100 mV; such drive voltage is by far lower than the standard half cell potential for gold (that is 1.5 V) in order to avoid artifacts due to redox reactions taking place between electrodes and salts dissolved in plasma. The LCR meter has been connected to the assembled empty device and an “open” calibration has been performed in order to eliminate the capacitive parasitic effects of connections. In these conditions the impedance measurement accuracy is 0.1 %.

Blood flows in the microchannel at a flow rate of Q = 750 µl/min in order to obtain a target shear rate of 3000 s^{-1}.

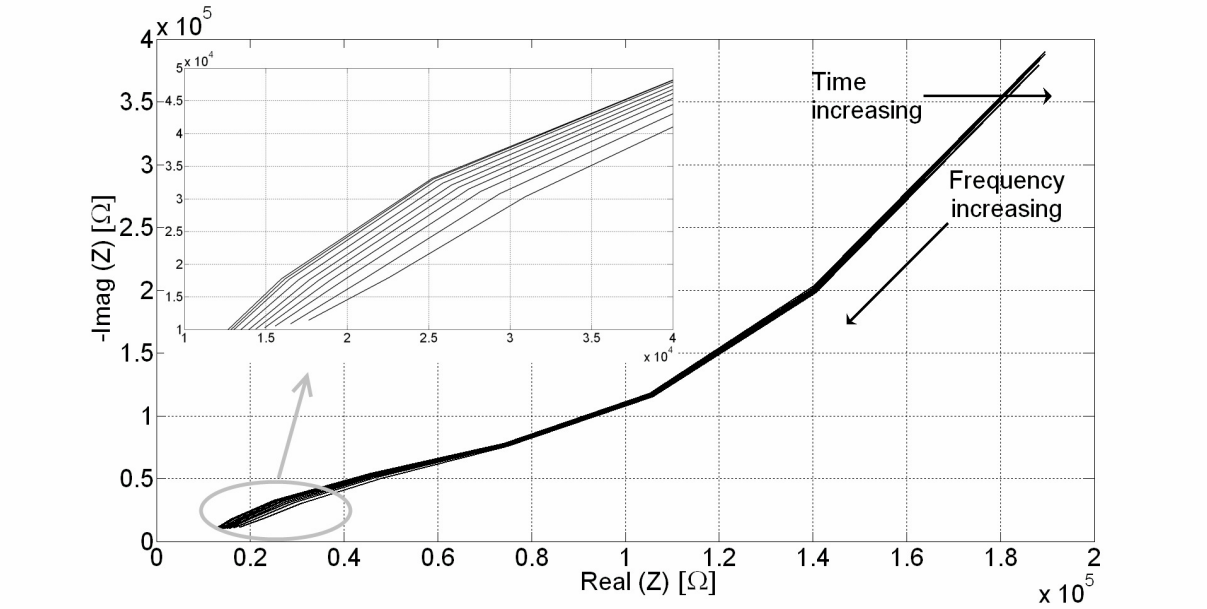


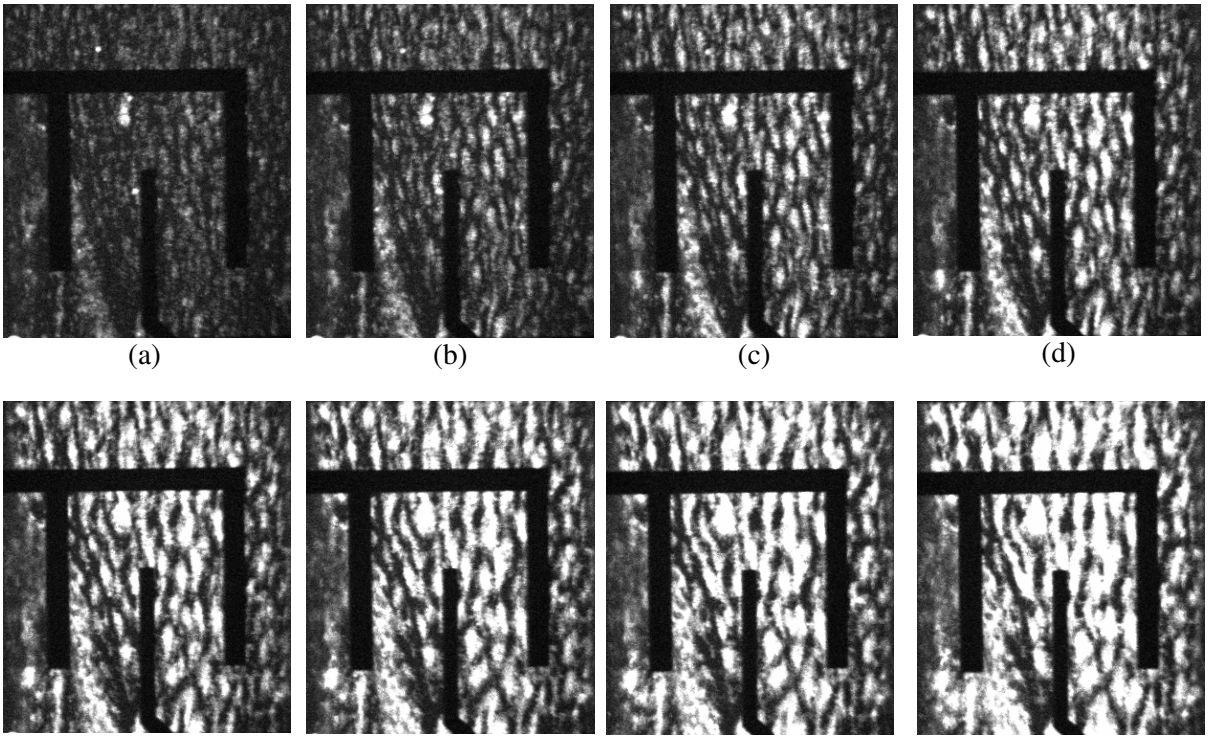
Figure 2: Impedance Spectroscopy during during thrombus growth; curves are 17 s time spaced

Figure 2 shows the Real - Imaginary plot of the impedance over a time interval of three minutes; the time resolution of acquired data is 1.7 s but for graphical reasons in Figure 2 each plotted curve is acquired every 17 s. As it can be seen in Figure 2, at low frequencies the interface electrode-blood (double layer) dominates the impedance behaviour masking the effects of thrombus formation. Since we already demonstrated that thrombus behaves like an insulator for frequencies below 300 kHz [3], the best frequency interval for the thrombus growth measurement is [100-300] kHz, in order to by-pass the double layer effects but not the cells membranes forming the thrombus. In this frequency range blood behaves mainly like a resistor whose resistance is increased by the thrombus growth, since the resistive cross section of blood is reduced proportionally to the thrombus height and width.

3. Experimental results

The measurements have been performed automatically acquiring data from the LCR meter and the CCD camera; the control panel has been set up in order to perform a two-wire measurement avoiding redox reactions and compensating the parasitic effects of cables and connections, as described in previous Section.

Figure 3 shows some significant frames acquired from the microscope during an experiment. The blood flows from the top to the bottom of each frame; the frames shown in Figure 3 (a) to 3 (f) are acquired 40 s, 60 s, 80 s, 100 s, 120 s, 140 s, 160 s and 180 s after the start of the experiment. The growing thrombus is represented by the white spots inside the investigation area. It is possible to see that, increasing time, single platelets are activated and start adhering to the collagen; then the aggregates increase both in base area and volume to form stripes in the direction of blood flow. With standard measurement systems (e. g. confocal microscope) the evaluation and measurement of the time evolution of growth is not possible, since the confocal scans can be performed only at the end of the experiment.

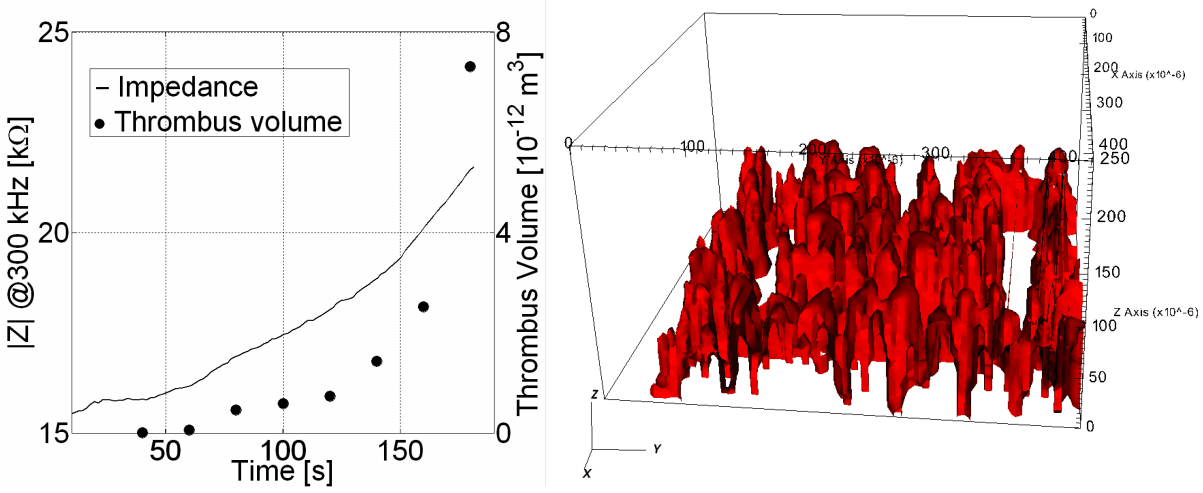


(a) (b) (c) (d)

(e) (f) (g) (h)

Figure 3: Acquired frames from microscope during thrombus growth, blood flows from the top to the bottom of each frame. Frames are acquired from the start of the experiment: (a) after 40 s; (b) after 60 s; (c) after 80 s; (d) after 100 s; (e) after 120 s; (f) after 140 s; (g) after 160 s; (h) after 180 s.

In Figure 4 (a) instead impedance data and reconstructed thrombus volumes are shown for all the frames depicted in Figure 3; the continuous line represents the impedance value (scale on left axis) and the circular markers represent the reconstructed thrombus volume (scale on right axis).



(a) (b)

Figure 4: (a) Impedance change over time measured at 300 kHz (line) and thrombus volume estimation (circles); (b) thrombus 3D model reconstruction

The uncertainty on the volume reconstruction can be overestimated approximating each reconstructed thrombus with a cylinder; this hypothesis is pejorative since thrombus shape looks like a paraboloid. The uncertainty of each thrombus base diameter D_i is related to quantization pixel of microscope and can be quantified in $u(Di)$ = 5 μm, while the uncertainty of the height h_i is related to the simulations steps and can be quantified in $u(h_i)$ = 5 μm. Since each thrombus volume can be overestimated as:

$$V_i = \pi D_i^2 h_i / 4 \quad (1)$$

Thus, propagating the uncertainty and considering height and diameter uncorrelated quantities since derived from two different measuring instruments, we obtain

$$u(V_i) = \sqrt{\left(\pi D_i\, h_i u(D_i)/2\right)^2 + \left(\pi D_i^2 u(h_i)/4\right)^2} \quad (2)$$

The uncertainties relevant to each volume reconstruction in Figure 4 are estimated less than 20%.

4. Discussions and conclusions

Here we presented a novel methodology, based on data fusion between optical and electrical information, which provides rapid information on the individual predisposition to form thrombi. This methodology will develop a quantitative mean to estimate the volume of thrombus during its formation, by monitoring the global electrical impedance of an aggregating whole blood sample. The perspective of the proposed device is the treatment of arterial thrombotic complication in cancer patients, due to the increased risk of haemorrhage induced by antithrombotic agents during the administration of prophylactic antiplatelet/anticoagulant therapies.

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