Evolution of the optical scattering properties of blood plasma during clot formation

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Abstract. Venous thromboembolism (VTE) is a common and serious disease which encompasses deep vein thrombosis (DVT) and pulmonary embolism (PE). DVT is created when a blood clot forms in the deep veins of the leg and when the clot migrates through the bloodstream, to lung arteries, it creates a PE. VTE is the third cardiovascular cause of death overall and is responsible for 30000 annual deaths in Europe. After biological and clinical investigation, nearly half of VTE cases have no known origin (idiopathic VTE). Among the patients developing idiopathic VTE, about 30% of them would have a recurrent thromboembolic event, 70% would not be subjected to any recurrence. A balance must be struck between the risks of recurrent thrombosis if anticoagulant treatment is stopped versus the risks of bleeding associated with continued anticoagulation therapy that can go up to the course of decades. The search for new biomarkers allowing to best stear the treatment of patients is thus of major interest. Recent studies seem to link clot's structure to a risk of recurrence. The aim of our work is to develop a sensitive optical method, in order to help with VTE patient's prognosis, measuring the evolution of the scattering coefficient of a plasma during *ex vivo* clot formation.

1 Introduction

Venous thromboembolism is a common and serious disease which encompasses deep vein thrombosis and pulmonary embolisms in which blood clot forms in the venous system. When a blood clot forms in the deep veins of the leg, and migrates through the bloodstream, to lung arteries, it creates a pulmonary embolism. PE is a serious medical condition with a death rate of about 10%. The estimated incidence rates of VTE among Europeans range from 104 to 183 per 100000 person/year [1] causing more than 30000 annual deaths in Europe [2]. After clinical investigation, nearly half of the events can be explained (immobilisation, cancer, inherited thrombophilia), the other half of VTE cases has no known origin (called idiopathic VTE). Among the patients developing idiopathic/non-provoked VTE, about 30% of them would have a recurrent thromboembolic event, 70% would not be subjected to any recurrence. This leads to difficult decisions in the population of non-provoked VTE; if the anticoagulation therapy used to treat VTE is very efficient, it also has major side effects, such as bleeding events, that can be deadly. A balance must be struck between the risks of recurrent thrombosis if anticoagulant treatment is stopped versus the risks of bleeding associated with an ongoing anticoagulation therapy that can go up to the course of decades. For the last 20 years, even if research has developed scores, made genetic discoveries, VTE patient's prognosis has not changed.

One field of research has focused on exploring *in vitro* clot by physico-chemical analysis techniques involving macroscopic and microscopic measurements such as permeability for pore size investigation, absorbance for lag phase (time to the start of protofibril aggregation) measurement by turbidimetry as well as maximum absorbance for the determination of average fibrin fibre thickness, imaging techniques for a visual evaluation of pore size and clot density in the fibre network with promising results [3]. In brief, it appears that VTE patients have *in vitro* denser fibrin clots. Developing clot analysis based on its physico-chemical properties seems promising not only to develop risk models for recurrence of venous thromboembolic disease and to offer personalised treatments, but also to guide research for a better understanding of the pathophysiological mechanisms involved. As a matter of fact, recent studies show that poor permeability of fibrin networks as well as resistance to lysis could be a novel risk factor for PE recurrence, especially in subjects that discontinued anticoagulant therapy after the first PE episode [4]. The aim of our research is to develop a sensitive optical method that consists of measuring the evolution of optical scattering properties of a plasma during *ex vivo* clotting.

2 Materials and Methods

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2.1. Sample preparation

Citrated platelet-poor plasma was obtained from the UMR 1304 – GETBO laboratory that has cohorts with biobank for 30 000 patients. The plasma used for the experiments is patient pooled homogenised plasma that was centrifuged at 2500g for 15 min at room temperature, and stored at -80°C until further analysis. A clot was induced in HEPES-buffered saline, tissue factor (INNOVIN®, Siemens Health Care Diagnostics, Order No. DAB B4212-40) and $CaCl_2$ (1M solution). Different clots were produced for different concentrations of fibrinogen. After adding 167µL of tissue factor and 50µL of $CaCl_2$ different proportions of HEPES/plasma were studied (Table 1).

Table 1. Volumes of plasma, HEPES-buffered saline, tissue factor and calcium chloride for studied samples which consisted of different proportion of plasma/HEPES

	Plasma/HEPES					
Volumes (µL)	50/50	40/60	33/67	29/71	20/80	14/86
Plasma	1254	836	627	501.6	313.5	209
HEPES	1000	1418	1627	1752.4	1940.5	2045
FT (1/333)	167.2	167.2	167.2	167.2	167.2	167.2
$CaCl_2$ (1M)	50.16	50.16	50.16	50.16	50.16	50.16
Total	2491.42	2491.42	2491.42	2491.42	2491.42	2491.42

2.2 Experimental set-up

Figure 1 illustrates the experimental set-up for the optical scattering measurement. A linearly polarised He-Ne laser beam (15 mW), operating at a wavelength of 632.8 nm, was directed onto the sample. The intensity of the laser was controlled by a half-wave plate and a polariser. The scattered light was captured by a powermeter console (THORLABS, PM100D) linked to a computer after spatial filtering. The averaging ratio was set at 1000 and the time interval between two consecutive measurement points was 10s.

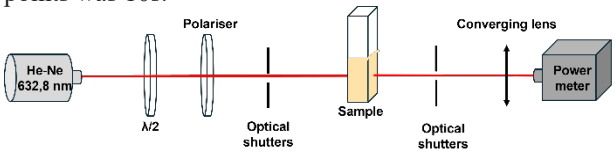

Figure 1. Experimental set-up of the measurement of the scattering coefficient. λ/2 is a half-wave plate.

The study is based on the Beer-Lambert law for a collimated transmission:

$$I(t) = I_0 e^{-\mu_s(t)d} \quad (1)$$

where μ_s is the scattering coefficient, d is the thickness of the optical glass cuvette. I_0 is the intensity measured for the HEPES-buffer saline solution and I(t) is the intensity of the transmitted beam.

3 Results and Discussion

The scattering coefficient was measured during clotting for different plasma/HEPES proportions (14/86, 20/80, 29/71, 33/67, 40/60, and 50/50). The intensity I_0 was measured for the HEPES-buffered saline solution. Then, the intensity I(t) was measured after adding plasma, tissue factor and $CaCl_2$. Analysis of clot formation's kinetics as a function of plasma concentration was carried out by analysing scattering coefficient time curves. Parameters such as the amplitude of variation of the scattering coefficient, the latency time for coagulation to start, or the rise time of μ_s coefficient have been studied.

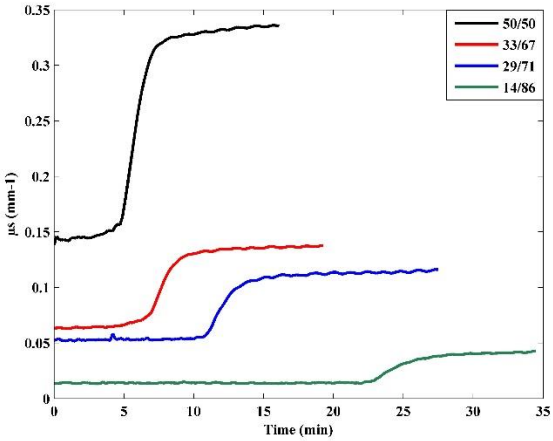

Figure 2. Example of scattering coefficient measurement during clot formation for different plasma/HEPES proportions

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