New technology for assessing platelet aggregation activity

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Abstract. In this paper, we discuss an application of the method of the Digital Laser Speckle Image Correlation method (DIC) for studying the aggregation function of platelets in whole blood with the maximum standardization of the pre-analytical and analytical stages of the study. Materials and images are provided, methods are explained.

1. Introduction

Currently, antiplatelet therapy is of high importance to prevent thrombotic complications [1,2]. Despite the different mechanism of action of antiplatelet agents (inhibitors of COX-1 platelet receptors, P2Y12 receptor blockers, GPIIb/IIIa receptor antagonists, phosphodiesterase inhibitors, etc.), ultimately, their task is to inhibit platelet aggregation. Due to the increase in cases of resistance and "perverted responses", the need for a personalized assessment of platelet aggregation activity is of great clinical significance.

However, the problem of individual selection of antiplatelet therapy is currently at the stage of clinical research. The main obstacle to the widespread introduction of such an approach is the presence of a variety of methods for determining platelet function with the often-encountered inconsistency of their results with each other. The variability of the results is due not only to the difference in the test methodology, but also to the clinical heterogeneity of patient groups, different antithrombotic therapy regimens, the unequal determination of the threshold value of the method for detecting drug sensitivity, and the difference in test execution techniques by different researchers [1–5].

To date, there are many methods for assessing platelet aggregation activity: assessment of changes in plasma optical density ("Gold Standard" – Born-O'Brien's turbidimetric method) [3], assessment in whole blood - by the method of impedance aggregometry (Multiplate® Analyzer) [1], classical thromboelastography (test platelet mapping), determination of thromboxane B2 in serum or plasma, determination of 11-dehydrothromboxane B2 in the urine, etc. [2].

The main problems in assessing platelet aggregation activity are a lack of correlation between different methods, variability of results, sample preparation time (40–50 minutes) and a lack of standardization of the study.

At the same time, a new well-proven method of measuring platelet aggregation activity in whole blood has been established – Low-Frequency Piezoelectric Tromboelastography (LPTEG Method) with the usage of the ARP-01M "Mednord" hardware-software complex produced in Russia (Registration certificate 2010/09767) [6,7]. The method allows to make an assessment in the Point-of-Care test mode

and does not require complicated sample preparation. The disadvantages of the method include the need for highly qualified personnel to correctly conduct the test and interpret the measurement results [8].

The aim of this work is to develop a method for evaluating the aggregation function of platelets in whole blood with the maximum standardization of the pre-analytical and analytical stages of the study.

2. Materials and methods

The technology proposed in this paper is based on the use of the Digital Laser Speckle Image Correlation method (DIC) [9–12]. The optical method is non-invasive and allows for measuring the temporal parameters of the processes by measuring the changes in the correlation coefficient of laser speckle images recorded on a digital camera. Previously, the team showed the possibility of using the DIC method for estimating the fibrin formation time using citrate blood plasma [11]. The results obtained [11] have provided an evidence to believe that this optical method can also be used to evaluate the blood cells aggregation activity in whole blood.

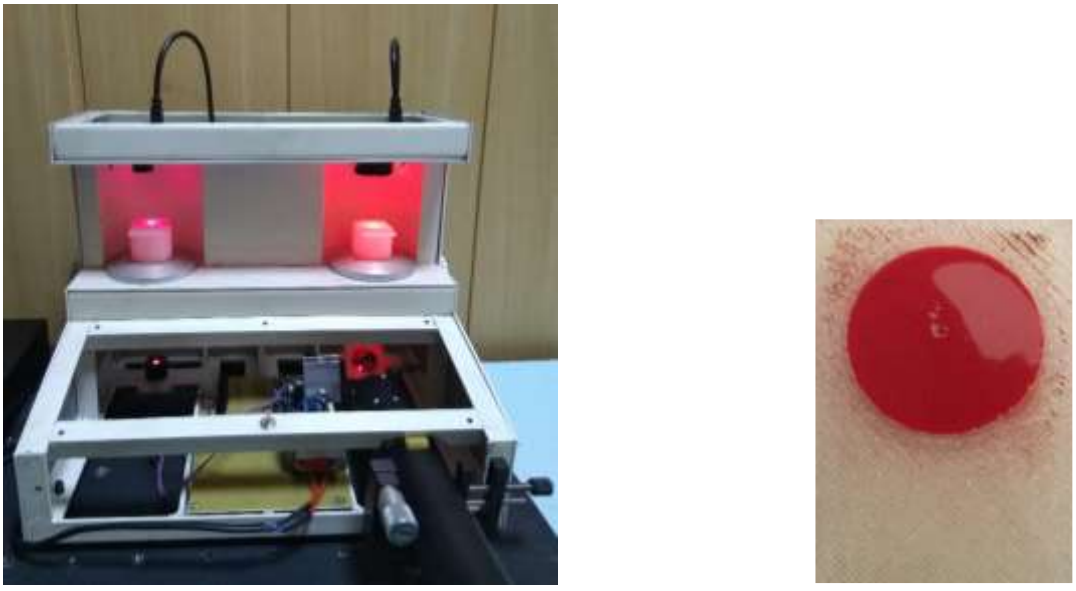
Figure 1 shows the appearance of an experimental setup for the study of blood clotting using the DIC method. The installation had two channels differing only in the radiation source. A laser diode with a power of 5 mW and a wavelength of 650 nm (left channel) and a He-Ne laser with a power of 5 mW and a wavelength of 632 nm (right channel) were used. Registration was carried out by the same monochrome VAA-136-USB USB-cameras. The frequency of registration of a digital camera is not a defining parameter, so the registration was carried out at a standard shooting frequency of 30 frames per second.

The cuvettes were made by 3D printing from transparent Watson-branded plastic manufactured by Best Filament. According to the results of a previous study [11], the optimal amount of analyte for the DIC method is 50-100 μl. Therefore, the cuvettes had a volume of ~100 μl with a diameter of 12 mm.

A typical view of speckle images is shown in Figure 2.

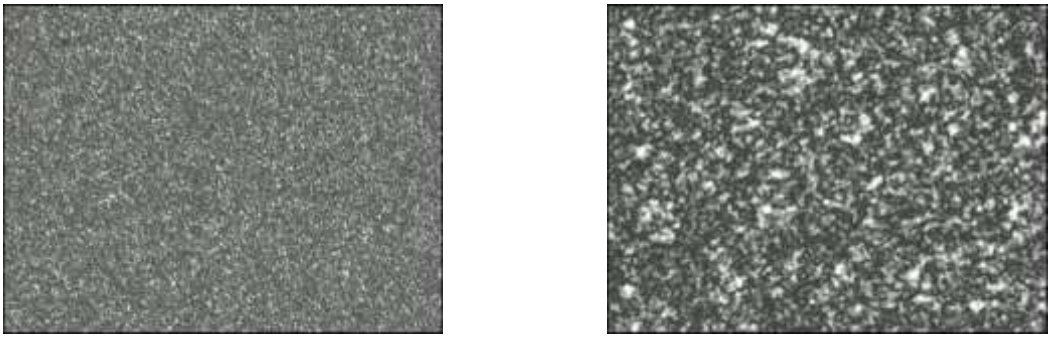
The calculation of the correlation coefficient was carried out using the correlation formula and the calculation algorithm described in [12], using the MatLab® software package.

As a control method for assessing platelet aggregation, the highly sensitive method of LPTEG was also applied, that also utilizes whole blood and has a high correlation with the gold standard by the Born-O'Brien turbidimetric method.



(a) (b)

Figure 1. Laboratory setup for a comparative study of the aggregation activity of the blood cells (a) and the experimental cuvette (b).



(a) (b)

Figure 2. Image view of speckle images obtained using a laser diode (a) and a He-Ne laser (b).

The study involved 4 volunteers of the Siberian population with different states of the hemostasis system who had previously signed a voluntary informed consent. The blood from the cubital vein was obtained into a three-component siliconized syringe (V = 1 ml) without applying a tow.
The blood was immediately (10–12 seconds) placed in a cuvette (V = 0.45 ml) made of the medical plastic (Mednord) and in two cuvettes printed on a 3D printer, and at the same time the registration of the platelet aggregation process was started.

The data from ARP-01M "Mednord" was processed in the specialized software X-Gemo-3M. Figure 3 shows the appearance of the device ARP-01M. Detailed information about the device is given in [13]. LPTEG curves were recorded to the T1 point - a point characterizing the aggregation activity of blood cells.

Figure 3. Low-frequency piezoelectric thromboelastograph ARP-01M "Mednord" (Russia).

The comparison of the obtained data was made in the SPSS Statistics 15.0 package.

3. Results and discussion

Figures 4 and 5 present the results obtained using two different methods: low-frequency piezoelectric tromboelastography (LPTEG) and laser speckle imaging (He-Ne laser and laser diode).

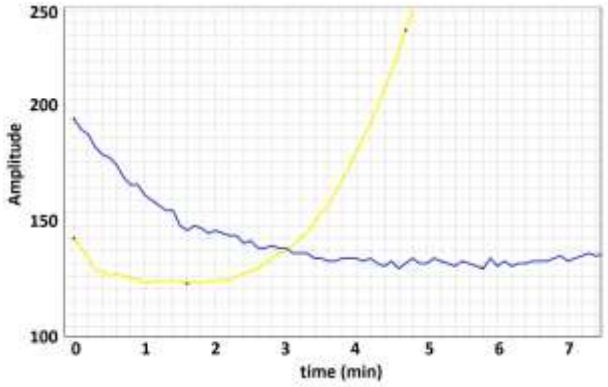
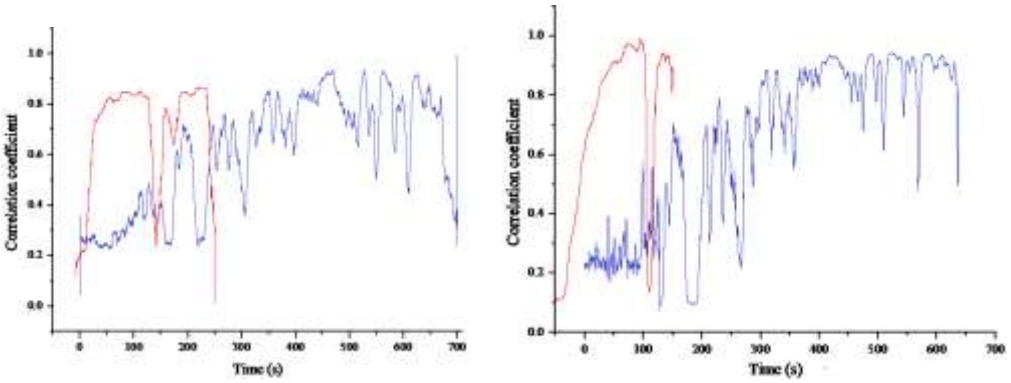


Figure 4. An example of data obtained by the LPTEG method, volunteers of the Siberian population: Patient 1 — yellow graph, Patient 3 — blue graph.



(a) (b)

Figure 5. An example of data obtained by laser speckle imaging, in Siberian volunteer s(Patient 1 – red graph, Patient 3 – blue graph), using a laser diode (a) and a He-Ne laser (b).

After conducting research, the data obtained were summarized in table 1.

Table 1. Obtained data.

Patient №	T1, min	He-Ne, sec	Diode, sec
1	1.6	61	92
2	2.1	103	-
3	5.8	354	418
4	2.9	146	-

Correlation analysis of the obtained results showed a high correlation of the proposed technique (both when using a laser diode and a He-Ne laser) with the method of LPTEG. The results of the correlation analysis is summarized in the table 2.

Table 2. The results of the correlation analysis.

		T1	He-Ne	Diode
T1	Pearson Correlation		0.989(*)	0.994(**)
	Sig. (2-tailed)		0.011	0.006
	N		4	4
HeNe	Pearson Correlation	0.989(*)		0.968(*)

	Sig. (2-tailed)	0.011		0.032
	N	4		4
Diode	Pearson Correlation	0.994(**)	0.968(*)	
	Sig. (2-tailed)	0.006	0.032	
	N	4	4	

*Correlation is significant at the 0.05 level (2-tailed).
**Correlation is significant at the 0.01 level (2-tailed).

4. Conclusion

The proposed method for assessing the functional state of the aggregation activity of blood cells has a strong correlation with the existing low-frequency piezothromboelastography method (> 0.9).
The method minimizes the errors of the preanalytical and analytical stages of the study, due to standardization of the intake (volume of the analyte, speed of the intake, thickness of the needle) and the use of native blood.

The results of tests demonstrates the perspective of development of the method of rapid assessment of the aggregation activity of blood cells.

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