Analysis of Platelet Anti-Coagulation by Optofluidic Time-Stretch Microscopy

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Thrombotic disorders, which are caused by the abnormal activation of platelets, have become a worldwide health issue in recent years. Although ethylenediaminetetraacetic acid (EDTA) is the most widely used anti-coagulator in clinical blood tests, for platelet aggregation detection, citric acid is usually used instead of EDTA as an anti-coagulator because EDTA breaks apart large platelet aggregations.[1] Conventional flow cytometry cannot be used to quantify the influence of EDTA on platelet aggregation because of the low spatial resolution and sensitivity. Here we present an *in vitro* label-free sensitive method for detecting platelet aggregates by optofluidic time-stretch (OTS) microscopy, which is able to conduct single-cell analysis at a throughput of 10,000 cells per second and a spatial resolution of 780 nm.[2] Specifically, we prepared samples with differing concentrations of collagen (agonist) in lysed blood, drawn with either EDTA or citric acid. Examples of acquired label-free bright-field images of platelets are shown in Fig. 1a and Fig. 1b. In addition to large aggregations, single platelets of 2-5 μm in diameter and small aggregations containing 2-3 platelets were observed. The size of the platelet aggregations in the EDTA samples remained the same when the concentration of collagen was increased, while the size of platelet aggregations in the citric acid samples increased with the collagen concentration. Furthermore, using machine-learning we quantitatively evaluated the size distribution of platelet aggregations in the EDTA and citric acid samples at a collagen concentration of 1 μg/mL. The results are shown in Fig. 1c.and they are consistent with the images in Fig. 1a and Fig. 1b, which show mostly small platelet aggregations in the EDTA samples and larger aggregations in the citric acid samples. These results indicate that our method has great potential for high-speed sensitive platelet aggregation detection. It is thus promising for the evaluation of thrombotic disorders in clinical settings.

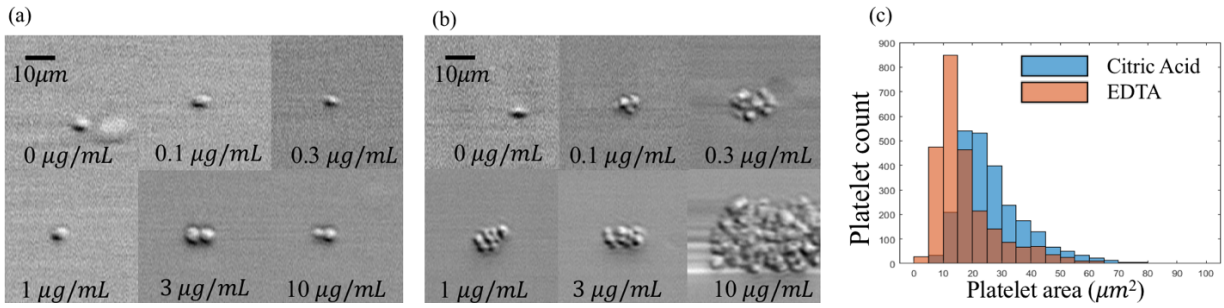

Fig 1. Experimental results. (a) Platelets in an EDTA anti-coagulated blood sample. (b) Platelets in a citric acid blood sample. (c) Histogram of platelet size in EDTA and citric acid samples at a collagen concentration of 1 μg/mL.

Reference

1. McShine, R.L. et al. Effect of EDTA on platelet count and other platelet parameters in blood and blood components collected with CPDA-1. *Vox Sanguinis*. **61(2)**,84 (1991).
2. Jiang, Y. et al. Label-free detection of aggregated platelets in blood by machine-learning-aided optofluidic time-stretch microscopy. *Lab Chip*. **17**, 2426 (2017).