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Label-free Microfluidic Sensing by Detection of Interaction-triggered Change in Blood Flow Characteristics

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Abstract

Microfluidic devices exploit combined physical, chemical and biological phenomena that could be unique in the sub-millimeter dimensions. The blood is a non-Newtonian fluid, containing particulate and soluble elements, which penetrates the whole body carrying a wealth of biomedical information. The design of microfluidic devices capable of extracting immediately this information is the current goal of development Point-of-Care (POC) medical devices. We examined the characteristics of blood flow in specially designed microfluidic devices having different geometric structure and material composition with the aim of defining suitable conditions for sensitive detection of changes in the interactions between particulate elements of the blood and the adequately modified surfaces of the microfluidic system. As a model experiment we demonstrated the fast analysis of the AB0 blood group system, applying respective antibody reagents and capillary blood samples with different blood groups. We showed that by tuning the hydrophilicity of the surface and capillary dimensions of the microfluidic system it is possible to detect precisely the red blood cell binding to the capillary walls by monitoring the flow rate characteristics in an autonomous microfluidic system. Our proof-of-concept results point to a novel direction in blood analysis in autonomous microfluidic systems and also provide the basis for the construction of a simple quantitative device for blood group determination.

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1. Introduction

Medical applications of microfluidics hold the promise of miniaturization and accelerated on-site testing for biomarkers. While classical assays of molecular biology and immunology can be scaled down for these systems, it is also of high interest to introduce novel principles taking advantage of the reduced dimensions and distinct physical phenomena observable on this scale. Since cells' dimensions are in the low micrometer range it seems intuitive to develop applications that utilize cells as components of the assay. We attempted to combine a self-driven microfluidic system and cell-molecule interactions in order to generate a simple but robust assay and readout system for Point-of-Care diagnostics. Here we provide proof-of-principle of the system using the human blood group markers as a model.

Nomenclature

POC	Point-of-Care
LOC	Lab-on-a-Chip
RBC	red blood cell

1.1. Autonomous microfluidics

Self-driven devices are ideal for POC testing as they require no external supply of power and are less prone to user-introduced error. Autonomous microfluidic devices exploit capillary forces for transferring liquid in the system [1]. Key factors affecting the performance of these devices are structure/geometry and material composition. Capillary forces depend on the interactions of the fluid, the gas phase and the capillary wall. Geometries modulate the contact angle of the proceeding liquid front thereby affecting liquid movement. Based on this knowledge complex systems with different functional units can be designed [2].

1.2. Red blood cells

Blood is a fluid tissue comprising particulate elements and dissolved molecules. It is in fact the most often sampled human tissue because of relatively easy availability and because of the wealth of information it carries. Of the particulate elements red blood cells are the most abundant with numbers in the range of 4 to 5 million cells per microliter. This accounts for approximately 50% of the blood volume and an enormous surface area owing to the doughnut shape of the cells. The diameter of a RBC is 7 micrometers, with a width of 2.5 micrometers in the ring and 0.5-1 micrometer at the center. The cell has a number of molecules embedded in the membrane; some of these are receptors for circulating ligands in the blood. RBCs carry oxygen bound to hemoglobin, in case of sever loss they need to be replaced to sustain this capacity. It has long been known that RBC transfusion is only possible between matched donors and acceptors, depending on their blood type. Blood type is a genetically determined property that appears as the quality of glycoproteins on RBCs and also the immunological recognition of these glycoproteins [3]. Two very important blood typing groups are the AB0 and the Rh group.

Table 1. The AB0 blood group.

Blood type	RBC antigen	Serum antibody
0	-	Anti-A and anti-B
A	A	Anti-B
B	B	Anti-A
AB	A and B	-

2. Experimental

2.1. Materials

The applied microfluidic capillary systems were fabricated by standard soft-lithography techniques applying SU-8 epoxy based negative photoresist pattern as molding replica and polydimethylsiloxane (PDMS – Dow Corning Sylgard 184) polymer as structural material. Autonomous flow of blood was achieved by adjustment of hydrophilic surface properties with embedding dimethylsiloxane ethylene oxide block copolymer (PDMS-b-PEO - Sigma-Aldrich) in the PDMS material. A 0.75 v/v% concentration of PDMS-b-PEO molecules was found to be optimal for the assay, considering that lower concentrations caused insufficient capillary transfer and higher concentrations significantly deteriorated the physical (optical, mechanical) properties of the polymer. A hydrogel coated glass slide (Nexterion Slide H, Schott) was used as the base substrate of the system. Appropriate surface areas of the slide were functionalized with the anti-A and anti-B blood typing reagents from Diagast. Blood was obtained by fingerprick and was anticoagulated with four different reagents: heparin, hirudin, sodium citrate and ethylene-diamin-tetraacetate. Even though these agents exert different effects and may distinctly influence fluidic properties we found no significant differences in the performance of our assay (data not shown).

2.2. Structural design

Our hypothesis implied that the binding of particulate elements of blood to the walls of a microfluidic system would result in retardation of the fluid motion. In order to be able to observe fluid movement as long as possible on the smallest possible footprint we designed a bifurcated winding channel system (Fig. 1. a). Blood sample is introduced via an inlet port, enters a common capillary channel (200 μm width), and then it is diverted into two channels of identical structure (200 μm width). We expected channel height to be a critical factor in the assay and tested various sizes in the range of a RBC diameter. We found that a height of 5 μm is suitable for the assay, with rather strict tolerance (0.5 μm).

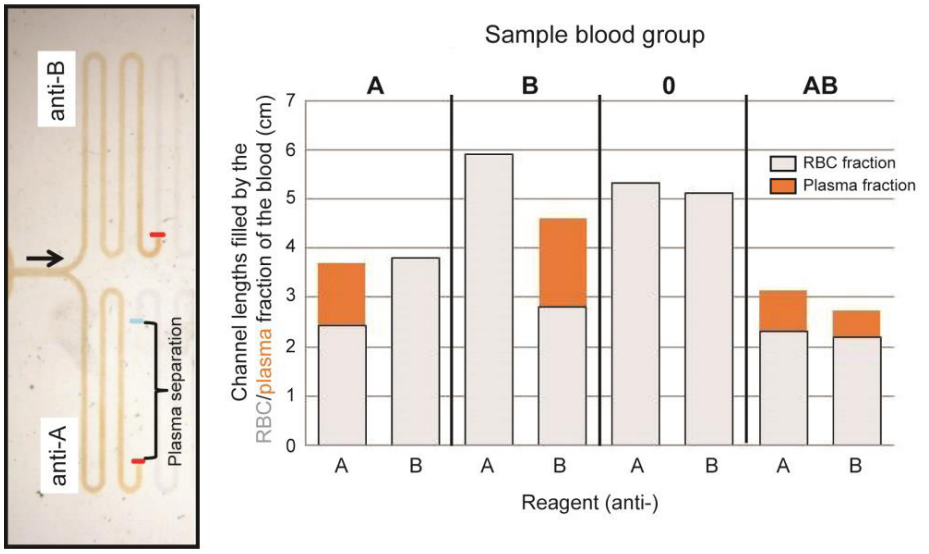

Fig. 1. Development of plasma front in samples. Image (left) and graph (right) shows plasma separation in case of different blood groups (A, B, 0, AB). Orange rectangles indicates the length of the region where plasma separated from RBCs

2.3. Changes in flow characteristics

Upon introduction into the inlet port blood autonomously starts to fill up the device, proceeding into the test channel with functionalized surface. One arm was functionalized with anti-A, the other with anti-B reagent. RBCs bearing the relevant antigens were captured by the reagents in a specific manner (data not shown). Fluorescently labeled type A RBCs were bound to anti-A surface, while type B RBCs to the anti-B surface, respectively. This binding continued during the assay resulting in an increasing area covered by stationary RBCs. By tracking RBC trajectories we found a significant increase in lateral movement due to collision of moving cells with stationary cells. Unexpectedly, we also observed the separation of moving RBCs at the liquid front from the plasma, resulting in RBC front and liquid front (Fig. 1. a). Importantly, this separation was strongly associated with the cell binding to the functionalized area. By monitoring this separation it was possible to identify the blood group of the sample, as shown in Fig. 1. b.

3. Conclusions

We have identified and optimized the physical conditions suitable to promote strong interactions, namely permanent binding, between RBCs and functionalized microfluidic channel surface resulting in altered flow properties of the test blood sample. This deviation is characterized by an overall retardation of blood flow and a concomitant and more pronounced retardation of the RBC component of blood. Using the model system of blood type group AB0 we also demonstrated that this phenomenon can be applied to reliably identify the blood group with absolutely no preanalytical steps. This assay is different from that of Noiphung et al. [4] since it is based on cell-capillary wall surface interactions, unlike the latter that is based on aggregation of RBCs.

We assume that this phenomenon can be exploited for the development of a range of other assays that utilize simple autonomous microfluidic systems and blood for medical diagnostics.

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