Review

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Emerging microengineered tools for functional analysis and phenotyping of blood cells

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The available techniques for assessing blood cell functions are limited considering the various types of blood cell and their diverse functions. In the past decade, rapid advances in microengineering have enabled an array of blood cell functional measurements that are difficult or impossible to achieve using conventional bulk platforms. Such miniaturized blood cell assay platforms also provide the attractive capabilities of reducing chemical consumption, cost, and assay time, as well as exciting opportunities for device integration, automation, and assay standardization. This review summarizes these contemporary microengineered tools and discusses their promising potential for constructing accurate *in vitro* models and rapid clinical diagnosis using minimal amounts of whole-blood samples.

Microengineered tools for functional blood cell analysis

Human blood circulating in the body reaches and exchanges information with every tissue through the vascular network and is therefore an important indicator of the functional status of the human body. Many life-threatening diseases either are directly caused by abnormalities of the blood or blood flow (e.g., ischemic heart disease, stroke, diabetes) or can be detected through careful examination of molecular and cellular biomarkers circulating in the blood (e.g., cancer, HIV/AIDS, tuberculosis) [1–4]. Because of their ready availability, blood cell analysis and phenotyping are arguably the most common and important tests used in the clinic to provide physiological or pathological information for disease diagnosis and staging, treatment selection, safety and efficacy monitoring, and drug-dose adjustment.

Complementary to complete blood count and morphological analysis, functional blood cell analysis is sometimes necessary as it provides direct information regarding the functional status of the human body. Red blood cell (RBC) fragility and deformability [5], white blood cell (WBC) immune response [6], and platelet aggregation [7] are among the most common functional tests performed on blood cells. However, the available techniques for assessing blood cell functions are limited, especially when considering the various types of blood cell and their diverse functions involved in different physiological and pathological contexts. Moreover, conventional tools for analyzing blood cell functions are bulky and costly, rely on complex manual operations and sample preparation, and are designed exclusively for research or clinical settings [8,9]. Due to these common technical limitations, traditional blood cell functional analysis and phenotyping tools remain difficult to standardize and do not meet the needs of modern clinical and health-care applications, including accurate and rapid testing of the diverse functions of blood cells, point-of-care diagnostics, and the construction of highly reliable *in vitro* models [10].

Recent advances in microengineering have offered researchers and clinicians an exciting new set of tools for accurate, fast, and affordable analysis of the cellular components of the blood (Box 1) [11,12]. The ability to precisely control and manipulate single cells in a defined environment has enabled an array of functional measurements that are difficult or impossible to achieve on conventional bulk platforms. Such miniaturized assays also provide the attractive capabilities of reducing chemical consumption, cost, and assay time, as well as exciting opportunities for integrating blood cell analysis with upstream blood sample preparation on a monolithic platform [13]. This review introduces recent achievements in microengineered tools for the functional analysis and phenotyping of blood cells. Examples of how microengineered tools are adapted for analysis of RBCs, WBCs, and platelets are discussed. Finally, we offer speculations on the research directions and potential opportunities for microengineered blood cell analysis tools to meet current and future challenges of clinical and laboratory diagnosis.

Functional analysis of RBCs

RBCs are the most abundant cells in human blood, with a normal concentration of around 5×10^9 cells/ml. With a

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Box 1. The microengineered toolbox

Laminar flow

Fluid flow in most microfluidic devices is laminar due to the small geometrical size of the devices. The stable and predictable flow field for laminar flow makes it easy to maintain a predefined shear rate, the magnitude of which can be tuned by adjusting flow rate or microchannel geometry. Laminar flow can also be manipulated to create complex flow patterns such as flow focusing [28,87] and hydrodynamic stretching [25,58] (Figure IA).

Constriction channels

Microfluidic constriction channels are microchannels whose width is smaller than that of cells passing through the channels (Figure IB). They have been extensively used as mechanical means to deform blood cells to assess their deformability. For ease of fabrication, almost all constriction microchannels have a rectangular cross-section, which differs from the circular blood-vessel shape. Despite this difference, constriction microchannels have been successful in retaining *in vivo* blood cell functionalities [88].

Microwell array

Microwell array is used for isolation and analysis of single blood cells (Figure IC) [89]. To ensure single-cell trapping, a blood cell suspension with a proper cell density is placed onto the microwell array and allowed to sediment into the microwells. One microwell array thus contains up to thousands of single cells, with each single cell trapped in an individual microwell. Each of the microwells creates a confined cellular environment that can effectively concentrate analytes and amplify detection sensitivity.

Microcontact printing

Microcontact printing (μCP) is a simple yet highly versatile method to pattern proteins on various kinds of substrate [90,91]. Briefly, a monolayer of protein is coated on a micropatterned elastomeric stamp. The stamp is then brought into direct contact with the target substrate, to which proteins can preferentially bind. Only proteins in direct contact with the substrate are transferred onto it (Figure ID). For blood cell functional analysis, μCP is mainly used for patterning adhesion proteins for cell adhesion and aggregation.

Micropost force sensor

The micropost force sensor was originally developed to measure cell traction force (Figure IE). It contains a regular array of vertical elastomeric posts with a post diameter down to 1–2 μm. The tips of the microposts are functionalized with adhesive proteins, with the post sidewalls passivated with nonadhesive molecules to ensure that cells adhere to the post tops. When a cell exerts lateral contractile force on the underlying posts, the posts will bend and the magnitude of the cell contractile forces can be inferred by the displacements of the post tips.

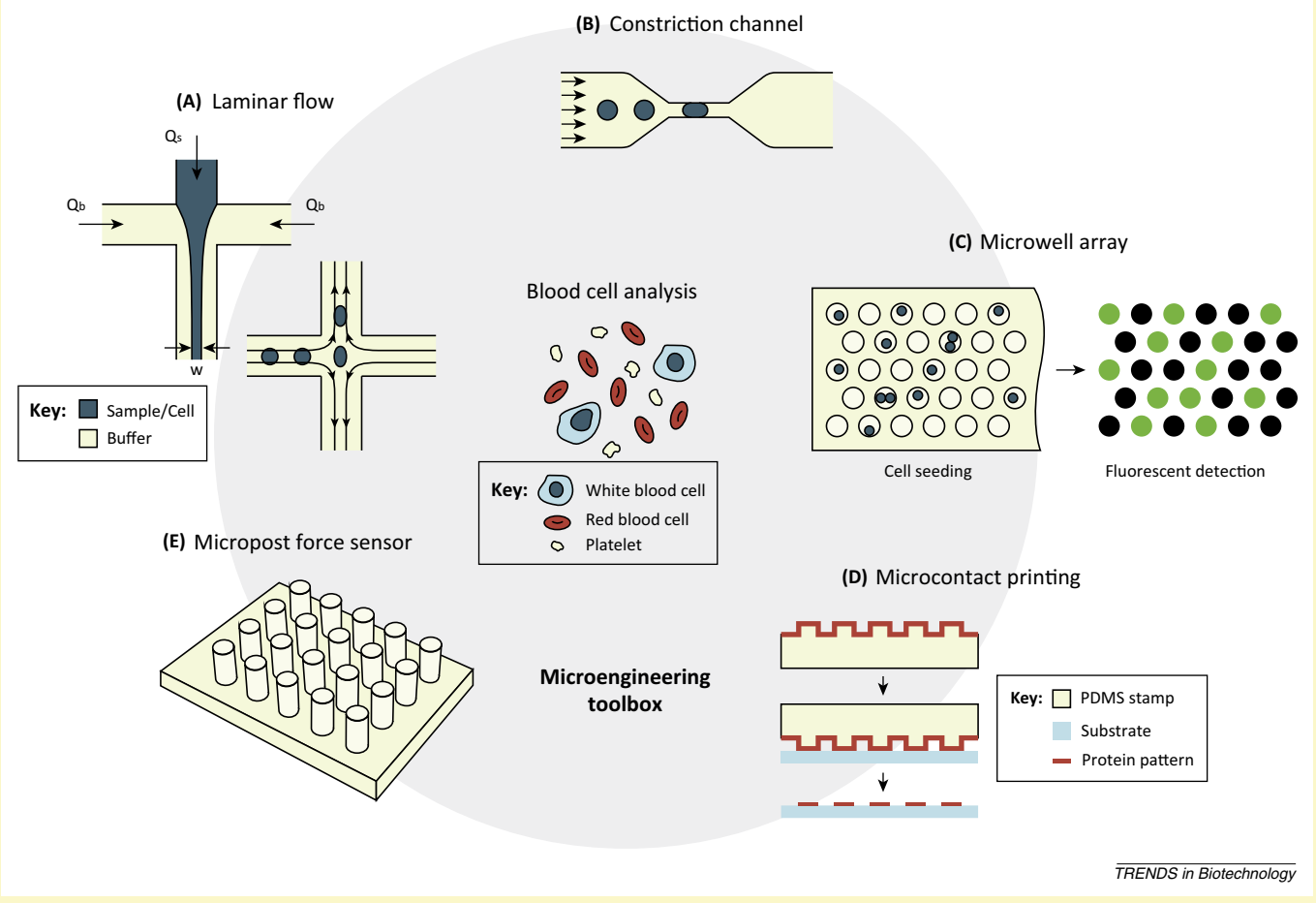

Figure I. The microengineered toolbox. **(A)** Microfluidic control of laminar flow. In one example, sample flow is focused by buffer flows to width *w*, which is determined by the ratio of buffer flow rate (Q_b) to sample flow rate (Q_s). In another example, extensional shear force was generated at the intersection of four perpendicular flows. **(B)** Microfluidic constriction channel for cell deformation assays. **(C)** Microwell array for simultaneous capture and analysis of thousands of single cells. Fluorescence-based biodetection [e.g., enzyme-linked immunosorbent assay (ELISA)] can be used to measure the amount of protein secreted by each single cell trapped in the microwell. **(D)** Microcontact printing for selective surface functionalization. The protein is absorbed on the micropatterned surface of a polydimethylsiloxane (PDMS) stamp before being transferred onto a substrate by direct contact of the two surfaces. **(E)** Micropost force sensor for measuring the contractile force of platelets and blood clots.