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Research highlights

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Wax-based chips for biological applications

Microfluidic techniques have been used in the last two decades for a range of scientific and engineering fields, but have lately become especially important in biology-based studies. In particular, immunoassays (*i.e.* biochemical tests that detect or measure the concentration of a substance in a complex environment by the use of an antibody) are noteworthy applications of microfluidics.[1] One limitation of this approach has been the lack of materials and processes that yield reliable, sturdy and biocompatible devices that are also simple to manufacture. For example, standard chip fabrication materials and processes often require harsh conditions (*e.g.* high temperature) that are incompatible with sensitive detection agents. Other processes (*e.g.* adhesive bonding of PDMS to glass) tend to produce chips prone to failure, such as leakage of fluids.

To address this challenge, Diaz-Gonzalez and Baldi[2] have recently developed a wax-based method for fabricating microfluidic chips suitable for immunoassays. They began by creating a poly(dimethylsiloxane) (PDMS) master mold *via* a standard soft lithography approach, then used it to mold melted wax on a dummy PDMS slab (Fig. 1a). This was accomplished by rapidly cooling the wax from 80 °C to 0 °C (Fig. 1b, c). Since the patterns were generated by molding a wax melt, both positive (protrusions) and negative (troughs) microfluidic structures as small as 25 μm could be manufactured with high fidelity. Next, the wax pattern was transferred onto a glass slide by exploiting the special material properties of wax (Fig. 1d). Namely, when heated to 40 °C, wax does not melt immediately, but first transforms from the solid phase into a plastic material. This property enabled adhesive bonding between the wax and glass, while preserving the shapes and dimensions of the wax pattern. Importantly, the glass slide required no pretreatment or surface coating for successful bonding. The researchers showed that the adhesive bond between glass and wax preserved its integrity, even when metal electrodes were patterned on the glass, since the plasticized wax could be molded by applying pressure. The resulting bond was similar in strength to PDMS–PDMS bonds generated *via* oxygen plasma. As the last step in the fabrication process, the dummy PDMS slab was peeled off without damaging the patterned shapes (Fig. 1e).

Diaz-Gonzalez and Baldi[2] used their wax-based chip to conduct an enzyme-based immunoassay. Prior to bonding with the wax pattern, the metal electrodes on the glass slide were coated with specific antibodies (detection agents). A solution containing enzymes was flowed into a simple microfluidic wax channel, where the enzymes bonded to the antibodies on the electrodes and a detectable current was generated. It is also noteworthy to mention that the antibodies were not damaged during the device fabrication, since gentle manufacturing methods were used, the wax-glass bond withstood the fluidic pressure (albeit a negative pressure was applied to pull rather than push the solution through the chip), and the generated current (on the order of nA) signalled high assay sensitivity.

This new wax-based fabrication method is simple and compatible with biological materials, so it could potentially open the path to a new class of microfluidic chips for biological applications. Furthermore, the proposed method does not require expensive equipment or extensive technical skill and has been successfully implemented with both natural

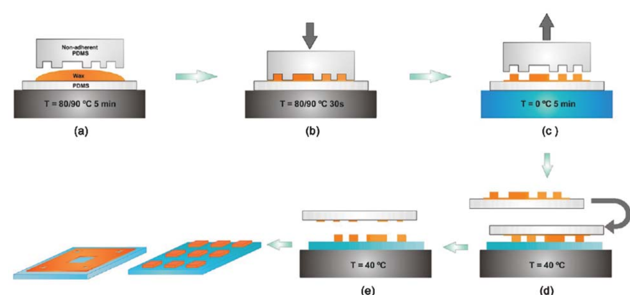

Fig. 1 Fabrication of wax-based chips. Figure reprinted with permission from Diaz-Gonzalez and Baldi.[2]

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beeswax and a paraffin-based wax, an affordable commercial product.

Digital microfluidic immunoassays with magnetic particles

A different strategy for conducting immunoassays on the microscale involves digital microfluidics (DMF). In DMF, liquids are not confined to microfluidic channels, but are manipulated in the form of nl–μl-size droplets by electrostatic forces generated by an array of insulated electrodes.[3] Immunoassays, which require the immobilization of antibodies on a surface and continuous immersion in a liquid, cannot readily be implemented with these DMF platforms.

Wheeler and colleagues from the University of Toronto have recently found a solution to this problem. By attaching antibodies to small particles suspended in a droplet of aqueous solution instead of the chip surface, Ng *et al.*[4] succeeded in conducting high-sensitivity immunoassays and keeping the antibodies functional throughout their experiments. Importantly, the particles they used had magnetic properties when exposed to a magnetic field (paramagnetism), so that they could be manipulated on the chip surface using an external magnet. Fig. 2 shows a schematic representation and photographs of a typical DMF procedure, in which the antibody-coated paramagnetic particles are captured and washed.

The researchers developed protocols for on-chip mixing of a sub-μl sample with the paramagnetic particles, washing of the particles, as well as for solvent exchange, which was needed for removal of unreacted antibodies. This was accomplished in a series of independent immunoassays on TSH, a marker of thyroid function, and estradiol, a type of estrogen produced in humans. The sensitivity of the assays (intensity of the fluorescent signal) was dependent on the antibody concentration, and on-chip particle pretreatment helped decrease nonspecific protein adsorption. The immunoassay results were sensitive enough to serve as reliable indicators of hypothyroidism and ovarian cancer. This was confirmed by a series of traditional immunoassays conducted on a well-plate.

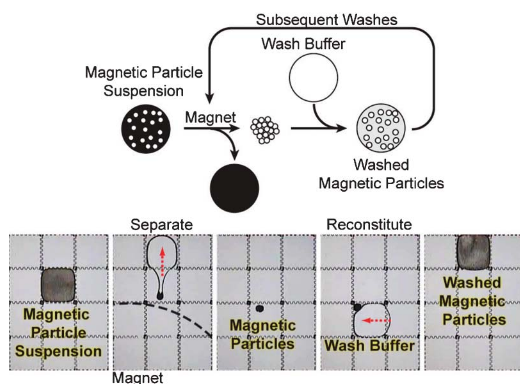

Fig. 2 A schematic representation and photographs of the washing or supernatant separation procedure. Figure adapted and reprinted with permission from Ng *et al.*[4]

There are multiple benefits of the presented approach for implementing DMF immunoassays: first, oil is eliminated as a carrier fluid, which prevents possible accumulation of proteins at the drop-oil interface. This is specifically helpful, as oil-based DMF applications are not readily compatible with cell culture and require special packaging to avoid leaks. Second, all sample preparation is carried out on-chip and can be automated by programming the electrode array. This makes the chip user-friendly and reduces the likelihood of user-generated errors. Third, unlike platforms that require immobilization of antibodies, the proposed method is compatible with multiple immunoassay platforms. In the future, a dedicated signal analysis unit based on absorption or fluorescence detection could be developed in order to generate uniform calibration data and to improve the measurement precision.

Inertial microfluidics for separating blood cells

Many diagnostic and therapeutic applications tend to rely on the quantification of leukocytes in a patient's blood as an indicator of inflammation and disease. To this end, multiple microfluidic methods have been developed to separate leukocytes from red blood cells, relying on cell lysis, inertial cell focusing and other methods.[5] However, the success (in terms of high throughput and resolution) of clinical applications of these methods is limited due to flow phenomena originating from cell–cell interactions.

In an effort to address this limitation, Wu *et al.*[6] have recently applied inertial microfluidics using a spiral microfluidic channel with a trapezoidal cross-section. Here, inertial forces cause buoyant cells or other particles to adopt an equilibrium position near the edge of a microfluidic channel. This is especially noticeable in spiral channels, as all cells of a particular type flow along a single lateral position and can be extracted through a fluidic outlet. The trapezoidal cross-section helps increase the efficiency of the process.

The PDMS device was molded from a poly(methyl methacrylate) master that was milled to generate a trapezoidal channel cross-section, with the shorter side of the trapezoid on the inside of the spiral and the longer side on the outside. First, fluorescent polystyrene beads of four different sizes (on the order of μm) were separated inside the device with high specificity. Then, a human whole blood sample was diluted by a factor of 2 and injected into the device. At a flow rate of 800 μl min^{-1}, roughly 90% of red and white blood cells were separated from each other after a single pass. Additional dilutions (*e.g.* 30-fold dilutions of 1 μl whole blood) and ten times lower flow rates further increased the cell separation efficiency. A key consideration in this test was the ratio of the

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dimensions of the trapezoidal channel. The highest cell separation efficiencies were achieved where the ratios of lengths were below 1.5.

Additional experiments showed that a solution of leukocytes obtained from whole blood after differential centrifugation could be separated into individual leukocytes after a single pass through the microfluidic device, yielding high accuracy and high precision quantification of different types of leukocytes in a blood sample. Another advantage of this device is the relatively large channel dimensions ($\sim$500 μm wide), which helps prevent clogging and accumulation of cells inside the channel. The proposed device is user-friendly and can process a single finger prick of blood ($\sim$1 μl) after only a two-fold dilution and without requiring any additional sample pretreatment. The inexpensive nature of the device material and the simple replication method make this approach a candidate for single-use cell-separation devices that can be used at the point-of-care, *e.g.* at a doctor's office.

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