DOCTORAL THESIS

Novel microfluidic platform for bioassays

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Abstract

Microfluidics have been created to acquire, operate, and process complex fluids in extremely tiny volumes with high efficiency and high speed, and without the requirement for an experienced operator. In addition, microfluidic systems also enable miniaturization and incorporation of different complex functions, which can help bring intricate diagnostic tools out of the laboratories. Ideally, these systems should be inexpensive, precise, reliable, robust, and well-suited to the medical diagnostic systems. Most of the microfluidic devices reported previously were based on devices made of polydimethylsiloxane (PDMS). PDMS is a material that dissolves in many common organic solvents. Meanwhile, it is also prone to absorb small molecules like the proteins, which is detrimental to a stable and reliable result. Current work focuses on bioassays that are badly needed in our life and these bioassays are addressed based on microfluidic platform with different materials. The translation of microfluidic technology into large scale implementations highly relies on new materials that address the limitations of PDMS.

Firstly, we fabricated two different microfluidic platforms for rapid antimicrobial susceptibility testing (AST). One was made of hydrogel, and the bacterial cells were cultured on the top of the device; the other was of polypropylene (PP), and bacterial cells were cultured inside the microchannels. Meanwhile, we developed a novel "barcode" sensor, a microscope-free method for cell accumulation and cell counting, as the downstream of the PP-based chips. As a result, AST can be accomplished

simply through an application on a mobile phone rather than using an expensive and sophisticated microscope.

Secondly, we presented a self-contained paper-based system for lead(II) ion detection based on G-quadruplex-based luminescence switch-on assay, comprising a novel type of paper-based chip and a matching portable device. Different from the reported paper-based devices, the paper substrate we chose was art paper, which is used for printing magazines. This type of paper could prevent the absorption of liquid into the paper matrix and hold the liquid in place for a period of time; and it could also be used for temporary liquid containing like a plastic substrate (such as polypropylene (PP) and polystyrene (PS)), but the surface of the paper is inherently hydrophilic. In such a design, liquid drops are suspended on the surface of the device in designed reservoirs, rather than absorbed into the paper; when the chip is tilted, the liquid drops will move to other reservoirs according to the guidance of channels defined on the surface. To differentiate it from reported μPAD devices that are fabricated with water-permeable paper, we name this new type of paper-based devices suspending-droplet mode paper-based microfluidic devices (SD-μPAD). Different from the conventional μPADs that use capillary force to drive liquid, our SD-μPADs uses wetting and gravity as driving force. To fabricate the superhydrophobic pattern on the paper device, we developed a new microcontact printing-based method to produce inexpensive and precisely patterned superhydrophobic coating on paper. The coating material is poly(dimethylsiloxane)

(PDMS), a hydrophobic and transparent silicone that has long been used for fabricating microfluidic devices. Importantly, the negative-relief stamp we used is made of Teflon, a non-stick polymer, so that the PDMS-coated paper could be peeled from the stamp flawlessly. After such fabrication process, the stamped area of the paper is coated with a textured PDMS layer that is decorated with arrays of micropillars, which could provide superhydrophobic effect and most effectively hold the droplets in place; the remaining area of the paper is still hydrophilic. As a demonstration of this new design, we developed a method using the reaction characteristics of iridium(III) complex for rapid, onsite detection of lead(II) ions in liquid samples. As the reagents have already been loaded onto the paper device during fabrication, the only reagent the users need to add is water. Because of the large Stokes shift of the iridium(III) complex probe, inexpensive optical filters can be employed, and we were able to make an inexpensive, battery-powered compact device for routine portable detection using a smartphone as a detector, allowing the rapid analysis and interpretation of results on site as well as the automatic dissemination of data to professional institutes, including tests even in poor rural areas in developing countries.

Thirdly, we upgraded our suspending-droplet mode paper-based microfluidic device (SD-μPAD), which is used for the detection of lead(II) ions in liquid solution. The reason is that our paper-based SD chips are not suitable for long reaction process (> 20 min) detection of biomolecules due to the potential permeation and

contaminating problems of art papers. Hence, we chose polypropylene (PP), a hydrophobic, cheap, and thermal stable material (< 110°C), as the material for the fabrication of the SD microfluidic chip. We established a convenient, low-cost, portable and reliable platform for monitoring $VEGF_{165}$ accurately, which can be applied for point-of-care (POC) testing. In this project, we also employed the label-free oligonucleotide-based luminescence switch-on assay on the microfluidic platform, which possesses the advantages of high sensitivity and high selectivity. Based on the detection of $VEGF_{165}$ in a three-step reaction process, we adopted a new design for the droplet transfer throughout the channels. This design could migrate the droplet through the chambers via controlling the orientation of the chip, which systematically combined the superhydrophobic force of the coating, the gravity of the droplet and the surface tension between PP and droplet. Therefore, traditional micro pump could be avoided and the total cost for the device could be substantially reduced. In addition, we developed an automatic, matched and portable device for the detection of $VEGF_{165}$, which assembled by a rotatable chip holder, a UV lamp, a filter, and a camera.

Finally, we developed a new whole Teflon membrane-based chip for the aptamer screening. Our article “Whole-Teflon microfluidic chips” introduced the fabrication of a microfluidic device entirely using Teflon materials, one group of the most inert materials in the world. It was a successful and representative introduction of new materials into the fabrication of microfluidic devices, which show dramatically

greater anti-fouling performance. However, even such device was inadequate for current purpose, as it is rigid and lacks convenient valve control functions for particle suspensions used in systematic evolution of ligands by exponential enrichment (SELEX). For this project, we propose a SMART screening strategy based on a highly integrated microfluidic chip. This new type of whole-Teflon devices, which are made of flexible Teflon membranes, offering convenient valving control for the whole SELEX process to be performed on chip and fulfilling the anti-fouling requirement in the meantime. The SELEX cycles including positive and negative selections could be automatically performed inside tiny-size microchambers on a microchip, and the enrichment is real-time monitored. The selection cycles would be ended after the resulted signal of the aptamers with high specificity reached a plateau, or no target aptamer is captured after a number of cycles of enrichment. Owning to the antifouling property of the chip materials, the loss of the sample is tremendously reduced. The SMART platform therefore is not only free of complicated manual operations, but also high-yield and well reproducible over conventional methods.

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