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Modern biotechnology: Origination of paper-based analytical devices

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Abstract

Nowadays most of the biotechnological processes can be performed on benchtop platforms without the need for an expensive, space-consuming equipment. In addition, the sensitivity and specificity of the conventional assays using biotechnological processes can be improved with the recent advancements in materials science and modern technology. One of the materials that aids chromatography, detection of biomarkers with response to a chemical/antibody/protein is "paper". Hence, analysis of samples i.e., detection, separation, amplification of biomarkers/biomolecules using paper as a substrate can be termed "modern biotechnology". This is a short review of paper microfluidics has benefitted a wide range of biotechnological/biomedical applications.

Keywords: Paper microfluidics; Paper pumps; Paper biotechnology; Capillary-based detection

Introduction

I have been working on the lab-on-chip devices that have miniaturized the size of the biotechnological equipment's to a benchtop platform for over a decade. The improved advancements in emerging technologies and their role in risk assessment [1], early detection and management of diseases [2] are possible due to the integration of inter disciplinary streams. The inculcation of various streams towards achieving the goal has inspired me to be in the field for a decade now as it involves constant learning of new techniques and new methods to develop platforms. I am involved in developing organ-on-chip models [3], biosensors, and microfluidic systems [4] for various applications including drug validation, risk assessment of diseases and sample processing for end users. The latest technology advancements for sample analysis is using paper, Particularly the filter paper and I would like to report the applications, expectations and limitations of using capillary forces to analyze the samples. Chromatography [5], identification of toxic components in the sample [6], amplification of genes [7] are some of the biotechnological processes that are performed using paper without the need for complex, space-consuming equipment's and this is termed "modern biotechnology." In the present editorial, I would like to give an overview of how paper microfluidics has benefitted a wide range of biotechnological/biomedical applications.

An alternative approach to conventional the biotechnological processes was started in 2007 by Whitesides *et al.*[8] although, paper-based assays have been used for few applications earlier. The hydrophilic and porous nature of cellulose enables the flow via capillary action without the need for an external pump or a power supply to transport fluids. Ever since paper-based analytical devices have used due its low-cost, quick fabrication and easy disposal method in industries to provide rapid results for diagnostics, environmental monitoring and chemical hazard testing applications [9]. Key features that make the paper a good candidate for such analysis is (i) use of biological samples in small volumes (ii) less time required for sample analysis and (iii) increased specificity and sensitivity of samples with low non-specific backgrounds. Three critical parameters considered while developing paper-based microfluidics are (a) pore size (size of particles retained in the substrate) (b) porosity (void volume of the membrane) (c) thickness of the membrane (to determine the volume, signal visibility and tensile strength of the analytes and substrates). Paper analytical devices can be fabricated in two ways: (i) physical blocking of pores to create hydrophobic boundary (ii) two-dimensional patterning/cutting. Physical blocking refers to patterning wax, organic solvents, PDMS, polystyrene, paraffin to create a hydrophobic barrierusing photolithographic, plasma treatment and printing (wax, screen, inkjet, flexography and inkjet) techniques. One of the example for comparison can be seen in fig (1), Where pore size, thickness and porosity of PVDF membrane can be seen. Patterning refers to the removal of unwanted paper by plotter knife and laser cutting, so the patterned area is a channel by itself. There are 2D and 3D capillary-based platform to carry out the different functions. Valves, fluidic timers, separation, thread mixing, fluidic batteries thermochroic displays are some of the examples of 3D platforms while multi step process disconnects, acoustic mixing, dilution, flow visualization, fluidic diode and text displays are the functions performed on 2D platforms as shown in figure (2).

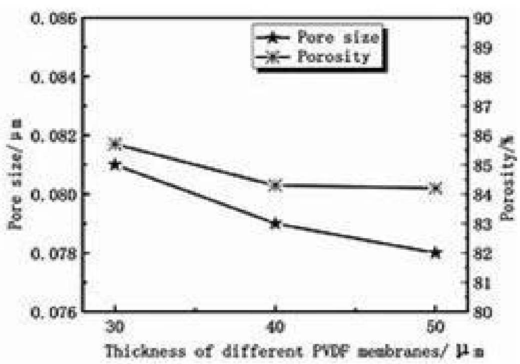

Figure 1: Comparison of pore size vs porosity vs thickness of membrane

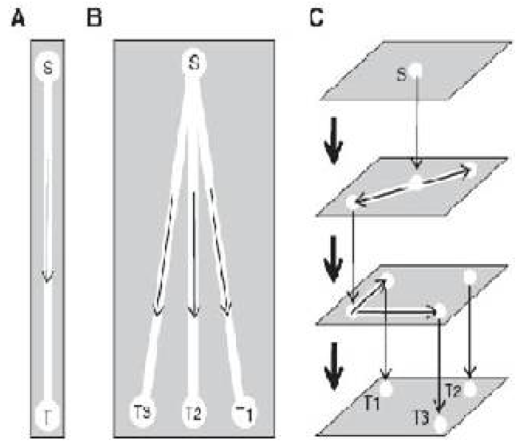

Figure 2: 2D AND 3D Capillary based platforms.

The most commonly used detection mechanisms for paper-based devices are colorimetric, electrochemical, nanoparticle, electrochemiluminescence, chemiluminescence, and fluorescence sensing methods for food, environmental and medical diagnostics. Mostly preferred method of detection for capillary-based microfluidics is colorimetric detectionas shown in figure (3) due to its easy read out without the need for external equipment. The outcome of a colorimetric assay can be classified into three categories: firstly, qualitative- gives a yes/no answer with a change of color. Secondly semi-quantitative - analysis uses a reference color chart fed to the system for estimating the amount of analyte present in the sample. Thirdly, quantitative measurement -the samples are processed using external equipment and software to quantify the signal intensities or hues. The analytes are quantified by screening the sample-introduced paper-strips to charge-coupled devices (CCD) (figure 4) and Complementary Metal-Oxide Sensors (CMOS) embedded in cameras and flatbed scanners. Recently used detector systems are reflectance-based, transmittance-based, and instrument-free measurements. Reflectance-based systems use a color adaption algorithm to quantify color intensities as in smartphone-based calorimetric detection. Flatbed scanners and smartphone integrated with image processing application have been used in laboratory testing and pesticide monitoring in water respectively. An attachment consisting of a light reflector, diffuser and plano-convex lens for illumination of the image placed in an acrylic holder is used to improve the contrast between the background and test strips and thus provide better efficiency, sensitivity, reproducibility of the smartphone-based colorimetric paper-based assay [10]. Transmittance-based measurements acquire cumulative density of light absorbing materials along the thickness of the paper and hence offers increased sensitivity. Efforts have been made by researchers to quantify biomarker for liver-specific functions in serum using a portable transmittance reader consisting of photodetectors and light emitting diodes. In instrument-free quantification, an external calibration with the standard colorimetric chart is used for semi-quantitative analyses of the sample. The analytes react with the predeposited reagents to form colored products as it moves along the channel due to capillary force. The color changes quantify the amount molecule present in the sample.

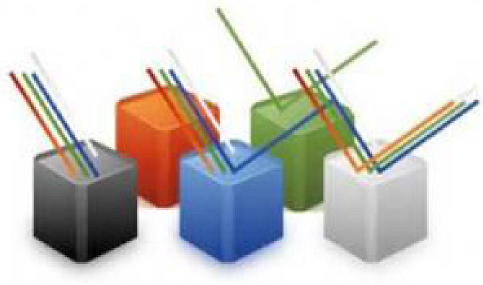

Figure 3: Absorption of particular wavelength of light during colorimetric.

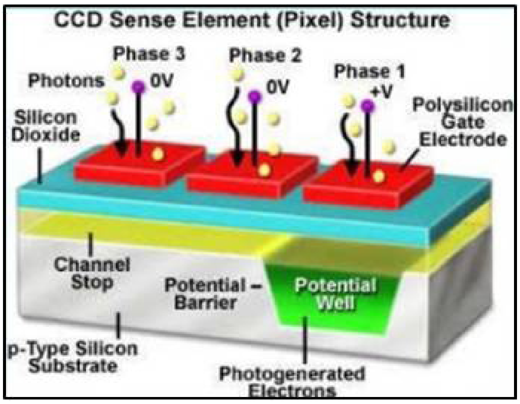

Figure 4: Charged couple device

The capillary-based detection platforms are used in biomedical, environmental, food safety and other applications. I would like to emphasize on the biomedical applications. Biomedical applications mostly use enzymatic reactions detection method due to the ability of the method to amplify the signal through catalytic properties. Enzymatic detection method had been used to detect clinically important biomarkers like phenylalanine, glucose and creatinine. 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-tetrazolium bromide (MTT) assay which was earlier analysed using a spectrophotometer and is now performed on a microfluidic paper-based analytical devices for detecting the (i) presence of certain biomarker responsible for (male fertility, (ii) to evaluate organophosphate poisoning, (iii) to detect biomarker for organ failure and cancer, lactate dehydrogenase. Another class of assay that biomedical applications take advantage of is the immunoassays where an antibody binds to specific antigen increasing the specificity of the assay. Conventional enzyme-linked immunosorbent assays (ELISAs) are now replaced with a paper-based analytical device for enhanced detection of analytes saving assay time and regents. The antibodies are physically attached to the paper-based analytical devices and analytes bind to the surface of antibodies generating color. Immunoassays have been used for monitoring cancer cells, detecting biologically relevant analytes, HINI and H3N2 viruses, cystic fibrosis was diagnosed by quantitative measurement of anions in sweat. Polymerization based amplification is employed for detection of *Plasmodium falciparum histidine*-rich protein 2 (P*f* HRP2) in malaria.

The advantages of using paper-based analytical devices are; (i) mass production of devices (ii) low cost as compared to other diagnostic devices (iii) has a wide range of applications ranging from biomedical applications to sensors (iv) easily disposed of by incinerating the paper. The paper-based analytical devices cover the ASSURED (Affordable, Specific, Sensitive, User-friendly, Rapid and robust, Equipment free and Deliver to the users who need them) criteria set forth by the World Health Organization and this makes it competent to other devices already in the market. Although, it is user-friendly and can be delivered to the people who need it the most; there are certain assays that need to be assessed by a medical expert as in case of telemedicine.

Although there have been many advantages of using paper-based analytical devices for many applications, there is still room for improvement in fabrication methods, As of now the cellulose production can be done with the wood available in abundance but going green as the vision for the safety of our own planet there are other sources of producing cellulose for the same, like cellulose extracted from agricultural waste and also cellulose produced from bacterial pellicle the sample read-out methods and sensitivity of the samples. While patterning wax is considered the easiest method to create hydrophobic boundaries, the boundaries so formed are not well-formed due to the non-uniform dissipation of heat on the surface of the hotplate. Besides, the use of organic solvents might be an issue as they can penetrate into the channel networks. Despite the fact that reflectance-based method allows detection of surface-bound proteins, it requires high concentrations of analyte to provide a detectable signal. Also, the smart phones that are used cannot provide ambient light to stabilize and measure the color information due to the built-in unknown automatic image correction operations. The prevalent drawback of using a transmittance light measurement is the low throughput due to the assay requiring at least 10min per sample.

Admitting the fact that the devices are simple, affordable and flexible, we have still opportunities for further developments in the field with regards to automation, sample readouts and bioanalysis. When paper-based systems are fully developed, one would want to see the paper-based platforms to (i) analyse many samples using one device (ii) allow multiplexing, i.e., run many assays (iii) analyze the control and unknown volumes of the sample automatically (iv) filter the samples and separate mixtures into individual components for analysis. The analytical devices can be inexpensive alternatives to a current diagnostic system and provide a useful diagnostic assay for a non-medical expert and the military in operations. These devices can also be used in basic research and hence may benefit laboratories and on-site diagnostics in developing and developed countries, as they are affordable, rapid and robust.

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