MICROFLUIDICS 2.0

Second Annual Workshop on Capillary Based Microfluidics for Bioanalysis

Poster Abstracts

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1. Development of a capillary-driven microfluidic biosensor for foodborne pathogens
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Rapid detection for pathogen with high selectivity and sensitivity are critical in food safety. Conventional identification methods are relatively complicated and time consuming. Here, we developed a miniaturized disposable biosensor capable of rapid detection at low cost. Using poly (methyl methacrylate) (PMMA) as a substrate, our system consists of self-priming microfluidics with sealed conjugate pads for pathogen capture, electrowetting valves for timely reagent delivery, and an absorbent pad as a pumpless alternative for additional fluid draw. The results demonstrated that the capillary-driven microfluidic device was rapid (< 30 minutes), required minimal sample volume (<10ul) and had appreciable sensitivity range (Limit of Detection is about 1fmol). The sensitivity, simplicity and portability of this novel device will allow for fast detection of food-borne pathogen at a low cost.

2. Fluorescent lateral flow assays for food safety
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Rapid analysis of food for pathogens, toxins and components will help producers ensure safe and affordable foods. We have developed advanced lateral flow assays using highly fluorescent nanoparticles. The assays were used to detect nucleic acid sequences from pathogens with a higher sensitivity and lower limit of detection than traditional methods. Fluorescent nanoparticles (~45 nm) were synthesized and modified with nucleic acid probes for use in the assay. In comparison to gold nanoparticles which had a limit of detection of approximately 5 fmols, the limit of detection for fluorescent nanoparticles was several fold lower at 0.027 fmol. The use of fluorescence allows rapid, low-cost and portable quantification of pathogens while providing extremely low limits of detection.

3. Watersoluble electrospun reagent containing nanofibers for on-chip storage
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The development of microfluidic diagnostics is often hampered by the necessity for on-chip storage of reagents. This work demonstrates the ability to electrospin reagents into water soluble nanofibers resulting in a stable on-chip enzyme storage format. Polyvinylpyrrolidone (PVP) nanofibers were electrospun incorporating horseradish peroxidase (HRP) at 20kV at a distance of 12 cm (100μL/hr). The fibers also included 25% w/w of sucrose to aid in enzyme stabilization. A one-step TMB kit was used to quantify the activity of HRP by placing the nanofiber mats into a microtiter plate and measuring change in absorption over time, the peaks of curves represented the activity of HRP. Following electrospinning, the activity peak for the HRP decreased by approximately 20%, and remained steady for the entire 45 days (room temperature) tested. No significant change in HRP activity was observed following electrospinning or after storage for 45 days at room temperature.
The fibers were also tested for activity on-chip for point-of-care diagnostics. Here, we developed a capillary flow microfluidic with on-chip material delivery, which combine lateral flow assay and microfluidic technology, and fluid transportation inside became automatic. Besides, a nanofibers mat contained biomaterial was integrated with this microfluidic. E. coli O157 detection was performed to demonstrated the application of this microfluidic, results shown that this microfluidic can successfully do the test, and the limit of detection was around 106, which indicated this microfluidic had potential in chemical or biomaterial detection, environment monitor.

4. Photoinitiated polymerization-based amplification under ambient conditions
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Although polymerization-based amplification (PBA) has demonstrated promise as an inexpensive technique for use in molecular diagnostics, oxygen inhibition of radical photopolymerization has hindered its implementation in point-of-care devices. The addition of 0.3–0.7 uM eosin to an aqueous acrylate monomer solution containing a tertiary amine allows an interfacial polymerization reaction to proceed in air only near regions of a test surface where additional eosin initiators coupled to proteins have been localized as a function of molecular recognition events. The dose of light required for the reaction is inversely related to eosin concentration. This system

achieves sensitivities comparable to those reported for inert gas-purged systems and requires significantly shorter reaction times.

5. Microfluidic platform for stress-induced rapid antibiotic susceptibility testing
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The spread of antibiotic resistance among pathogenic bacteria calls for the development of more efficient and rapid methods for antibiotic susceptibility testing. Current clinical methods for testing antibiotics rely solely on the quantification of the observed amount of bacterial growth in the presence of antibiotics. These methods are slow, as they require prolonged bacterial growth (at least 18-24 h). We developed a novel microfluidic platform for rapid antibiotic susceptibility testing based on stress-activation of biosynthetic pathways that are the primary targets of antibiotics. We chose methicillin-susceptible and resistant strains of Staphylococcus aureus (MSSA and MRSA) as model systems because of their clinical importance. One of the primary targets of antibiotics is the bacterial cell wall, a mesh-network of peptidoglycans surrounding the bacterial cell. We initiated a bacterial response to the antibiotic via direct damage to the bacterial cell wall. Bacteria, covalently bound to the bottom of a microfluidic channel, were subjected to a mechanical shear stress created by flowing liquid through the channel. The liquid contains both culture media (± oxacillin) and an additional chemical stress, e.g. the bacteriocidal agent lysostaphin. As cell wall biosynthesis in the susceptible strain is inhibited in the presence of antibiotic, damaged susceptible bacteria are unable to repair their cell walls and therefore die in response to the applied stress. In contrast, resistant strains are able to successfully repair their cell walls and thus survive. Bacterial cell death was assessed using a SYTOX Green dead-cell fluorescence stain. A pair of susceptible (Sanger 476) and resistant (MW2) S. aureus strains was used to develop phenotype separation criteria based on the fluorescence values after 60 minutes of exposure to stress and antibiotic. The clinical feasibility of the method was successfully tested in determining the susceptibility to antibiotics of 16 representative S. aureus strains. Due to the nature of microfluidic devices, our method can be easily adapted for simultaneous testing of multiple bacterial strains and antibiotics.

6. Research at the Intersection of Life Sciences and Engineering: Developing Microfluidic Platforms for Diagnostic Applications
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Our Life Sciences Engineering team has developed two completely integrated chips for the detection of bacteria from liquid samples. We have developed a molecular diagnostic that automates sample preparation, nucleic acid amplification, and detection in a low cost lab-on-a-chip format. The system conducts bacterial lysis, nucleic acid isolation and concentration, polymerase chain reaction (PCR), and end-point fluorescent detection of the target amplicon. We utilized a novel porous polymer monolith embedded with silica that has been shown to lyse bacteria and isolate nucleic acids from clinical samples. The PCR thermal cycling was achieved with a ceramic heater and air cooling, while end-point fluorescence detection was accomplished with an optical spectrometer. The integrated functionality of the chip was demonstrated using Bacillus subtilis as a model bacterial target. We have also extended our system to function as an enzyme-linked immunosorbent assay (ELISA) platform. Using remote valve switching, sample is propelled from a reservoir on the chip over a capture column filled with antibody-adsorbed polystyrene beads. Biotinylated detection antibody is then passed over the column, followed by streptavidin-labeled horseradish peroxidase (HRP). For bacterial quantitation, a chemifluorescent HRP substrate is then pushed over the column and into the detection well where fluorescence is measured. The method performs all necessary ELISA steps (starting from antigen incubation) in a fraction of the time required for standard off-chip protocols (1 h vs 18–36 h). Functionality of the chip has been demonstrated using Escherichia coli O157:H7 as a model antigen.

7. Iodine Quantification in Fortified Salt Using a Paper Analytical Device
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Iodine deficiency is the leading preventable cause of cognitive impairment in children, and nearly all countries have a salt iodization program to fortify it to a level of 20-40 ppm iodine. However, some of these countries lack

resources to monitor iodine levels properly. We are developing an inexpensive method to measure iodine in fortified salt. The paper analytical device (PAD) contains reagents to measure iodide and iodate; it was tested on salt samples by volunteers at Notre Dame University in the spring of 2012. The PAD detected iodizing agents with a sensitivity of 86% and specificity of 99%. Quantification was achieved only 53% of the time. To improve the quantification results, the PAD is being redesigned and retested.

8. A multi-step immunoassay using dry, patterned reagents in a two-dimensional paper network format
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Low-cost point-of-care devices appropriate for low-resource settings require the use of dried reagents to reduce user steps, remove the need for a cold chain, and facilitate device automation. Traditionally, conjugate pads have been used to store dry reagents in both conventional microfluidic and lateral flow-based devices. Our earlier work demonstrated novel methods for printing reagents on porous substrates to enable controlled spatial and temporal concentration gradients of reagents rehydrating during capillary flow within a porous device. Here we present an application of those methods in the implementation of a signal-enhanced immunoassay for the malaria antigen Plasmodium falciparum histidine-rich protein 2 (PfHRP2), using reagents patterned and stored on a single porous membrane. The multi-component gold enhancement system that we used for signal amplification loses functionality if the components are mixed prior to drying. Thus, components must be stored dry in separate regions and recombined on the device for use in the assay. Here, we show that gold enhancement reagents printed separately can be combined upon rehydration to yield a bolus of complete gold enhancement solution, which is then able to enhance the gold signal generated in the PfHRP2 assay. We achieved 3.2-fold signal enhancement after 60 minutes, using just 6 µl of printed gold enhancement reagents. Reproducible signal was visible at concentrations as low as 5 ng/ml. This is a clear demonstration of the viability of patterning and drying reagents onto a paper device for dry reagent storage in paper-based assays.

9. A Centrifugal Force-Based Method of Characterization of Membranes Used in Paper Microfluidics
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Capillary force-driven microfluidic systems such as two-dimensional paper networks (2DPNs) are showing great potential for performing sophisticated diagnostic tests. The flow rate, one of the most important design criteria, is described for a constant width strip by Darcy's law: $u_s = k_s \Delta P / \mu L_c$ (where u_s: superficial fluid velocity; k_s: superficial permeability; ΔP: pressure differential; μ: viscosity; L_c: distance travelled from fluid source). ΔP is the capillary pressure developed by the fluid/membrane combination: $\Delta P = 2\gamma\cos(\theta)/r_m$ (where γ: surface tension; θ: fluid/membrane contact angle; r_m: mean pore radius). Obtaining values needed to calculate the capillary pressure is nontrivial; these values will also change after chemical modifications of the matrix surface. Manufacturer-provided flow metrics for most paper-like materials used in microfluidics are insufficient to predict flow rates in multi-material 2DPNs. Two important properties are left intertwined when describing membranes by their capillary rise time (by most manufacturer standards the number of seconds for water to travel 4 cm from a fluid source into a dry, constant-width strip): (i) ΔP pulling the fluid into the dry material and (ii) the fluidic resistance that increases with distance from the fluid source. Both are important parameters for programming the function of 2DPNs. These can be separated by noting how high fluid rises in a porous material under the influence of gravity. Unfortunately, for small-pore materials, the equilibrium height can be several meters, making the experiment both very slow and inconvenient. We demonstrate a centrifugal device to independently measure ΔP for relevant membranes.

10. On-Paper Purification of Nucleic Acids for Point-of-Care Applications
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Molecular diagnostics often rely on complex sample preparation techniques that necessitate expensive laboratory equipment and trained technicians. Due to these requirements, these tests are rarely available at the point-of-care (POC), keeping diagnostic results from populations who need them most. Paper-based devices are inexpensive, easy to manufacture, and disposable making them ideal candidates for POC tests designed for low resource settings. This work reports a simple, inexpensive, and disposable system for on-paper purification of nucleic acids (NAs), a key component in molecular diagnostics. This system is developed for lateral flow in a

porous membrane coupled with a “chargeable” linear polysaccharide, chitosan, that utilizes a reversible pH-triggering method to selectively capture NAs. This method expands on the original work by Cao et al. by moving the entire process to commercially-available porous membranes, such as nitrocellulose. Add!
itionally, we have shown direct patterning of chitosan onto porous membranes. This technique also substantially concentrates the sample, resulting in a multi-purpose device in which capture and concentration occur simultaneously. The synchronized combination of these events provides a unique system that can be used for a wide range of NA targets. The concentrated NAs are ready to use in various downstream processes without further purification.

11. Pre-programmed, self-powered circuits built from microfluidic capillary elements
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Capillary-based microfluidic devices enable self-powered flow of liquids, making them useful for point-of-care applications. However, such devices are often limited to the filling and draining only one liquid at a time. This limits their usefulness in complex fluidic operations that require multiple chemical delivery steps. Here we present two new capillary valves, programmable retention burst valves and low aspect ratio trigger valves, that provide enhanced control over fluid flow in microchannels. Programmable retention burst valves sequentially drain liquids according to local increases in the capillary pressure of multiple channels. Meanwhile, low aspect ratio trigger valves passively halt liquid flow by combining an abrupt enlargement of a two-level stop valve with a hydrophobic cover. We integrated these valves with previously developed capillary fluidic elements to enable autonomous and sequential delivery of liquids in a capillary-based microfluidic system. Circuit masters with two levels of thickness were fabricated in SU-8, replicated into PDMS, and sealed against a flat PDMS cover. To characterize fluid flow, the circuit was filled with dilute aqueous solutions of food dye and visualized under a microscope. Combining programmable retention burst valves and low aspect ratio trigger valves with existing fluidic elements enabled autonomous and sequential delivery of multiple reagents. This device could be used to autonomously deliver reagents in complex fluidic operations such as multi-step sandwich assays.

12. Synthesis of Perfluorinated Nanoemulsion Composite Hydrogels Using Stop-flow Lithography
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Nanoemulsions are metastable dispersions containing nanoscale liquid droplets in another immiscible fluid. They are often utilized in biological and cosmetics applications. However, nanoemulsions are thermodynamically unstable due to high surface tension between the dispersed and continuous phases, and will phase separate over time. Their degradation over time is attributed to molecular diffusion of the dispersed phase through the continuous phase, or Ostwald Ripening (ω), in an effort to minimize the system’s free energy. Previously, our lab has demonstrated encapsulation and loading of nanoscale oil droplets within a hydrogel gel matrix using stop-flow lithography[1] (SFL) in microfluidic PDMS devices[2]. Here, we explore how the encapsulation of perfluorinated oil droplets in a hydrogel affects their stability. Perfluorocarbons have the unique ability to highly solubilize oxygen.

1. An, H. Z.; Helgeson, M. E.; Doyle, P. S. Nanoemulsion Composite Microgels for Orthogonal Encapsulation and Release. Adv. Mat. 24: 3838-3844. (2012).
2. Dendukuri, D; Doyle, P. S. The Synthesis and Assembly of Polymeric Microparticles Using Microfluidics. Adv. Mat. 21: 4071-4086 (2009).

13. Determination of Nitrite in Saliva Using Microfluidic Paper-based Analytical Devices
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The development of novel analytical strategies is critical to improve early diagnosis and follow treatment monitoring. In this regard, point-of-care platforms avoid transferring ill patients to central locations, provide fast responses, decrease the overall cost of the treatment and can significantly improve the success of the treatment. Among others, paper-based microfluidic devices are one of the most versatile and efficient platforms to design diagnostic tools. In comparison with traditional paper-based devices (such as reactive strips or ELISA), paper-microfluidics are faster, require smaller samples, are far more efficient and can perform multiple and complex

biochemical reactions without cross-contamination or external power. Moreover, instead of giving a negative/positive response, paper microfluidic devices can provide a visual reading that is proportional to the concentration of the target marker. Considering the potential impact that this technology could have in oral health (e.g. periodontitis), the objective of this work was the development of microfluidic paper-based analytical devices (μPAD) to identify and quantify levels of nitrite in saliva. Devices were fabricated by wax printing and allowed the detection using a colorimetric reaction (Griess's method). Results related to the design, characterization, and application of these devices will be discussed along with a general overview of the advantages of this emerging technology.

14. A Paper-Based Electrochemical Device for Salivary Analysis in Low-Resource Settings Grace Wu, Boston University
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Diagnosis and detection is one of the most effective means of controlling matters that adversely affect public health and safety. Yet, in the developing world, most gold standard diagnostics remain widely inaccessible due to cost and lack of infrastructure. One strategy to increase access to health and safety devices has been through the development of point-of-care (POC) diagnostics that are low-cost, portable, and easy-to-use for on-site analysis. In particular, paper has recently been in the spotlight as such a POC platform. Compared to conventional POC tests made of glass or plastic substrates, paper itself is even more thin, light-weight, portable, disposable, and can store biological and chemical molecules for analytical measurement within its fibrous network. Several paper-based tests, dubbed μPADs for "micro-Paper Analytical Devices", have demonstrated high sensitivity and specificity detection of proteins, bacteria, and metals for applications in disease diagnosis, health monitoring, and food and water safety. To expand the capability and robustness of μPADs, we propose to develop a reliable and quantitative paper-based biosensor for salivary analysis. Additionally, a quantitative analysis will be performed to study and improve the long-term viability of paper devices. Saliva is an ideal sample for POC testing as it is easy to collect and less expensive than blood collection. The model analyte to be measured in saliva will be ethanol, the consumable form of alcohol, which can be detected electrochemically. This was chosen because inexpensive and reliable methods to measure alcohol concentration are not readily available in resource-limited areas. A speedy, easy-to-use, and low-cost method to screen subjects can be useful in emergency hospital settings, as well as road safety campaigns.

15. Patterning Microarrays in all Three Dimensions
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Protein microarrays can improve our understanding of disease and lead to drug target and biomarker discovery—but fabricating them is not easy! Clinical sample volumes are small and contain very few target molecules, which cannot be amplified. Sandwich assays promise highly specific detection, but suffer from cross reactivity, which limits multiplexing. We developed a fabrication approach that addresses these issues by: 1) performing multiple tests on very small sample volumes 2) fractionating the sample onto specific test sites 3) and eliminating detection antibody cross reactivity.
We designed a stacked 3D microarray to simultaneously separate and pattern fractions of biological samples. The samples are distributed into microchannels in the x-y plane and sorted in the z-direction by multiplexed affinity columns. The layers in the 3D stack are wax-patterned, paper-based microarrays [1, 2]. Each layer is functionalised with a different capture antibody. The paper layers are aligned by eye and held together with non-permanent adhesive to form multiplexed channels 500 μm in diameter. After the protein samples are pulled through the 3D arrays, the paper pieces can be easily peeled apart to reveal 2D patterns of a specific protein. The arrays are then analysed using fluorescence-based readout.
This inexpensive and simple approach makes protein microarrays more accessible and we believe it could be a useful tool for detecting trace biomarkers in small clinical samples.

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2. Liu H.; Crooks R. *J. Am. Chem. Soc.* **2011**, *133*, 17564–17566

16. Electrophoretic Screen and Molecular Imprinted Silica Particles as Antigen Assays
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Currently infectious diseases are the leading cause of death in developing countries due to the lack of resources and poor infrastructures. When the infrastructure is not available, the need of a portable device is necessary to reach to those that cannot receive proper medical attention. The project objective is to develop a portable immunoassay that can perform in underserved areas where there is no access to laboratory equipment. This device should offer an optimized array of assays for the targeted area and must withstand ambient temperatures without degrading. To achieve simplicity of the assay, a microfluidic device is required to achieve blood separation. After blood separation, the analyte will flow through a second microfluidic device containing an array of capillaries, each of these testing for different targets. Finally the biggest challenge of our objective is stability in ambient temperature. This is because immunoassays require antibodies which, depending of the type, require refrigeration. As an alternative to antibodies we will be exploring molecular imprinted silica particles. Using antigens as silica particle templates, the particles will recognize the molecule of interest by shape specific interactions. Currently we have tested silica particles imprinted with human chorionic gonadotropin (HCG). These particles agglomerated under the presence of HCG, but not under PBS. We will now test molecular imprinting of different proteins, and the design of the microfluidic device will begin.

17. Paper SERS sensors for trace chemical detection

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Surface-enhanced Raman spectroscopy (SERS) is a highly sensitive analytical technique, but is currently confined to the laboratory setting. This is a consequence of the high cost and short shelf-life of conventional SERS-active substrates, which are fabricated on rigid surfaces such as glass, silicon and polymers using expensive microfabrication techniques. Paper is a widely available and relatively inexpensive material that has been utilized for sensing applications. Due to its natural hydrophilicity, paper can be leveraged for sample manipulation, such as analyte separation and concentration. In this work, we demonstrate that paper is also a promising alternative to rigid materials for SERS applications. The coupling of paper sensors with SERS results in an inexpensive and simple-to-use analytical tool that promises rapid, portable and high sensitivity analysis at the point of sample. Paper SERS sensors are fabricated very simply by inkjet-printing of gold and silver nanostructures onto paper. These paper SERS sensors have been utilized in the form of spot-on assays, swabs, dipsticks, filters and paper chromatography. Apart from a portable Raman system, no additional equipment is required. Using these devices, detection of 10 attomoles of analyte molecules has been achieved. The assays are quantitative and have low signal variability. Paper SERS sensors have many potential applications, including law enforcement, food safety, and border protection – areas where rapid and highly sensitive detection of chemical and biological threats are of the essence. Additionally, by bio-functionalizing the devices, point-of-care biological assays are possible.