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AES-1

Characterizing the Dielectric Properties of Human Mesenchymal Stem Cells Using a Quadrupole Microfluidic Device

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Characterizing the dielectrophoretic response of stem cells at various stages of growth and in the presence of promoters is an important first step in designing an electrokinetic microdevice to separate stem cell types [1]. Dielectrophoresis is a technique utilizing nonuniform electric field to polarize cells based on the polarizability and dielectric properties of their membrane, cytosol, and other structurally dominant organelles. The DEP force is also tunable via adjustments to the electric field magnitude and shape and the surrounding medium [2, 3]. Hemapoetic stem cell identification has been accomplished via antibody tagging of unique cell-surface antigens followed by flow cytometry and fluorescence-activated cell sorting, but adaptation to other types of stem cells has been limited [1]. Therefore, there is a need for an identification technique that does not require tagging and is versatile enough to identify and separate all types of stem cells. Dielectrophoretic separations are advantageous because identification of stem cells can be coupled with separation in a microfluidic device. In this work, a microfluidic device with gold quadrupole electrodes, microchannel and a pumping system will be used to characterize the DEP response of human mesenchymal stem cells. Mesenchymal stem cells are multipotent adult stem cells and can differentiate into osteoblasts, adipocytes, chondrocytes, astrocytes, and myoblasts based on environmental promoters [4]. The mesenchymal stem cells will be characterized at frequencies ranging from 100kHz to 80MHz in a medium with conductivity ranging between 0.01 and 0.5 S/m [1]. These experiments will map out the cross-over frequency of the human mesenchymal stem cells in different stages of growth and in the presence of promoters. COMSOL simulations and MATLAB will be used to fit the data to a single or multishelled spherical DEP polarization model in order to back out structural polarizability and conductivities of components of the human mesenchymal stem cells. The broader implications of electrokinetically identifying differences in stem cells lie in purification and control for tissue engineering and cell therapy applications [4].

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AES-2

Monitoring Hydroxyl Radical Production In Mitochondria with Micellar Electrokinetic Chromatography

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Reactive oxygen species (ROS) are thought to be a significant contributing factor to the aging process at a cellular level. Of particular interest are the ROS generated by the mitochondria, as research has pegged these to be a significant source of oxidative damage. Current methodology to investigate ROS production is heavily biased towards fluorescence microscopy. This method, however, lacks low limits of detection and the ability to distinguish between components fluorescing at the same wavelength. To overcome these drawbacks, we are developing sensitive capillary electrophoresis techniques with laser-induced fluorescence detection (CE-LIF) for ROS analysis. The treatment of cells and mitochondria with probes selective for a ROS of interest and subsequent separation will enable us to monitor low levels of specific ROS present in biological samples. The current focus of our work is the use of 2-[6-(4 -hydroxy)phenoxy-3H-xanthen-3-on-9-yl]benzoic acid (HPF) to monitor the production of hydroxyl radicals. In vitro controls have verified the selectivity of the probe, and the analysis of isolated mitochondria from L6 myoblasts shows our ability to measure basal and stimulated levels of hydroxyl radicals. The initial CE-LIF method development will be presented here, as well as results from control mitochondria. The application of HPF combined with CE-LIF to look at hydroxyl radical levels in mitochondria isolated from adipocytes will also be discussed.

AES-3

Separation of Particles Using Contactless Dielectrophoresis and Electrokinetic Flow

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Dielectrophoresis (DEP), the motion of a particle in a non-uniform electric field, has become a robust method for analyzing nano-particles, cells, viruses, and DNA based on their physical and electrical properties. A new technique, contactless Dielectrophoresis (cDEP), isolates cells from contact with the electrodes. This is achieved by using fluid electrodes which are isolated from the sample channel by thin insulating membranes. The absence of contact between electrodes and the sample fluid inside the channel prevents bubble formation and avoids any contaminating effects the electrodes may have on the sample. This technique has demonstrated the ability to isolate THP-1 human leukemia monocytes from a heterogeneous mixture of live and dead cells as well as discriminate between cells of different metastatic potential. Recently, this technique has been improved for operation at frequencies as low as 1 kHz, allowing us to manipulate cells using both positive and negative DEP. Typically, this technique relies on the use of pressure driven flow which creates a non-uniform flow velocity and requires the use of a syringe pump. This work presents the analysis and evaluation of the combination of cDEP with electrokinetic flow to isolate and enrich particles.

AES-4

Mixing Enhancement in Microfluidic Devices Using Contactless Dielectrophoresis (cDEP)

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Mixing in microfluidic devices has an important role in numerous biological and chemical applications. Rapid mixing is necessary in many lab-on-a-chip (LOC) and Micro Total Analysis Systems (μTAS) devices for chemical processes, including specific applications such as chemical reactions, and for biological processes, such as enzyme reactions, DNA or RNA analysis, and protein folding.
Despite the small length scale of microdevices, rapid, efficient mixing is difficult to achieve. Due to small length scale the Reynolds number, the ratio between inertia and viscous forces, is small. Thus mixing without any intentional stretching and folding of interfaces is dominated by molecular diffusion, which takes a long time relative to the typical operating timescale of these microdevices. To address this need, various techniques for enhancing mixing in microdevices have been proposed, including both active and passive techniques that are based on phenomena such as chaotic advection. There are many active mixing techniques, including those that use pressure, magnetohydrodynamic, acoustic, or thermal disturbances to enhance mixing. Electrokinetic mixing has proven to be an efficient method for actively mixing solutions or microparticles.

DEP, the motion of a particle in a suspending medium due to the presence of a non-uniform electric field, has shown great potential for particle separation, manipulation, and identification of micro- and nano-scale particles. However, studies in the literature using DEP for mixing enhancement are fairly limited.

In this study, we have investigated mixing in microdevices based on a new technique, termed contactless dielectrophoresis (cDEP). In cDEP, an electric field is created in a microchannel by electrodes that are inserted into two side channels filled with conductive solution. These side channels are separated from the main channel by thin insulating barriers that exhibit a capacitive behavior. An electric field is induced in the main channel by applying an AC field across the barriers. Not having direct contact between the sample and the metallic electrodes eliminates issues that plague conventional DEP such as bubble formation, electrode delamination, and sample contamination. Furthermore, the fabrication process is relatively simple because it is not necessary to pattern micro-electrodes in the main channels. This method thus is well suited to traditional mass fabrication techniques such as hot embossing and injection molding.

We consider four variations of a device that consists of two “mixing chambers” located on opposite sides of a 50 mm or 100 mm rectangular microchannel. The chambers are either rectangular or semi-

circular, and they are either placed symmetrically on opposite sides of the channel or are staggered with an axial spacing of 300 mm. In all cases, the depth of the chambers is 50 mm to match the channel depth.

In our cDEP device, there are electrode channels which are separated from the mixing chamber by 20 mm insulating barriers. The electrode channels are filled with a conductive solution, and electric field gradients are generated in the microchannel using electrodes that are inserted into two electrode channels. The capacitive nature of the barrier between the chambers and the electrode channels generates a nonuniform electric field when an AC signal is applied. In some locations, the electrode channels are far from the mixing chamber to prevent their electric effect on the mixing chamber, thereby creating an electric field similar to that given by three isolated electrodes.

Before applying the electric field, the two inlet streams flow side-by-side through the devices with negligible diffusive mixing. After applying the electric field, the beads suspended in the bottom fluid are manipulated by the induced DEP force. The mixing chambers exhibit a resulting secondary flow. This secondary flow is observed to stretch and fold the fluid, which is an important characteristic of rapid mixing. The stretching and folding in each device occurs between the symmetrically placed mixing chambers. In devices with two separate mixing chambers, the first mixing chamber begins the stretching and folding, and then the second chamber amplifies this stretching and folding and enhances mixing.

We quantified the effectiveness of these devices by evaluating mixing index, which is equal to 1 minus the ratio of the standard deviation of the concentration after mixing to the standard deviation of the concentration before mixing. We ran mixing experiments for voltages between 0 and 300 Vrms (0, 50, 100, 150, 200, 250, 300 Vrms) at a constant frequency of 600 kHz and a flow rate of 5 μL/hr. We found that both devices with rectangular mixing chambers achieve a maximum mixing index of 80%. However, the maximum mixing index obtained in circular mixing chamber was 57%. This occurs because the rectangular shape of mixing chamber decreases the velocity of particles inside the mixing chamber, which give more time to DEP to influence the particles and create mixing. However, circular mixing chambers cannot have the same effect on particle's trajectories; thus, we do not observe efficient mixing.

We have also considered the dependence of mixing on the flow rate for a fixed voltage and frequency. The mixing efficiency decreases by increasing the flow rate, which is equivalent to decreasing the flow time scale. The results also showed that a higher mixing index can be obtained at higher voltages for a given flow rate.

In addition to mixing beads with a fluid, these microdevices can also be used for mixing two fluids when one of those fluids contains beads. To prove this claim, we suspended beads in a water-based blue dye solution and pumped the sample into one of the microdevice inlets. Deionized water was pumped through the other inlet. After applying an AC signal of 300 Vrms and 600 kHz, the dye solution got mixed with the deionized water due to the secondary flow generated by the DEP force on the beads.

AES-5

Rapid Estimation of the Most Probable Number (MPN) of Viable Bacteria in Water Samples

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Various regulatory agencies such as the USDA, FDA and EPA require that the number of viable bacteria present in certain samples of food or water be less than a certain specified number. For instance the US Pasteurized Milk Ordinance requires "Grade A" pasteurized milk to have a total viable bacterial count of ≤ 20,000 CFU/ml, and the EPA requires that the concentration of coliforms in primary recreational water bodies not exceed 5 CFU/ml.

The enumeration of VIABLE bacteria is typically done using a statistical technique known as the Most Probable Number (MPN) method. The method involves progressively diluting an aliquot from the sample of interest into microbial growth media (repeated in triplicate) and keeping a track of which dilutions show evidence of the presence of even a single VIABLE bacterium. Currently, the latter (whether a vial contains any viable bacteria, or not) is determined by culturing the vials for 1-2 days, and looking for the development of turbidity in the sample (either manually, or using a spectrometer). It is known that if there is even a single viable bacterium present, it will give rise to hundreds of millions, which will turn the solution turbid.

Since the concentrations of bacteria in the sample have to rise to millions of CFU/ml before they can be detected via turbidity measurements, it takes a long time (typically 1-2 days) before the MPN can be determined. This delay has many adverse economic (and even, health) consequences.

In contrast, our method promises to deliver MPN results in less than 1 day. The principle underlying our method of detection is the polarizability of viable bacterial cells. In the presence of an alternating electric field, there occurs a build-up of charge at the membrane, causing the cells to act like capacitors. As the bacteria multiply in number, there will be a corresponding increase in the charge stored in the interior of the suspension (its "bulk capacitance"), and this increase in bulk capacitance over time serves as a signature for the presence of viable bacteria.

We were able to quantify viable E. coli in Tryptic Soy Broth (TSB) in <6 hours as opposed to 1-2 days taken by the current methods and also have a cut off time of ~10 hours which significantly reduces the wait time for industries.

This method can thus have a significant impact on many modern water and food quality applications.

AES-6

Theoretical and Experimental Approach On Simultaneous and Continuous Separation and Concentration of Particles On an iDEP Platform

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Dielectrophoresis (DEP) is an electrokinetic technique successfully employed for the manipulation of particles in microfluidic devices. DEP is defined as the movement of dielectric particles due to an induced polarization in the presence of non-uniform electric fields. In this work, non-uniform electric fields are achieved employing insulating structures between a set of electrodes. This approach is known as insulator-based dielectrophoresis (iDEP). In the present work, performance of a simultaneous and continuous separator and concentrator of a mixture of particles is studied theoretically and experimentally. A two-particles mixture of polystyrene beads with different sizes are employed to evaluate the performance of the separator and concentrator on a DC-iDEP platform device. A computational model was developed in order to predict particles pathlines and estimate trapping regions where particles are concentrated. Theoretical and experimental results are compared in the present study.

AES-7

Dielectrophoretic Response of Perfluorocarbon Oil-Core, Chitosan, Poly-L-Lysine, CaPO4-Shell Nanoparticles

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Dielectrophoresis (DEP) is useful to assemble, discriminate, and manipulate particles based on a particle's polarizability and interactions with a surrounding medium under non-uniform electric fields. Core-shell nanoparticles are traditionally synthesized for drug delivery [1], nanoreactors [2,3], and diagnostic markers [4], and can be constructed with various liquid core and various solid shell materials. These engineered nanoparticles facilitate systematic investigation of DEP polarization mechanisms and are much simpler than biological cells. Perfluorocarbon oil in a phospholipid micellar structure were used for the core, along with three shell materials (chitosan, Poly-L-lysine, and CaPO4) which were chosen for their dielectric properties and known synthesis procedures. We report the frequency-dependent, and medium conductivity-dependent responses of ~250nm core-shell nanoparticles. Particle-particle interactions were quantified by tracking pearl-chain assembly of the nanoparticles in two dimensions. Experiments were conducted within a 100nl chamber housing 100um wide Au quadrupole electrodes spaced 25um apart. Frequencies from 100kHz to 80MHz at 10Vpp were tested and the frequency-dependent motion was quantified via video microscopy image intensity profiles in two perpendicular planes. Experimental COF results were compared with spherical shell models of the real part of the Clausius-Mossotti factor as a function of frequency, conductivity of the shells, and medium conductivity. This work exhibited two dimensional nanoparticle patterning into pearl-chains and then bands, which were subsequently probed for coherent light scattering via spectral techniques.

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AES-8

Facile Isolation and Recovery of Biological Molecules with Chemically Triggered Degradable Polyacrylamide Gel Electrophoresis

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Polyacrylamide gel electrophoresis (PAGE) has been widely used in the analysis and separation of biological molecules, including proteins, DNA and RNA. For further analysis and manipulation of separated biological molecules, it is sometimes necessary to recover them from polyacrylamide gels. Most commonly, biomolecules are isolated from polyacrylamide either by diffusion or electroelution. The poor diffusion of large biomolecules makes the methods slow and low-yielding. An alternative approach that has been proposed to reduce recovery time and increase yield is to incorporate degradable crosslinkers into the gel. Although degradable electrophoresis gels have been used to isolate biological molecules with high yield, they often require very limiting, harsh conditions, such as, acidic or basic buffer systems. To solve these limitations, we have developed novel crosslinkers consisting of α-azido ether functionality which can be degraded by bio-orthogonal chemical triggers. Here, we show that large biological molecules such as plasmid DNA can be separated by PAGE containing the new crosslinker, N3-EG2, and liberated with high yield via a chemical trigger. In addition, we find that the degradation conditions do not affect the activity of the biological molecules. We believe that N3-EG2 crosslinkers could be widely applied to biological molecule recovery and combined with other technologies like proteomics.

AES-9

Morphological Effects On the Electrostatic Potential In a Divergent and Convergent Channel for Microfluidic Applications

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A number of researchers have proposed combining electroosmotic flow (EOF) and pressure-driven flow as a means of controlling the motion and separation of bioparticles in diverging- converging microchannels[1]. In order to get better understanding of role of electrostatic potential on fluid motion, analytical study of the electrostatic potential in divergent and convergent channels has been conducted in this study. Distribution of electrostatic potential is given by the solution of 2D Poisson-Boltzmann Equation (PBE) with both long channel and Debye–Huckel approximations. As a product of the investigation, one can assess the behavior of the electrostatic potential inside of a convergent-divergent section. Three key parameters have been identified to describe the electrostatic potential behavior: the angle ($\propto$) of the convergent (or divergent) section (related to the walls of the channels) that handles the "magnitude" of the deviation with respect to a straight channel; the ratio of the wall potentials, R, which handles the symmetrical/non-symmetrical aspects of the electrostatic potential, and the ratio of the width to the length (γ) that controls the "shape" of the channel section. Results of this study will be shown by using a series of portraits that capture the key behaviors of the electrostatic potential with respect to the three parameters described above.

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AES-10

Experimental Validation of a DNA Pre-Conditioning Strategy Developed Using Brownian Dynamics Simulations

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We have developed a new pre-conditioning strategy to improve DNA stretching by electrokinetic flow in a microfluidic device using Brownian dynamics simulations. The core of the strategy is to "pre-stretch" DNA in the direction orthogonal to its final stretching direction. This allows DNA to be pre-stretched while requires no additional strain to be provided. Although we have shown in simulations that this strategy can improve the degree of DNA extension considerably even at medium Deborah number, an experimental validation is yet to be performed. In this study, experiments on the newly developed pre-conditioning strategy and some of its variation are performed. We compare in details the predictions and experimental results in the averaged maximum DNA extension, its distribution and the mechanism causing the preconditioning effect. It is found that the new strategy provides moderate improvement on the degree of DNA extension. The cause of the discrepancy between simulations and experiments will be discussed.

AES-11

Analysis of Channel Morphology for Electrophoresis of Biomolecules: Effect of Axial and Orthogonal Fields

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Research involving polymer gels with embedded nanoparticles of varying properties is quite attractive because of the multitude of potential applications, including separation of biomacromolecules for clinical diagnostics, tissue scaffold growth etc. Moreover, analysis of the effects of the pore length and diameters on the transport of biomolecules is an important aspect to be studied either analytically or computationally as shown by previous efforts [1]. In this research, we propose to computationally analyze different pore models (associated with gel materials) and study the effect of geometry on the transport properties. Results will illustrate, for example, the role of pore-scale in conjunction with the effect of secondary electrical fields.

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AES-12

Design and Simulation of an Automated Rare Blood Cell Detector

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In recent years the demand for using circulating tumor cell (CTC) counts in cancer management has increased. With the ability to detect small numbers of rare cells in blood and the motivation to non-invasively monitor therapeutic progress, CTC detection technology would play an important diagnostic role. However, the current detection process can be performed only in specialized laboratories with the analysis requiring two to four skilled technicians. The device's price can reach seven figures, limiting the availability for cancer detection. Analysis requires weeks (or even months), making it difficult to detect disease at an early stage.

Our goal is to design a portable point-of-care device consisting of three syringes, a static mixer, a magnetic filter, a microfluidic "chip" and a waste receptor. Compared with current detection systems, our device is much smaller, lower in price, and requires only one operator. The device would be available for use in clinics, nursing homes or residences for detection of most cancers (pancreas, ovarian and melanoma) in an early stage.

Our recent research focuses on the simulation of the cell detector. CFD software is utilized to simulate the static mixer's operation, mixing the blood sample, buffer and reagents. CFD simulation results are then compared to the results of laboratory experiments. Following the static mixer simulation, the magnetic filter is simulated, incorporating magnetic particle motion. The results of the simulation are compared to experimental results with the results used to optimize the size and configuration of the magnet filter.

AES-13

Optimal Design of Microfluidic Capillary Networks for Rapid Gel Free DNA Separation

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10 to 100 times reductions in run time over the current state-of-the-art gel electrophoresis DNA length based separation are made possible through the mathematical optimization of a novel separation technique known as micelle end-labeled free solution electrophoresis (ELFSE). In gel electrophoresis the gel sieving matrix, necessary to make DNA velocity length dependent through friction, performs at typical lab speeds of 400 bases in 1 hour. The absence of a sieving matrix in aqueous solution will ensure that DNA of all lengths will migrate at the same velocity without another friction causing agent. In micelle ELFSE this friction is generated by a dilute low viscosity solution of micelles allowing for unique, superior trade-offs in operating conditions, like electric field strength, capillary length, and micelle size, necessary for large increases in throughput. These trade-offs depend on the separation platform. Capillary arrays and microfluidic devices are examined as candidates for the fastest separation mode.

Length based separation is achieved when DNA populations of different lengths migrate at different velocities. Small deviations in DNA population velocity cause ideal Dirac-delta signals to widen into Gaussian signals which can overlap and become unresolved. These small deviations in DNA velocity are due to micelle polydispersity, diffusion, and other mass transfer effects as described by a moment analysis which renders a model similar to the Van Deemter equation in chromatography to describe the Gaussian width. Micelle polydispersity is typically the largest signal widening effect which is reduced over time by time averaging through transient binding of the micelle to the end of alkylated DNA. Because of the necessity of DNA to sample different micelles over time, fast run times and high resolution are conflicting objectives that are difficult to mitigate without a rigorous optimization approach.

Non-linear programming techniques are utilized to solve the dual objective optimization problem. The model is non-convex and requires a global optimization code like BARON to generate the pareto front. BARON identifies standard form non-convexities to relax into a convex underestimate proceeding through a spatial branch and bound to reduce the gap between the convex underestimate with the non-convex local solution to finally converge to the global optimum. The model is written to expose these standard form non-convexities (bilinear, linear fractional, and concave univate terms) for fast convergence of BARON.

Typical lab scale separations are performed using either a capillary array in a bench top system or in a curved channel in a microfluidic device. Single capillary, parallel capillaries, serpentine microfluidics, and spiral microfluidics are examined; as well as finish-line versus full channel snap shot detection

modes. Snap shot is the fastest detection mode possible as every population of DNA smaller than the length of read is guaranteed to be resolved in channel once the length of read is resolved, owning to the monotonic nature of the resolution. Electric field strength and channel length are found to be strong functions of the micelle polydispersity. A reduced parameter combination of the electric field, channel length, and micelle polydispersity is used to collapse the optimization results for different levels of polydispersity onto a single curve. Minimized run times are shown to have significant improvement over gel based separations. Specifically 27%, 74%, 84%, and 90% reduction in run time out to 400 bases over gel for single capillary, 2 parallel capillaries, serpentine microfluidics, and spiral microfluidics respectively. Even more improvement may be realized through the use of an electro-osmotic counter flow to slow down fast moving, hardly resolved long DNA.

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AES-14

Degradation of Kappa-Casein In Aqueous Solutions by Pulsed Corona- Based High Oxidation Method: Further Studies

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One of the key physical parameters for successful degradation of humic acid, for the case of direct Pulsed Corona Discharges was determined to be the electrode-to-electrode gap. In this, we are texting this parameter in the degradation of kappa-casein and reporting results of its effects on degradation. Non-thermal high oxidation method, a novel oxidation technique based on localized electrical discharge has been used successfully to degrade high molecular weight organic compounds (dye solutions, humic acids and fulvic acids) before. In this research, a pulsed corona discharge was applied to reduce kappa-casein in aqueous solutions and the effects of pH, and frequency were investigated.

AES-15

Microfluidic Platforms for Solid Form Screening of Pharmaceuticals

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Screening of pharmaceutical parent compounds (PC) to identify solid forms with appropriate physicochemical properties is critical in drug development [1]. Traditional screening methods often use automated robotic systems that consume significant amounts of material, ~5 mg per condition, using methods such as microbatch, temperature control, antisolvent addition, and solvent evaporation [2]. Microfluidics has the potential to screen PCs using much smaller amounts of material, ~5 µg per condition. The availability of such technology would enable high throughput screening for suitable solid forms of PCs at a much earlier stage, when only very small amounts of PC (*e.g.*, ~10 mg) are available.

Here, we report microfluidic technology that enables the screening for solid forms (salts, cocrystals, polymorphs) of PCs via diffusional mixing, antisolvent addition, or evaporation, as well as subsequent on-chip characterization of the solid forms using Raman analysis.

Previously, microfluidic platforms for crystallization screening have been reported, for example to identify crystallization conditions of proteins via free interface diffusion (FID), and for polymorph screening via control over solvent evaporation rate [see *e.g.*, 3-5]. However, typically these microfluidic platforms are not compatible with a wide range of solvents and they do not allow for *in situ* characterization, aspects that drastically limit their applicability in pharmaceutical screening.

Here, we present microfluidic platforms that are compatible with a much broader range of solvents, allow for combinatorial mixing of PC and counterion (salt or cocrystal formers) solutions in arrays of 24 or 48 sub-microliter wells (90 to 200 nL/well), and allow for on-chip analysis of the solid forms formed, e.g. by Raman spectroscopy. Depending upon the desired method of crystallization (*e.g.*, FID, antisolvent addition, or evaporation), the microfluidic chip is comprised of different layers and well geometries. Incorporation of an X-ray transparent and solvent resistant material such as cyclic olefin copolymer (COC) and thiolene, and minimizing the thickness of PDMS layers to less than 150 µm drastically improves solvent compatibility, minimizes solvent absorption over long term evaporation experiments, and renders the chips compatible and Raman spectroscopy.

For chip validation, we screened for salts of tamoxifen and ephedrine using controlled solvent evaporation, we screened for salts of ephedrine and cocrystals of caffeine using diffusional mixing and we studied the effect of different drug concentrations and different drug/antisolvent ratios on polymorphism of indomethacin. On-chip Raman spectroscopy was used to identify different salts and cocrystals. This microfluidic technology is presently being evaluated by our collaborators at Abbott Laboratories for its potential to replace their existing, robotic solid form screening approach.

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AES-16

X-ray Compatible Microfluidic Platforms for Screening, Crystallization and *De Novo* Structure Determination of Proteins

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Protein crystallography is an important aspect of structural biology that provides information on the 3D structure of proteins and insight into their function. This intensive, multi-step process includes expression, purification, and crystallization of protein material, followed by harvesting of the crystals for X-ray analysis. Some of these steps have been improved recently, but major bottlenecks still exist in finding optimal crystallization conditions and in avoiding crystal damage while manually harvesting the oftentimes small, delicate crystals for X-ray analysis [1].

Here we present a microfluidic platform that allows for screening of (crystallization) conditions, together with on-chip X-ray analysis of the crystals formed. Not only do these chips eliminate crystal handling, they also allow for room temperature data collection from multiple small crystals, as opposed to collecting data from a single large crystal which requires cryocooling to avoid radiation damage. Using these chips, we have been able to fully resolve the structure of a novel bacterial lyase.

The microfluidic platform presented here retains the integrated fluid handling capabilities of traditional multi-layer devices necessary for high throughput crystallization, while achieving X-ray transparency, thus enabling *in situ* analysis. Our hybrid chip consists of three layers of materials: cyclic olefin copolymer (COC) for the control layer, a thin polydimethylsiloxane (PDMS) fluid layer for valve actuation, and a bottom substrate of COC / Duralar. The attenuation of X-rays by the assembled layers is minimal and does not affect diffraction data. While X-ray compatible microfluidic chips have been developed before, they have not been able to take advantage of the integrated fluid handling and high throughput capabilities of traditional multilayer PDMS chips [2-4].

We use 3x2-cm$_2$ array chips of 24 to 96 individually addressable wells, ~50 nL in volume each, to screen multiple crystallization conditions. Each screen uses less than 5µL of protein solution. Once a suitable condition has been identified, it is repeated in every well to obtain a large number of isomorphic crystals. We then mount the whole chip in the X-ray beam and collect diffraction data. By merging small wedges of data collected from each of these small crystals at room temperature, we can determine the structure of the protein, whereas traditionally a full dataset is collected from a single, large cryocooled crystal. ***We fully validated this methodology by determining the structure of a novel bacterial lyase PhNA to 2.07 Å resolution*** using singlewavelength anomalous diffraction phasing methods that make use of data collected entirely on-chip at room temperature. Our approach bypasses issues associated with the need to grow large, high quality crystals, crystal damage during handling, cryocooling, and radiation exposure and enables data collection at biologically relevant temperatures. ***This is the first example of an entirely hands-free screening, crystallization, and de novo structure determination of proteins within a microfluidic chip.***

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AES-17

Quantification of pH Gradients and Implications in Insulator-Based Dielectrophoresis of Biomolecules

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Insulator-based direct current (DC) dielectrophoretic (iDEP) devices can potentially replace traditional alternating current (AC) dielectrophoretic devices for many cellular and biomolecular separation applications. iDEP devices employ large DC fields, where electrode reactions and electrokinetic ion transport mechanisms become significant enough to affect ion distributions in the nanoliters of fluid in the microdevice. Most commonly encountered of these electrode reactions is the electrolysis of water. With most electrode materials, when an electric field is applied, H+ and OH- ions are generated at anode and cathode surfaces, respectively. These ions have high diffusional and electrophoretic mobilities. Therefore, their diffusional and electrokinetic transport can result in H+ and OH- concentration gradients within the microfluidic channel. This leads to what is termed a "natural pH gradient," and can be exploited for applications such as isoelectric focusing (IEF). However, natural pH gradients can also cause unexpected fluid behavior in micro- or nanofluidic systems by causing spatial changes, such as nonuniform wall surface charges. In addition, analyte properties may depend on pH, and the formation of natural pH gradients may not be desirable.

This work shows natural pH gradient formation in an iDEP microdevice with Pt wire electrodes, under conditions applied during iDEP protein manipulation experiments. pH changes were quantified by measuring the fluorescence intensities of pH sensitive dye FITC Isomer I and the pH insensitive dye TRITC and correlating the FITC/TRITC fluorescence intensity ratio to pH. A dependence of natural pH gradient formation on the phosphate buffer solution concentration was observed under 100 V/cm electric fields. When the channel was filled with relatively low concentration solutions (Conductivity: s=0.01S/m or 0.05 S/m), pH was found to drop below 4 in 5 to 10 minutes. However, this behavior was more consistent in the case of 0.01 S/m solution. pH gradient formation was not observed in the case of a relatively high concentration solution (s=0.10 S/m), due to the higher buffering capacity of the solution. pH was observed to drop dramatically in <1 min under 3000 V/cm electric fields with all buffer solutions. It was also observed that when the Pt wire electrodes had been in use for prolonged times, pH gradient formation did not occur under 100 V/cm electric fields, even with the 0.01 S/m buffer solution. However, 3000 V/cm electric fields still caused rapid pH changes in the microchannels when the used Pt wire electrodes were employed, regardless of the solution concentration.

This work shows that pH gradients can form in iDEP devices, possibly affecting the operation of such devices. It is shown that pH gradient formation is influenced by electric field strength, buffering capacity of the medium, and the properties of the electrode surface. Based on this work, pH gradient formation can be prevented by using solutions with high buffering capacity, by passivating the electrodes prior to use, or by using electrode materials on which water electrolysis reactions do not occur.

AES-18

Changes in Cross-Over Frequency (COF) Based on ABO-Rh Antigen Expression

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Human blood samples of known blood type were suspended in a 0.9S/m dextrose buffer and subjected to an alternating current dielectrophoretic field in a quadrupole device. Previous work has shown that the expression of ABO-Rh antigens on the erythrocyte surface will change their dielectrophoretic signature at 1MHz. This work aims to determine the cross-over frequency as well as the regions of nDEP and pDEP based on ABO-Rh blood type and directly compare these results to those of β-galactosidase modified blood cells. The enzyme β(1-3)-galactosidase was used to cleave the ABO antigens at their galactose bonds and thereby create a uniform surface antigen expression. This enzymatic reaction was optimized by agglutination results as well as UV-Vis and HPLC testing of the reaction effluent. The dielectrophoretic signature of erythrocytes were tested in a quadrupole device from 100kHz to 80MHz. The lower cross-over frequency for the blood types tested (A+, A-, B+, B-, AB+, O+ and O-) varied between 30.5MHz and 47.5MHz (range of 17MHz). The corresponding lower cross-over frequency for the enzymatically modified blood types varied between 36.5MHz and 41.5MHz (range of 5MHz). A higher cross-over frequency was sometimes observed in the region of 70-80MHz and was reduced by the presence of the Rhesus factor. These results suggest that the antigen expression influences the cell's ability to polarize in alternating current field and that the lower COF is controlled by the ABO surface antigen expression and the higher COF is controlled by the transmembrane Rhesus factor.

AES-19

A Multiplexed Microfluidic Platform Utilizing Optimized Normally Closed Valves for Investigating Microbial Gene Expression

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The development of integrated microfluidic platforms that can sustain live samples (*i.e.* cells) for prolonged periods of time while also allowing for cell growth in enclosed compartments has been a challenge [1]. The need for continuous actuation of the typical normally-open pneumatic valves in these chips is particularly cumbersome.

Here, we report the development and utilization of microfluidic platforms that utilize arrays of normally closed (NC) microvalves. These chips allow for quantitative biological measurements, including microbial gene expression and stimulus-driven response in living cells, as presented here.

Microfluidic array chips with NC valves have several advantages, for example, in applications where portability is highly desired and prolonged on-chip analysis in constrained analytical chambers is needed. To extensively increase the advantages of the NC microvalves for biological studies, we studied the optimization of these valves with respect to their integration and use in complex microfluidic array chips. We developed a set of design rules, which allowed us to assemble a novel microfluidic platform that incorporates these valves to compartmentalize ~50-pL cell growth chambers. With respect to NC valve optimization, we identified appropriate dimensions for reliable operation at minimum actuation pressures. These new chip configurations based on NC valves minimize the loss of solvent, while drastically easing portability because pneumatic lines can be removed after filling. These features enable quantitative biological studies for several hours to be performed on-chip in a high throughput and combinatorial fashion. The NC valves also facilitate the periodic supply of nutrient media, as well as the exposure of the cells in to different chemical stimuli loaded in adjacent compartments. *The latter two features set our work drastically apart from alternative approaches reported by others that typically require continuous flow* [2,3].

We used this new microfluidic array chip to investigate microbial gene expression, and we are extending these studies to the single cell level. First, we monitored the controlled growth of *E. coli* cells tagged with a green fluorescent protein (GFP) reporter in a microchamber. The observed growth dynamics within the platform are comparable to growth measurements performed at the macro-scale. Next, we used this chip and the observed growth dynamics to study the phenomenon of bistability in gene expression in the bacterial lactose metabolism network [4]. At certain concentrations (<3 μMol or >30 μMol) of lactose analog, which acts as a chemical trigger, bistability is manifested as a homogenous

population of "on" (induced) and "off" (uninduced) cells in the microchamber. Presently, we are extending these experiments to elucidate multi-stable gene expression profiles that are associated with bacterial responses to diverse metabolites. This would open up the use of this platform to explore the effects of antibiotics, hormones, and toxins on cell growth/death.

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