

Medical Electronics

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High-throughput Arrayed Comets for DNA Damage and Repair

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Sponsorship: National Institute of Environmental and Health Sciences

DNA damage has long been known to contribute to cancer, aging, neurological disorders, and heritable diseases. Although effective methods for assessing DNA damage levels have been available for decades, and it is well established that information about DNA damage levels is highly useful both in the clinic and in population studies, measurements of DNA damage are far from routine. The core experimental method for this work is the well-established comet assay, in which cells are embedded in agarose, exposed to buffers that dissolve the cell membrane and denature DNA, and subjected to electrophoresis. The governing principle is that damaged DNA migrates more readily than undamaged DNA, which tends to stay tightly wound within the nucleoid.

A major goal was to increase throughput by using patterning techniques to enable analysis of cells within a defined array. We optimized a patterning method that captures cells in microwells [1], [2] that are molded directly into agarose gel (Figure 1). This method enables capture of single or multiple cells, controlled by well size, and produces analyzable comets from any amount number of cells (Figure 1). The tunable well size and the biocompatibility of agarose also enable studies on any cell type. This platform has proved effective for studying multiple irradiation and chemical exposures on a single slide. Figure 2 demonstrates X-ray dose response studies, where 10 doses were measured on a single slide, and 3 different cell types were studied. We have further improved throughput with automated image analysis software and are using fluorescent labeling to multiplex conditions, cell types, and biomarkers. We will continue to increase the versatility and through-put with automated chemical spotting. We hope ultimately to provide an assay that is simple and easy enough to be useful in a broad range of clinical, epidemiological, and experimental gene-environment interaction studies.

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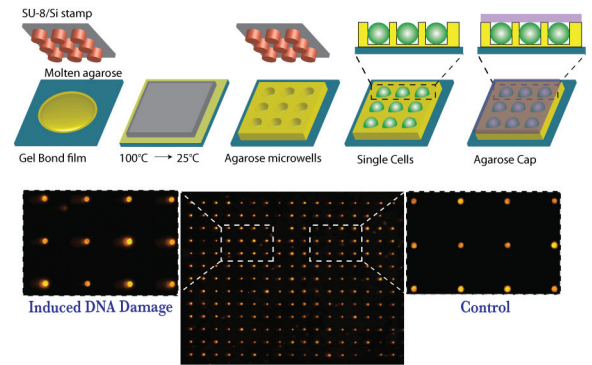


FIGURE 1: Fabrication of the comet-chip. Microwells are molded directly into agarose gel using a lithographically patterned Si/SU-8 stamp. Cells are loaded into the wells by gravitational settling, and an agarose capping layer encapsulates the cells. Lower panels show patterned comets from an experiment where cells are treated with PBS or H₂O₂.

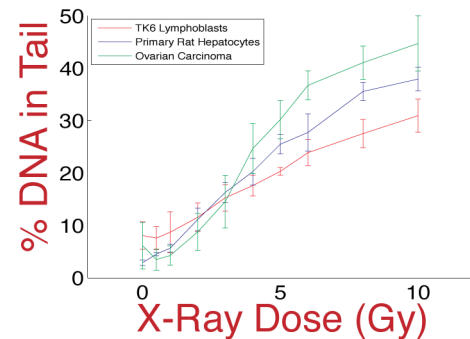


FIGURE 2: Dose response of cells treated with X-ray radiation. Colored lines represent different cell types. Ten X-ray doses were applied to each comet-chip.

Development of Modeling Tools for the Human Cardiovascular Circulatory System

T. A. El-Moselhy, L. Daniel
Sponsorship: NSF

Studying certain medical conditions, such as hypertension, requires the simulation of the whole cardiovascular system, including arteries, veins, and various organs treated as vascular beds. Such a requirement renders full 2- or 3-D coupled fluid-structural simulations impractical due to their high demand in computational resources. The computational efficiency of lower dimensional models comes at a cost of sacrificing correctness. For instance, 1-D artery models cannot accurately model bends and bifurcations in the major arteries that significantly alter fluid flow, and 0-D vascular bed models cannot provide spatial information. One way to overcome the computational complexities associated with high dimensional models without sacrificing the accuracy is to develop techniques that generate automatically reduced order models from fluid-structural simulators for complex arterial segments, such as bends and bifurcations, to faithfully reproduce the flow and pressure at each end of such segments.

As a first step toward generating reduced order models for arterial segments, we developed a 2-D fluid-structure interaction solver to accurately simulate blood flow in arteries with bends and bifurcations. Such blood flow is mathematically modeled using the incompressible Navier-Stokes equations. The arterial wall is modeled using a linear elasticity model [1]. Our solver is based on the immersed boundary method (IBM) [2]. The numerical accuracy of our solver stems from using a staggered grid for the spatial discretization of the incompressible Navier-Stokes equations [3]. The computational efficiency of our method stems from using Chorin's projection method for the time stepping, coupled with the fast Fourier transform (FFT) to compute the pressure [2]. We have validated our results versus reference results obtained from MERCK Research Laboratories for a straight vessel of length 10cm and diameter 2cm. Our results for pressure, flow, and radius variations are within 3% of those obtained from MERCK.

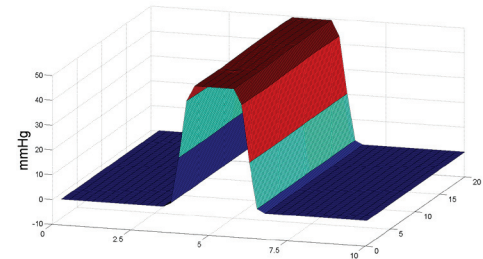


FIGURE 1: Pressure build-up inside artery at time 0.165 sec. Almost uniform pressure observed inside straight artery and almost zero pressure observed outside the artery.

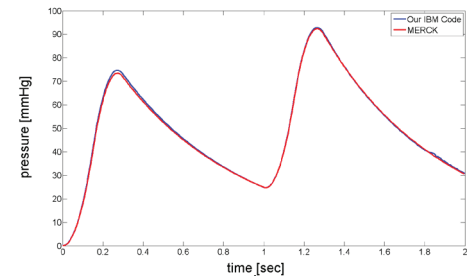


FIGURE 2: Pressure at the middle of a straight 10cm artery as a function of time. Simulations performed using our immersed boundary method code and compared to reference results obtained from MERCK.

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Electrokinetic Accumulation of Enzymatic Product Enhances Detection Sensitivity in Microfluidic ELISA

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Sponsorship: Agency for Science, Technology and Research, Singapore

We present a method to enhance detection sensitivity of microfluidic bead-based ELISA by accumulating fluorescent product molecules using nanofluidic electrokinetic preconcentration. Bead-based ELISA integrated on a microchip has been shown to provide a lower detection limit and require a shorter assay time [1], but signal intensity does not increase with time as the reaction product is continuously flushed away. We circumvent this limit by utilizing a nanofluidic electrokinetic preconcentrator to accumulate fluorescent product molecules downstream of the reaction zone and show that signal intensity increases with time, therefore greatly improving the detection sensitivity.

The experimental procedure is explained below. The sandwich immunoassay consisting of antibody-coated beads, carbohydrate antigen CA19-9 in serum, and HRP-coupled secondary antibody is conducted in a microcentrifuge tube. After extensive washing, the functionalized beads are physically trapped in front of pillar structures within the microchannel. Downstream of the self-assembled bead array is a nanochannel fabricated

using the method in [3]. When voltages are applied at the reservoirs, electroosmosis induces flow of the substrate solution across the beads. Fluorescent product is continuously generated as the enzymes in the bead array catalyze the reaction between the substrate molecules. At the vicinity of the nanochannel, electrokinetic trapping lead to accumulation of the charged fluorescent product molecules and clear increase in signal intensity.

The results are shown in Figure 1. When the antigen concentration is 0 U/mL, the intensity ratio between the product and fluorescein tracer is 1:3. This ratio becomes 1:2 and 2:1 when the antigen concentration is increased to 0.05 U/mL and 0.5 U/mL respectively. Without preconcentrating product molecules, the detection limit of bead-based micro ELISA is 5 U/mL. Therefore, this method enables us to detect at least 100X fewer enzyme molecules and increase the dynamic range by 2 orders of magnitude.

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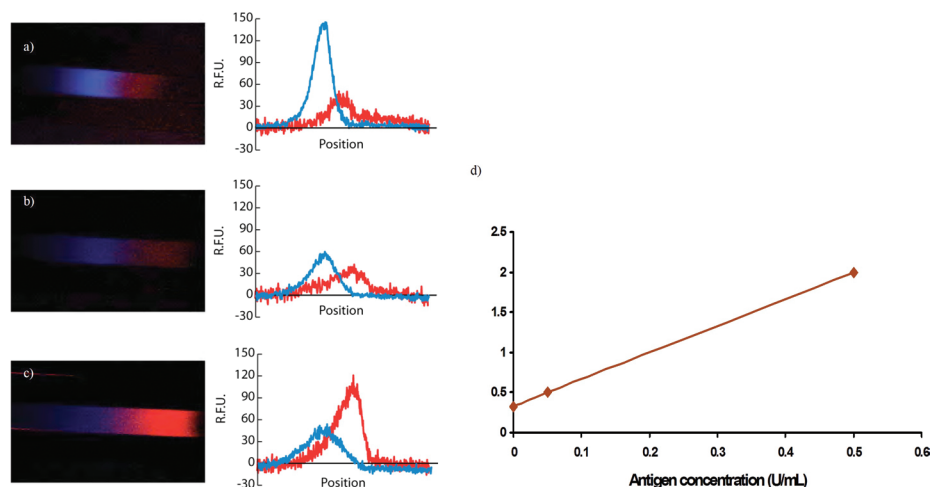


FIGURE 1: Experimental results: Fluorescence intensity of product molecules (red) and tracer (blue) for antigen concentration a) 0 U/mL b) 0.05U/mL c) 0.5 U/mL and d) linear relationship between intensity ratio and antigen concentration.

Microfluidic-based Preparative Protein Separation by Free-flow Isoelectric Focusing (FF-IEF)

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Sponsorship: Army Research Office - ISN, Koch Center for Cancer Research, Department of Biology

Isoelectric Focusing (IEF) is the first step for 2-D gel electrophoresis and plays an important role in sample purification for proteomics research. Microfluidic-based IEF has been developed recently in the analytical scale but is less feasible for protein samples with milliliters in volume and milligrams in mass. In this work we have overcome these limitations of traditional IEF with a novel microfluidic free flow IEF (FF-IEF) device in a preparative scale for continuous protein separation in the liquid phase. The 5- by 7-cm device with 24 outlets was fabricated by soft lithography through a PDMS/glass feature (Figure 1). Novel design of the triangular-shape separation channel facilitates the establishment of the pH gradient with not only a corresponding increase in separation efficiency but also a decrease in focusing time. Multiple outlets of 24 fractions facilitated the sample collection and solution handling. We showed the reproducible establishment of the pH gradient from 10 to 4 in a linear fashion.

The electric field and potential efficiency across the separation channel were optimized with consideration of Joule-heating removal. After the shaping of the PDMS prior to the device binding, a functionalized polyacrylamide gel region at the bottom of the device was selectively controlled to adjust the ratio of the applied potential across the separation channel. Energy

consumption across the functionalized polyacrylamide gel was investigated and selectively controlled to adjust the potential efficiency between 15-80% across the triangular separation channel. The length of the polyacrylamide gel was found to have a critical effect on the electric fields and could block as much as 99% of applied voltage. The device can achieve constant electric fields as high as 370 ± 20 V/cm through the entire triangular channel given the separation voltage of 1800 V, enabling a powerful micro-device for different separation environments.

Protein samples with a dynamic concentration range between $\mu\text{g/mL}$ and mg/mL could be loaded into the micro device at a flow rate of 1 mL/hr and residence time of ~ 12 min. We have demonstrated the improved separation using the FF-IEF system over the traditional 2D gel electrophoresis on a protein complex of 9 proteins and 13 isoforms. Post-device sample concentrations result in a 10-20-fold increase, which allows for isolation and detection of low-abundance proteins. This preparative micro-device would also benefit proteomics research by retaining high molecular weight proteins, providing a higher yield of protein that has a broader range in pI, and saving 98% of the experimental time compared to a conventional IEF IGP gel strip (30 min instead of 23-36 hrs).

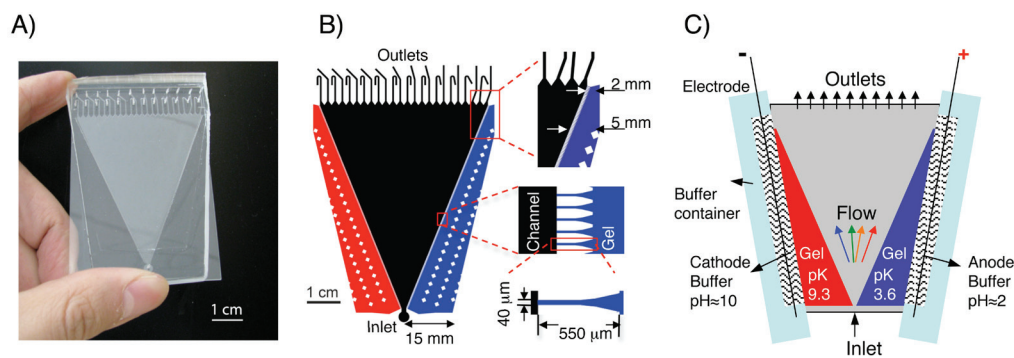


FIGURE 1: A) A FF-IEF device. B) The FF-IEF device design. C) Schematic drawing of microfluidic FF-IEF system. A functionalized pH gradient cathode (pK=9.3) and anode (pK=3.6) polyacrylamide gels were polymerized into the gel regions that were connected to the separation channel. Polyacrylamide gel sections both have 5mm in width on the top from the first post to the side and 15 mm on the bottom from the inlet to the side. A poster structure (squares) was included in the gel regions for support. The device is 5 cm by 7.5 cm with the center triangular separation channel of 4.6 cm in top width and 5.6 cm in height. All channels and channels have a depth of 160 μm .

Microfluidic Studies of Cancer Invasion

I. K. Zervantonakis, S. Chung, W. J. Polacheck, R. D. Kamm
Sponsorship: Draper Laboratory

Cell invasion is a central component of cancer metastasis, enabling tumor cells to escape from the primary organ and form metastases in distant sites, which are the cause for 90% of cancer deaths. The importance of the tumor microenvironment, including biochemical and biophysical factors, is well recognized in the metastatic cascade as an important regulator of the invasive phenotype. Microfluidic devices offer a unique tool for designing and performing *in vitro* assays that allow for control of these microenvironment cues while enabling high-quality imaging of cellular behavior. Our microfluidic device design for the cancer invasion assays is based upon previous work over the past years in our lab [1]-[3]. The design consists of independently addressable microfluidic channels interconnected through 3D matrices, wherein tumor cells can be seeded, while establishing interstitial flow and/or chemoattractant gradients through the matrix (Figure 1).

During the previous year we have used breast cancer (MDA231) and human glioblastoma (U87MG) cell lines in collagen type I matrices to perform chemotaxis and interstitial flow assays. We found that in the presence of an epidermal growth factor (EGF) gradient, both

cancer cell lines extended long protrusions and migrated preferentially in the direction of the gradient. However, although both cell types demonstrated 3D cell motility, multiple individual U87MG cells invaded the gel, while the MDA231 showed a more collective cell-migration strategy (Figure 2). In the interstitial flow experiments, we embedded MDA231 cells inside the 3D matrices and assayed for their alignment to the flow streamlines. The results demonstrated that a slow interstitial flow of $3\mu\text{m/s}$, comparable to flow velocities in tumors *in vivo*, resulted in alignment of the tumor cells that extended long protrusions along the streamlines, contrary to the control, where the cells were randomly orientated in the gel. Both results demonstrate that chemoattractant gradients and interstitial flow are critical regulators of tumor invasiveness and have important implications for the dissemination of tumor cells from the primary site. Ongoing work includes quantification of tumor cell invasion using time-lapse confocal microscopy for tracking single cells and measuring their migration characteristics. Furthermore, we are performing assays for probing tumor-endothelial cell interactions in the context of cancer angiogenesis and intravasation.

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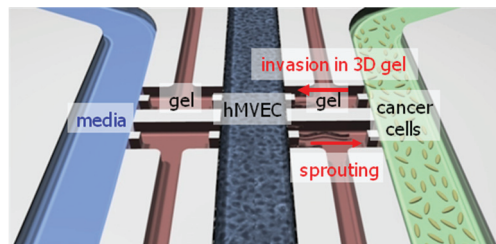


FIGURE 1: Microfluidic device design for studying cancer invasion and tumor-endothelial cell interactions. Cells are seeded in independently addressable channels, interconnected through 3D matrices across which chemoattractant gradients and interstitial flow can be established.

Immunostaining (hMVEC:green, MDA231: red, DAPI:blue)

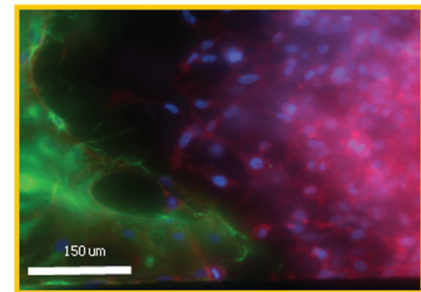


FIGURE 2: Immunostaining of tumor (red) and endothelial (green) cell nuclei (blue) inside the device. Cells are in direct contact, with a few cancer cells migrating individually at the front and an expanding tumor mass at the back

Integrated Microfluidics for Screening Stem Cell Microenvironments

W. G. Lee, W. Y. Sim, B. Chung, A. Khademhosseini
Sponsorship: NIH

Embryonic stem cell (ESC) differentiation is a potentially powerful approach for generating a renewable source of cells for tissue engineering and regenerative medicine [1]. It is known that the microenvironment greatly influences ESC differentiation and self-renewal. Most biological studies have aimed at identifying individual molecules and signals. However, it is becoming increasingly accepted that the wide array of signals in the ESC microenvironment can interact in a synergistic and antagonistic manner based on their temporal and spatial expression, dosage, and specific combinations. This interplay of microenvironmental factors regulates the fate of ESC in terms of decisions to proliferate, self-renew, differentiate, and migrate. Despite this complexity, the study of stem cell cues in a systematic manner is technologically challenging, expensive, slow, and labor intensive. Here we propose to develop an enabling technology based on a high-throughput integrated microfluidics that can overcome many of these challenges. To test the proposed system, we will characterize the integrated device in elucidating specific aspects of mesodermal differentiation in a systematic manner. By providing a way of testing combinatorial microenvironments for directing stem cell differentiation, this approach promises to be of great benefit for cardiac cell and tissue engineering.

To demonstrate the feasibility of the device, we first visualized the functional elements of the device such as fluidic channels, control channels, and microwells (Figure 1). This device was fabricated by multi-layer soft lithography. The fluidic channel made by positive photoresist (AZ 4620) is 30- μm -thick with a round shape, and the pneumatically actuated control channel fabricated by negative photoresist (SU-8 2150) is 40 μm thick. To obtain a round profile of a fluidic channel, the positive photoresist (AZ 4620) was reflowed at 200 $^{\circ}\text{C}$ for 2 min after development. Specifically, the middle layer contained a 20- μm thin RTV615 membrane for the fluidic channel. The crossing of control channels over fluidic channels formed an on-chip barrier valve.

To characterize the cell culture environment for the device, we seeded mouse ESCs into the fluidic channel of the device, docked into specific microwells, and then cultured EBs for 5 days (Figure 2). In our experiments, we cultured gooseoid (Gsc)-ESCs, which can show green fluorescent protein (GFP) for mesendodermal stages of the EBs. Note that no GFP indicates undifferentiated cells or ectoderm stage of the EBs. In summary, Gsc-ESCs were well-docked into the microwells, resulting in good EB formation. After a 5-day culture, GFP images were obtained from the EBs within the microwells.

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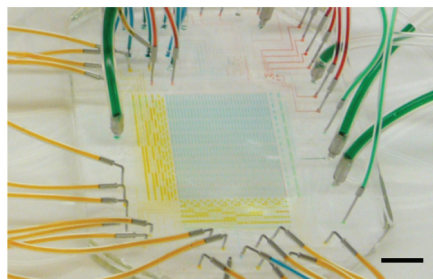


FIGURE 1: Visualization and compartmentalization of functional elements of the microfluidic screening device. Note fluidic pathways (green), multiplexed valves (yellow), vertical compartments and mixers (red), and horizontal compartments (blue). Scale bar is 10 mm.

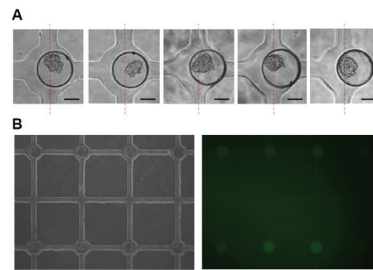


FIGURE 2: Characterization of EB formation of Gsc-ESCs and 5-day culture of the EBs within the microwells of the microfluidic screening device. Note that the GFP (green color) indicates mesendodermal differentiation of the EBs. (A) Observation of EB formation (1 day after cell loading). (B) Phase contrast and fluorescent images of 5-day cultured EBs. Magnification of the objective lens is 10 X. Scale bar is 100 μm .

A Micro Cell chip for Studying Effects of Mechanical Stimulation on the Fate of Mouse Embryonic Stem Cells

W. Y. Sim, W. G. Lee, A. Khademhosseini
Sponsorship: NIH

Regenerative medicine and tissue engineering are potentially important technologies in creating transplantable tissues for health and medical care. In particular, bone and cartilage regeneration by using embryonic stem cells (ESCs) is an area of great interest for improving the quality of life.

Recently, physical stimuli such as compression, shear stress, strain, stretch, and hydraulic force have been shown to play important roles in the chondrogenesis and osteogenesis of stem cells [1]. Mechanical stimuli enhance the differentiation of stem cells and improve the mechanical properties of tissue. It is important to understand how cells respond to variations in mechanical forces. Since mechanotransduction mechanisms are still not well understood, there is a great demand for developing new technology that enables precise control of the cellular microenvironment.

Here we present a micro cell chip integrated with various sizes of microwells for the study of effects of mechanical stimulation on the fate of mouse embryonic stem cells (mESCs). This cell chip is designed not only to culture mESCs loaded into variously sized microwells (diameters: 150-, 200-, 300-, 400-, 500- μ m), but also to apply dynamic compressive stimulation for chondrogenesis and osteogenesis of mESCs. This device is based on the pneumatic actuator with a flexible diaphragm. It consists of one air chamber, twelve cell chambers, and a microvalve system; it is fabricated by multi-layer soft lithography (Figure 1). Specifically, it consists of seven layers of RTV615 material and laser-machined

poly(methyl methacrylate) (PMMA) substrates. The device is initially filled with a solution containing mESCs and embryoid body (EB) culture medium by a syringe pump. After confirmation of the cell aggregation in each microwell (Figure 2), mESCs are exposed to cyclic compressive stimulus (frequency 1 Hz, pressure 5 kPa) for 10 minutes every 12 hours for 7 days. During the mechanical stimulation, the inlet and outlet of each cell chamber is closed by an integrated microvalve system to prevent an undesired shear stress on the cell chamber.

To evaluate the differentiation of mESCs under mechanical stimuli, we are currently conducting cell viability tests and immunocytochemistry on chondrogenesis and osteogenesis. This micro cell chip may provide a convenient and effective tool for directing the differentiation of ESCs into bone or cartilage.

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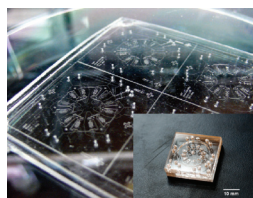


FIGURE 1: Photograph of a micro cell chip integrated with various sizes of microwells and microvalve system for the study of mechanical stimulation effect on the mESC fate. Three RTV 615 layers (microwells + microfluidic chambers and channels + microvalve). (Inset) Photograph of the fabricated device after oxygen plasma bonding with RTV 615 and PMMA layers. Scale bar is 10 μ m.

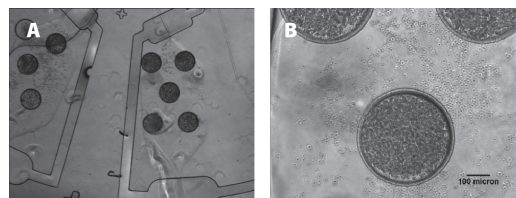


FIGURE 2: Phase contrast images of the EB formation after cell loading in 300 μ m-diameter microwells. (A) Microvalve is located on the inlet and outlet of cell chambers. (B) Closed-up image of a microwells. Scale bar is 100 μ m.

Measurement of Mass, Density, and Volume of Yeast through the Cell Cycle

A. K. Bryan, A. Goranov, A. Amon, S. R. Manalis
Sponsorship: NIH

Differences in cell density (mass per unit volume) have traditionally been used for cell synchronization and purification, but advances in density measurements may be used to investigate how cell size, both mass and volume, are regulated during cell growth and division [1]. As shown in Figures 1 and 2, we have validated two methods for measuring cell density and observing density changes in real time. In the first method, cell density is measured by combining single-cell mass and volume data from the suspended microchannel resonator (SMR) and Coulter counter to calculate cell density. The second method measures density changes in living cells by comparing cell density to fluid density as each cell transits the resonator. We used these techniques in *Saccharomyces cerevisiae* to identify an energy-dependent G1/S density change that requires START, TOR complex 1 function, and actin dynamics. In conjunction with these density measurements, FACS and bud emergence data suggest that bud formation may be required for this density shift. These density measurement techniques are applicable to most non-adherent cells and subcellular particles, and they offer cell-friendly and time-resolvable methods that extend beyond cell-cycle studies.

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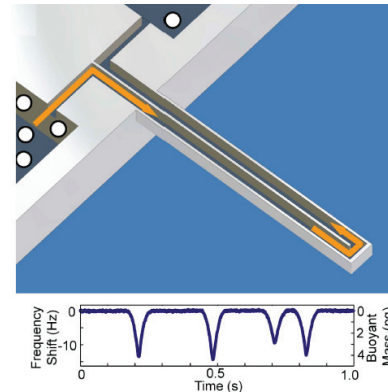


FIGURE 1: Illustration of instrument and cell measurement. Cells flowing through the cantilever displace a volume of fluid equal to their own volume and change the cantilever's resonant frequency proportional to this change in cantilever mass. The flow rates are controlled by pressure. The frequency shift as cells flow through the microchannel is dependent on the position of the cell along the cantilever and the buoyant mass of the cell. The absolute maximum frequency shift for a cell (four of which are shown) occurs at the cantilever tip and this is proportional to the cell's buoyant mass. The system returns to a stable baseline upon each cell's exit from the microchannel.

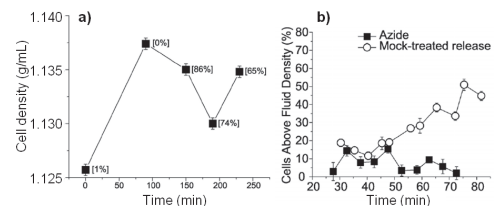


FIGURE 2: a) Density of formaldehyde-fixed cell populations synchronized by centrifugal elutriation. Bud counts are reported as percent budded in brackets next to each measurement. Cells begin to enter S-phase between 60 and 120 min. Error bars are the standard deviation for the technique as measured with NIST particle standards. b) Real-time single-cell relative density measurement. Cell state, distinguished by cell density, is determined by the direction of frequency shift. For a fluid density near that of the cells, G1 cells have a negative buoyant mass (positive frequency shift), and cells entering S-phase at a later timepoint have a positive buoyant mass (negative frequency shift). The proportion of cells in each state is directly correlated to the percent of cells below or above fluid density, and the change of cell state was recorded as it occurred for samples released from a G1 arrest.

Microscale Continuous Cell Culture

K. S. Lee, R. J. Ram
Sponsorship: NSF

For systems biology, the models are more often limited by the absence of experimental data than by available computational resources. Unfortunately, there is still great difficulty in making the leap from genetic and biochemical analysis to accurate verification with conventional culture growth experiments due to variations in culture conditions. Measurements of metabolic activity through substrate and product interactions or cellular activity through fluorescent interactions are generally highly dependent on environmental conditions and cellular metabolic states. For such experiments to be feasible, continuous cultures [1], [2] utilizing control strategies must be developed to measure relevant chemical concentrations, introduce chemical inputs, and remove waste. An integrated microreactor system with built-in input fluid metering for environmental control will enable controlled and programmed experiments capable of generating reproducible data.

The design of a microchemostat for providing environmental control is given in Figure 1. The chip is fabricated out of polycarbonate, utilizing PDMS

membranes for actuation and pumping [1]. Mixing and oxygen delivery are performed through actuation of the membranes situated between the fluidic and actuation layers of the growth chamber sections. Initial growth results are shown in Figure 2. Culture experiments are performed with *E. coli* FB 21591 in 1 g/L glucose and 100 mM MES defined medium supplemented with 100 ug/ml kanamycin. Cell density is measured with an optical sensor set up to measure forward scattering through the growth chamber at 640 nm in a path length of 2 mm. The system is first run in batch culture to demonstrate cell viability within the reactor and then diluted and run in continuous mode with a dilution rate of 0.158 hr^{-1} . After continuous culture, the system is run in batch again to demonstrate cell viability and a reduced growth rate induced by continuous operation.

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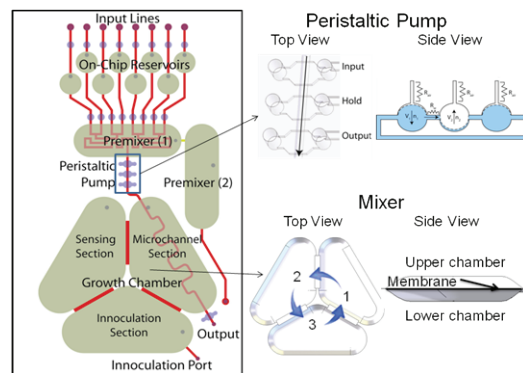


FIGURE 1: Schematic and picture of the microchemostat device. Eight inputs lines provide control over the chemical composition of the medium within the growth reservoir. Fluids are introduced through a peristaltic pump that injects liquid into the growth chamber. Fluids are first premixed in the premixer to reduce chemical gradients between the growth chamber and input fluids.

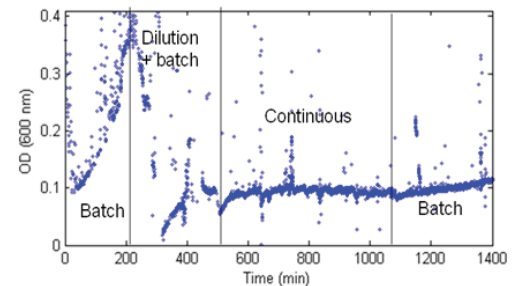


FIGURE 2: Plot of the cell density over time for a growth on a defined glucose medium. The chip is run in two modes; first batch growth, followed by continuous mode at a dilution rate of 0.158 hr^{-1} , and finally at batch growth again to demonstrate cell viability after continuous operation.