

Receptor expression changes as a basis for endothelial cell identification using microfluidic channels†

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Microfluidic channels functionalized with adhesive ligands are versatile platforms for cell separation in a variety of applications. However, not much is known about how the adhesiveness of targeted cell types can vary within such channels due to the combined influence of fluid shear forces and exposure to ligands. Using microfluidic channels and the tetrapeptide ligand arg-glu-asp-val (REDV), we demonstrate how such dynamic changes can provide a basis for the identification of three distinct phenotypes of endothelial cells: human umbilical vein endothelial cells (HUVECs), human microvascular endothelial cells (HMVECs), and endothelial colony forming cells (ECFCs). This distinction is accomplished by characterizing changes in the adhesion profiles of the three cell types in REDV-coated microfluidic channels induced by soluble REDV and fluid shear forces. The significance of this technique lies in the ability to distinguish very similar cell-types without fluorescent label-based staining or flow cytometry.

Introduction

Adhesion-based microfluidic cell separation techniques have been utilized for a wide range of applications, from cancer diagnostics to tissue engineering.^{1–4} This separation approach typically involves the functionalization of a microfluidic channel with molecules that bind to one or more cell types and the capture of cells from a flowing stream. Recent work by various groups has demonstrated the versatility of this approach in terms of cell types targeted^{1,4–7} and design of functionalized capture surfaces^{8–11} (e.g. ‘capture and release’ coatings¹²). A major advantage of the adhesion-based separation approach, particularly from the standpoint of clinical applications, is label-free operation.¹ Traditional cell separation approaches, such as fluorescence- and magnet-activated cell sorting (FACS and MACS), by contrast, require pre-processing through labelling of sample cell suspensions with fluorescent or magnetic tags.

The efficacy of adhesion-based microfluidic cell separation is governed by two factors: the affinity of cells to the ligands utilized for capture (typically antibodies or peptides) and the magnitude of fluid shear forces.¹³ The design of adhesion-based microfluidic cell separation systems is generally carried out with the implicit assumption that cells are quiescent during the separation process. In other words, it is assumed that no changes occur within the cells that would substantially affect affinity (due to receptor up/down-regulation, for example). While this assumption probably applies to the majority of applications where adhesion-based microfluidic separation has been utilized, not enough is known about how such changes might occur, particularly with respect to sensitive cells such as stem/progenitor cells and cells known to respond to shear forces. When the

capture of such cells is the objective, experiments must be carried out to ensure that changes in adhesiveness of the target cells during the separation process are well-characterized *a priori*. On the other hand, such dynamic changes, when properly characterized, may have applications of their own.

We describe a cell-identification approach for endothelial cell subpopulations that is label-free and takes advantage of unique responses of cell receptors to microfluidic shear forces. The cell types examined in the present study are human umbilical vein endothelial cells (HUVECs), adult human microvascular endothelial cells (HMVECs), endothelial colony forming cells (ECFCs) extracted from human umbilical cord blood, and normal human dermal fibroblasts (NHDFs). The conventional method of EC characterization is by fluorescent labeling of surface markers^{14–17} and analysis by flow cytometry or microscopy.^{18,19}

Materials and methods

Materials

Ethanol (200 proof), cover slips (35 × 60 mm, no. 1), microcentrifuge tubes, and cell culture flasks were purchased from Fisher Scientific (Fair Lawn, NJ). 3-Mercaptopropyl trimethoxysilane was obtained from Gelest Inc. (Morrisville, PA) and the coupling agent GMBS (*N*-γ-maleimidobutyryloxy succinimide ester) was obtained from Pierce Biotechnology (Rockford, IL). SU-8-50 photoresist and developer were obtained from MicroChem (Newton, MA); silicone elastomer (Sylgard 184) and curing agent were obtained from Dow Corning (Midland, MI). Phosphate buffered saline (PBS; 1×, without calcium or magnesium) was purchased from Mediatech (Herndon, VA). HUVECs, HMVECs, ECFCs, NHDFs, endothelial basal medium-2 (EBM-2), fibroblast cell basal (FCB) medium, singlequot kit supplements and growth factors, HEPES buffered saline solution, trypsin neutralizing solution, and 0.25% trypsin/EDTA solution were purchased from Lonza (Walkersville, MD).

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Blocking monoclonal antibody against the α_4 integrin (HP2/1) was purchased from Abcam (Cambridge, MA). Blocking monoclonal antibody against the β_1 integrin (AIB2) was purchased from the Developmental Studies Hybridoma Bank at the University of Iowa (Iowa City, IA). The peptide arg-glu-aspartic acid (REDV), along with REDV conjugated to fluorescein isothiocyanate (FITC) were purchased from American Peptide (Sunnyvale, CA). Quantum FITC-5 MESF kits were purchased from Bangs Laboratory (Fishers, IN).

Device fabrication

The design of the microfluidic device followed previously described soft lithography techniques.^{20,21} Negative masters for device fabrication were manufactured at the George J. Kostas Nanoscale Technology and Manufacturing Research Center at Northeastern University. For master fabrication, 2-dimensional projections of each device type were drawn using AutoCAD printed at high resolution on transparencies (FineLine Imaging, Colorado Springs, CO). The photomask was then used to generate a negative master. A silicon wafer was coated with SU-8-50 photoresist to a thickness of approximately 70 μm and exposed to ultraviolet light (365 nm, 11 mW cm^{-2}) with the transparency overlaid using a Quintel 2001 mask aligner. Following curing, the unexposed photoresist was removed using SU-8 developer, and the feature height verified using a Dektak surface profiler (Veeco Instruments, Santa Barbara, CA). The first of two microfluidic devices used in this study has a geometry that ensures linear variation of shear stress along its length. The design of this device stems from the theory of Hele-Shaw flow and design equations derived by Usami *et al.*²² The second type of device is a straight channel device with dimensions of 1 mm \times 50 mm \times 0.07 mm (width \times length \times height).

Polydimethylsiloxane (PDMS) replicas were generated using silicone elastomer and curing agents in the ratio of 10 : 1 (w/w). This mixture was poured onto the negative master and allowed to degas, then cured at 65 $^\circ\text{C}$ for 2 h. PDMS replicas were peeled off the wafers prior to punching inlet and outlet holes with a 19-gauge blunt-nose needle. The replicas and glass slides were exposed to oxygen plasma (100 mW with 8% oxygen for 30 s) in a PX-250 plasma chamber (March Instruments, Concord, MA) and then immediately placed in contact with each other. The irreversible bonding between PDMS and glass was completed by baking for 5 min at 65 $^\circ\text{C}$. Surface functionalization was performed immediately after the baking step.

Surface modification

A 4% (v/v) solution of 3-mercaptopropyl trimethoxysilane in ethanol was prepared under a nitrogen atmosphere and flowed into the device. This was left to react undisturbed for 30 min. The unreacted silane was flushed out with ethanol and a 0.28% solution of GMBS in ethanol was then flowed into the device. The GMBS was left to react for 15 min. Following this reaction period, ethanol was used to flush out the unreacted GMBS, followed by flushing with PBS. A solution of 0.1 mg mL^{-1} of REDV in PBS was then flushed into the device. Following a 30 min period, the devices were flushed with PBS and either used directly in experiments or stored at 4 $^\circ\text{C}$.

Cell culture

HUVECs, HMVECs, and NHDFs were cultured in 75 cm^2 tissue culture flasks at 37 $^\circ\text{C}$ in a humidified atmosphere with 5% CO_2 and 95% air. Cells were cultured in vendor-supplied media (Lonza, Walkersville, MD). HUVECs were cultured in EBM-2 with the following added supplements: hydrocortisone, hFGF-B, 0.39%; VEGF, 0.097%; R3-IGF-1, 0.097%; ascorbic acid, 0.097%; heparin, 0.097%; FBS, 1.97%; hEGF, 0.097%; GA-1000, 0.097% (v/v). HMVECs were cultured in EBM-2 with the following added supplements: hEGF, 0.094%; hydrocortisone, 0.038%; GA-1000, 0.094%; FBS, 4.72%; VEGF, 0.094%; hFGF-B, 0.38%; R3-IGF-1, 0.094%; ascorbic acid, 0.094% (v/v). NHDFs were cultured in FCB medium with the following added supplements: hFGF-B, 0.098%; insulin, 0.098%; FBS, 1.95%; GA-1000, 0.098% (v/v). ECFCs were cultured in collagen-coated 75 cm^2 tissue culture flasks at 37 $^\circ\text{C}$ in a humidified atmosphere with 5% CO_2 and 95% air. ECFCs required EBM-2 with the following added supplements: hydrocortisone, hFGF-B, 0.35%; VEGF, 0.089%; R3-IGF-1, 0.089%; ascorbic acid, 0.089%; heparin, 0.089%; FBS, 10.6%; hEGF, 0.089%; GA-1000, 0.089% (v/v). All cells were grown to 80% confluence and isolated for experiments by trypsinization using a 0.25% Trypsin-EDTA solution. Cells were used for 3 passages only. Cell suspensions were centrifuged at 190 $\times g$ and then resuspended in serum-free medium (EBM-2 for ECs and FCB for NHDFs) at a concentration of 5 $\times 10^5$ cells mL^{-1} for flow experiments.

Hele-Shaw flow experiments

Cells were flowed through the Hele-Shaw devices at a flow rate of 50 $\mu\text{L min}^{-1}$ (which represents a shear stress range of 0.53–2.00 dyn cm^{-2}) using a Harvard Apparatus PHD 2000 syringe pump (Holliston, MA). Homogeneous suspensions of each cell type were examined and cell adhesion along the device axis measured by placing a field finder (with 1 mm \times 1 mm grids) under the device. At selected points along the device axis adhered cells were manually counted under a Nikon Eclipse TE2000 inverted microscope. Three cell counts were taken and averaged at each location, with each location representing a 1 mm square. All flow experiments were performed at room temperature.

Straight channel flow experiments

Cell suspensions with concentrations of 5 $\times 10^5$ cells mL^{-1} were incubated in various concentrations of REDV (0, 50, 75, and 100 $\mu\text{g mL}^{-1}$ in serum-free and supplement-free culture medium) in closed centrifuge tubes with vented caps for 30 min at 37 $^\circ\text{C}$ in a humidified atmosphere with 5% CO_2 and 95% air. This process was followed by centrifugation at 190 $\times g$ and resuspension in serum-free medium at a concentration of 5 $\times 10^5$ cells mL^{-1} . Cells were flowed into straight channel device at flow rates of 4.7 $\mu\text{L min}^{-1}$ and 5.5 $\mu\text{L min}^{-1}$ for 32 min. These flow rates correspond to 0.9 dyn cm^{-2} and 1.1 dyn cm^{-2} , respectively. Cell adhesion within the devices was measured using a field finder (with 1 mm \times 1 mm grids) placed under the microfluidic chamber. Cell counts were taken at locations corresponding to $\frac{1}{4}$, $\frac{1}{2}$, and $\frac{3}{4}$ \times the total channel length.

Antibody blocking flow experiments

Anti- α_4 and anti- β_1 blocking antibodies were used at concentrations of $0.7 \mu\text{g mL}^{-1}$ individually to block HUVEC integrin receptors. HUVECs at concentration of $1 \times 10^6 \text{ cells mL}^{-1}$ were added to 1 mL of $0.7 \mu\text{g mL}^{-1}$ anti- α_4 or anti- β_1 (cells and antibodies in serum- and supplement-free medium) and incubated for 30 min at 37°C in a humidified atmosphere with 5% CO_2 and 95% air. These cells were centrifuged and resuspended in REDV of concentrations 0, 50, 75 and $100 \mu\text{g mL}^{-1}$ in serum-free and supplement-free medium for 30 min at 37°C in a humidified atmosphere with 5% CO_2 and 95% air. Following incubation the cells were centrifuged and resuspended in serum-free and supplemented-free medium to a concentration of $5 \times 10^5 \text{ cells mL}^{-1}$. Cells were flowed into the straight channel devices at a flow rate of $5.5 \mu\text{L min}^{-1}$ for 32 min. This flow rate corresponds to 1.1 dyn cm^{-2} . Cell counts were taken at locations corresponding to $\frac{1}{4}$, $\frac{1}{2}$, and $\frac{3}{4}$ \times the total channel length.

Fluorescence intensity calibration for binding site evaluation.

Quantum FITC-5 MESF kits comprising 6 populations of microspheres, *i.e.* five populations having different known levels of FITC and one blank population, were used for this calibration. A standard curve relating fluorescence intensity to standardized MESF values from quantum MESF beads was generated by flowing the beads into the flow-cytometer (Beckman Coulter Quanta SC) and recording the fluorescence intensity.

REDV-binding site enumeration following REDV-pre-incubation. HUVEC suspensions with a concentration of $5 \times 10^5 \text{ cells mL}^{-1}$ in serum-free and supplement-free medium were incubated in varying concentrations of REDV (50, 75, and $100 \mu\text{g mL}^{-1}$) for 30 min. This step was followed by centrifugation at $190 \times g$ for 5 min. The supernatant was discarded and $300 \mu\text{g mL}^{-1}$ of REDV-FITC (a saturating concentration level) was incubated with $5 \times 10^5 \text{ cells mL}^{-1}$ of these cells for 30 min. Cell suspensions were centrifuged at $190 \times g$ for 5 min and the cells were resuspended in PBS. Next, these cells were run through the flow-cytometer and the median intensity peak measurement was recorded. Comparison of the median fluorescence intensity peak value with the calibration curve allowed evaluation of the number of unoccupied REDV-binding sites.

REDV-binding site enumeration following REDV-preincubation and microfluidic flow. HUVEC suspensions with a concentration of $5 \times 10^5 \text{ cells mL}^{-1}$ were incubated in varying concentrations of REDV (50, 75, and $100 \mu\text{g mL}^{-1}$) for 30 min. Following incubation, the cells were centrifuged and resuspended in serum-free and supplement-free medium to a concentration of $5 \times 10^5 \text{ cells mL}^{-1}$. Cells were flowed into a device containing immobilized REDV at a flow rate of $5.5 \mu\text{L min}^{-1}$ for 32 min. Cells from the outlet of this device were collected and incubated in $300 \mu\text{g mL}^{-1}$ of REDV-FITC for 30 min. Cell suspensions were centrifuged at $190 \times g$ for 5 min and resuspended in PBS. The cells were then introduced into the flow cytometer for measurements of unoccupied REDV-binding sites as described above.

Statistics and data analysis

For each flow condition studied, 5 repetitions of experiments were performed unless otherwise specified. Error bars represent standard errors of the mean (standard deviation/ \sqrt{n} , where $n = 5$). One-way analysis of variance (ANOVA) was performed to investigate the relationship between cell types. This statistical analysis was executed using KaleidaGraph 4.0. A p value ≤ 0.001 was considered significant.

Results

Initial characterization of cell adhesion was carried out with microfluidic devices designed to generate Hele-Shaw flow. As described in prior work by our group, this device is shaped with a flared geometry that provides a linear gradient of shear stress along the flow channel axis.^{9,22} This geometry provides a versatile platform for the evaluation of cell adhesion to surface-immobilized ligands as a function of shear stress.

Fig. 1 shows the adhesion profiles of the four cell types of interest on surfaces coated with the tetrapeptide arg-glu-asp-val (REDV). This tetrapeptide is known to selectively bind to endothelial cells²³ and this selectivity is reflected in Fig. 1 by the significantly greater adhesion of all three endothelial cell (EC) types relative to NHDF adhesion ($p < 0.001$) over the entire range of shear stress examined. It is important to note that cell adhesion in Hele-Shaw devices represents a composite measurement that incorporates the effects of ligand-receptor binding affinity and the extent of receptor expression. For example, cells of one type with low affinity to a given ligand but with a large number of binding receptors may have an adhesion profile similar to cells of a different phenotype with greater affinity to the same ligand but fewer receptors. (Static cell adhesion assays also involve this kind of composite measurement.) In Fig. 1, all three EC types show very similar adhesion

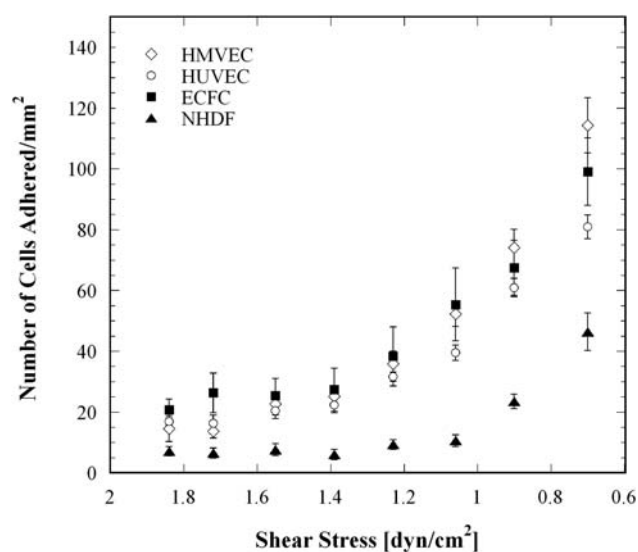


Fig. 1 Adhesion as a function of shear stress observed with endothelial cells and fibroblasts on REDV-coated surfaces in microfluidic Hele-Shaw devices. Error bars denote standard errors for each point based on 5 repetitions.

profiles through the entire range of shear stress examined and hence no distinction between the different EC types can be made using this data.

Fig. 2 shows the results of an experiment carried out by incubating each cell type with varying concentrations of REDV in solution, followed by removal from this solution and flow through an REDV-coated microchannel at a shear stress level of 0.9 dyn cm^{-2} (flow rate of $4.7 \mu\text{L min}^{-1}$; channel dimensions of $50 \text{ mm length} \times 1 \text{ mm width} \times 0.07 \text{ mm height}$). Here, NHDFs serve as a control because they do not adhere selectively to REDV and are not known to undergo receptor expression changes upon shear exposure. The first data point for each cell type (corresponding to $0 \mu\text{g mL}^{-1}$ soluble REDV) represents adhesion measurements made with cells flowed through REDV-coated channels with no prior incubation. Comparing the adhesion levels at 0.9 dyn cm^{-2} in Fig. 1 to that of the $0 \mu\text{g mL}^{-1}$ soluble REDV point in Fig. 2 indicates an apparent discrepancy. This difference in magnitude can be attributed to the difference in the volume of cells available in the syringe initially. Cells are introduced with an initial volume of 0.6 mL in the Hele-Shaw device while cells of the same concentration are introduced into the straight channel chamber with an initial volume of 0.15 mL . In our flow experiments the syringe pump is oriented vertically, allowing cells to settle at the entrance of the syringe. A higher volume of cells in the syringe means that more cells will settle hence a higher population of cells will enter into the device. Additionally the flow rate in the straight channel chamber is much lower resulting in some cells being retained in the syringe.^{20,24}

When incubated with $50 \mu\text{g mL}^{-1}$ REDV solutions, a dramatic drop in cell adhesion is observed for all three EC types (Fig. 2). This drop is expected as occupation of receptors on the cells by soluble REDV would make these receptors unavailable for

binding to REDV immobilized within the microchannels. The expected trend with increasing concentrations of the incubating solutions would therefore be a monotonic decrease in cell adhesion. However, with soluble REDV solution concentrations of 75 and $100 \mu\text{g mL}^{-1}$, an unexpected increase in HUVEC adhesion is observed. HMVECs and ECFCs show a similar trend of increasing adhesion above $75 \mu\text{g mL}^{-1}$ of soluble REDV.

The same experiment was carried out using a flow rate corresponding to a shear stress level of 1.1 dyn cm^{-2} . The results, shown in Fig. 3, indicate markedly lower adhesion at the non-incubated condition relative to Fig. 2. However, the most striking difference between the experiments carried out at 1.1 dyn cm^{-2} versus 0.9 dyn cm^{-2} is the dramatic increase in adhesion observed beyond a soluble REDV solution concentration of $50 \mu\text{g mL}^{-1}$. Specifically, for HUVECs and ECFCs, the pre-incubated cells are observed to be even more adhesive than their non-incubated counterparts when the soluble REDV concentration is above $75 \mu\text{g mL}^{-1}$. At the highest soluble REDV concentration of $100 \mu\text{g mL}^{-1}$, the adhesion values for each EC type are significantly different ($p < 0.001$).

The adhesion of ECs to REDV is known to occur *via* the $\alpha_4\beta_1$ integrin.²⁵ To examine if the same integrin is involved in the increased EC adhesion observed following incubation with soluble REDV, a series of experiments was carried out using antibodies known to block the α_4 and β_1 sub-units. In these experiments, HUVECs were first blocked with saturating concentrations of either anti- α_4 or anti- β_1 and then subjected to incubation with a solution of REDV (concentration range 0 – $100 \mu\text{g mL}^{-1}$) before flowing through an REDV-coated microchannel at a shear stress level of 1.1 dyn cm^{-2} . The results of these experiments are shown in Fig. 4 with the HUVEC data from Fig. 3 for comparison. These results indicate that blocking the α_4

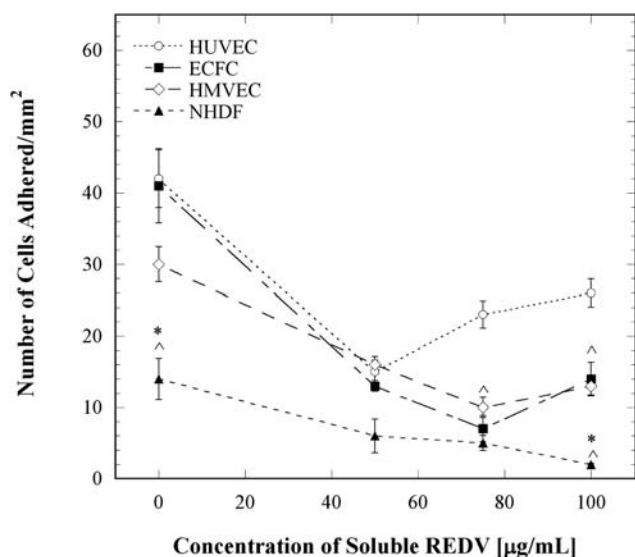


Fig. 2 Cell adhesion comparison between cell types in REDV-coated straight microchannels at 0.9 dyn cm^{-2} . ^ denotes significant difference with $p < 0.001$ relative to HUVECs at specified REDV concentrations and * denotes significant difference with $p < 0.001$ compared to ECFC and HMVEC at specified REDV concentrations. Error bars denote standard errors for each point based on 5 repetitions.

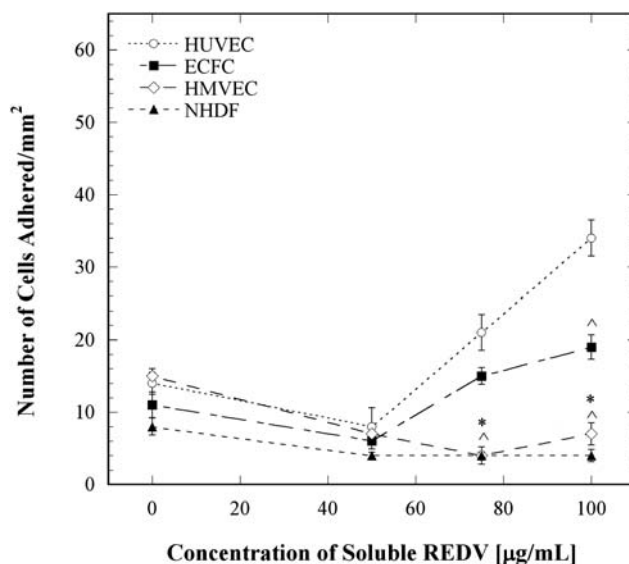


Fig. 3 Cell adhesion comparison between cell types on REDV surface at 1.1 dyn cm^{-2} . ^ denotes significant difference with $p < 0.001$ compared to HUVECs at specified REDV concentrations and * denotes significant difference with $p < 0.001$ compared to ECFC at specified REDV concentrations. Error bars denote standard errors for each point based on 5 repetitions.

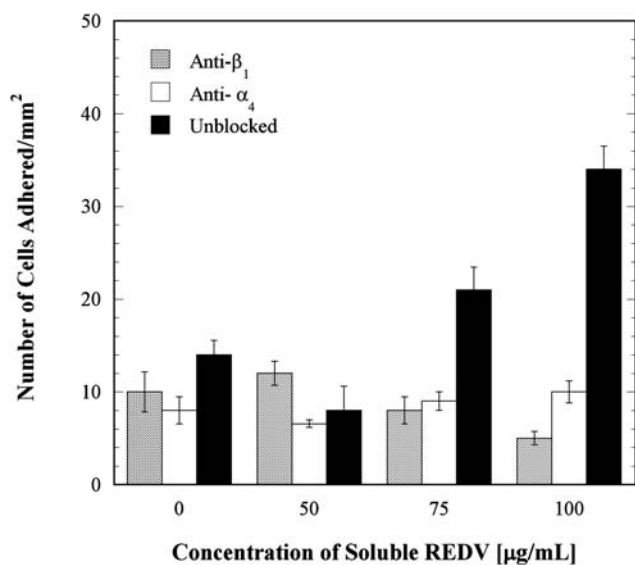


Fig. 4 Adhesion of HUVECs within REDV-coated channels at a shear stress of 1.1 dyn cm^{-2} after blocking with anti-integrin blocking antibodies. Measurements with anti- α_4 and anti- β_1 at varying REDV concentrations (shaded and white bars) are not significantly different from each other within each REDV concentration level as well as across the range of REDV concentrations examined ($0.2 < p < 0.9$). Error bars denote standard errors for each point based on 5 repetitions.

and β_1 sub-units on the HUVECs results in total suppression of the enhancement observed in cell adhesion following incubation with soluble REDV.

Further experiments were carried out to count the number of unoccupied REDV-binding sites on HUVECs *via* flow cytometry following incubation with soluble REDV and following flow

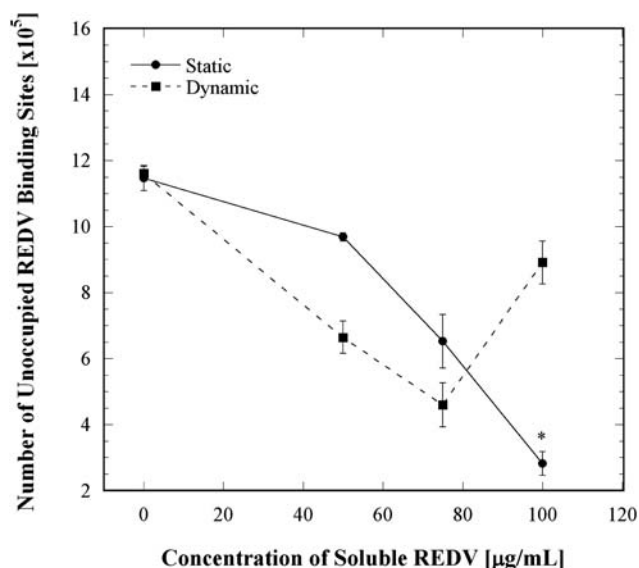


Fig. 5 REDV-binding site quantification with HUVECs prior to (static) and after flow through microchannels at a shear stress of 1.1 dyn cm^{-2} (dynamic). * denotes significant difference with $p < 0.001$ compared with the dynamic data point for 100 µg mL^{-1} soluble REDV. Error bars denote standard errors for each point based on 3 repetitions.

through the microchannels. In the first set of experiments, the number of unoccupied REDV-binding sites was measured following incubation with soluble REDV. The results from these experiments are shown in Fig. 5 (static). HUVECs in this group showed a monotonic decrease in the number of unoccupied REDV-binding sites present on the cell surface as the concentration of soluble REDV was increased. It is noteworthy that even with the highest concentration of soluble REDV, a large number of unoccupied REDV-binding sites remain on the HUVECs.

In the second set of REDV-binding site quantification experiments, HUVECs were incubated with soluble REDV and then flowed through REDV-coated straight channel devices at a shear stress level of 1.1 dyn cm^{-2} . Binding site measurements were carried out with the cells that flowed through the channel without being captured. The results from these experiments are shown in Fig. 5 (dynamic). Incubation with progressively higher concentrations of soluble REDV lowers the number of unoccupied REDV-binding sites, but only up to a point. At an incubation level of 100 µg mL^{-1} of soluble REDV, HUVECs subjected to flow have significantly more REDV-binding sites than cells not subjected to flow ($p < 0.001$). This second set of experiments was repeated with straight channel devices with no surface coating and the results of these experiments was identical to those performed with REDV-coated devices. (This consistency is expected because the cells analyzed have little or no contact with the flow channel surface.)

Discussion

The present study was motivated by our desire to characterize shear-induced receptor expression changes in cells subjected to microfluidic flow and capture. ECs were chosen for this study based on their well-known responsiveness to fluidic shear forces. This responsiveness is typically manifested in the form of receptor upregulation following prolonged exposure to shear.^{26,27} The nature of these responses has been elegantly summarized in a recent review by Young and Simmons.²⁸ Our experimental protocol called for incubating the cell suspension of interest in a solution of known REDV concentration to occupy a set number of REDV-binding sites on the cells prior to flowing these cells into an REDV-coated microchannel. The expectation was that such occupation of binding sites using solutions of increasing REDV concentration would lower cell adhesion within the REDV-coated channel, ultimately leading to zero adhesion when all REDV-binding sites were occupied. We further hypothesized that the extent of cell adhesion suppression would be different among the different EC cell types thereby providing a means to distinguish them from one another.

The data shown in Fig. 2 and 3 indicate a counterintuitive increase in cell adhesion for all three EC types upon incubation with increasing concentrations of soluble REDV and the magnitude of this increase is different for each EC type. A series of experiments was carried out to determine if the unexpected increase in cell adhesion was due to (i) the activity of an integrin receptor other than $\alpha_4\beta_1$, (ii) soluble REDV alone, (iii) the combination of soluble REDV and shear exposure within REDV-coated microchannels, or (iv) shear exposure alone.

The results in Fig. 4 indicate that the $\alpha_4\beta_1$ integrin plays a critical role in the enhanced adhesion observed in Fig. 2 and 3 and eliminates the possibility that soluble REDV engages or activates a different integrin to generate the observed response.

The unexpected increase in cell adhesion in Fig. 2 and 3 has a clear dependence on the amount of soluble REDV presented to the cells as well as the magnitude of shear stress to which the cells are exposed. To examine if incubation with soluble REDV alone was giving rise to the increased adhesiveness observed, we measured the number of unoccupied REDV-binding sites before and after incubation. As the data in Fig. 5 (static) indicate, incubation with increasing concentrations of soluble REDV reduces the number of available REDV-binding sites on HUVECs following a monotonic trend.

By contrast, when the number of REDV-binding sites is measured after pre-incubation with soluble REDV and after flow through an REDV-coated microchannel, the trend is not monotonic (Fig. 5; dynamic). Exactly the same trend is observed when these cells are flowed through an un-coated microchannel (data not shown), indicating that it is the shear exposure, rather than the surface-immobilized REDV, that is responsible for the observed increase in the number of REDV-binding sites. However, this increase in the number of unoccupied REDV-binding sites is only observed for cells incubated with 100 $\mu\text{g mL}^{-1}$ of soluble REDV. It is surprising that despite removal of the cells from contact with soluble REDV *via* centrifugation following the incubation step, these cells still retain a 'memory' of the incubation as they are exposed to a shear stress level of 1.1 dyn cm^{-2} . This 'memory' is mediated critically by the $\alpha_4\beta_1$ integrin, as indicated by our results in Fig. 4.

Taken together, our results indicate a highly synergistic interplay between soluble REDV used in 'pre-conditioning' the cells, the $\alpha_4\beta_1$ integrin, and relatively small levels of shear stress exposure. All three elements are required to generate the response observed in Fig. 2 and 3. We therefore postulate that pre-incubation with high ($\sim 80\text{--}100 \mu\text{g mL}^{-1}$) levels of soluble REDV engages a critical number of $\alpha_4\beta_1$ integrins such that any subsequent exposure to shear encourages the rapid surface presentation of more REDV-binding integrins. The short time frame of this event (residence time of the cells in the straight channel devices is ~ 45 s) is both unexpected and surprising. Upregulation of receptors in cells is typically preceded by events such as gene expression, protein production, and trafficking of the newly-formed receptors to the cell membrane, and such a sequence of events is unlikely to occur within 45 s. Hence, the observed enhancement of cell adhesion would be more attributable to phenomena such as trafficking of vesicle stores or changes in the number of activated receptors. The latter possibility can be ruled out based on the results shown in Fig. 4. Here, saturating levels of anti-integrin blocking antibodies are incubated with the HUVECs; the unbound antibody is removed by centrifugation; the cells are subsequently exposed to varying levels of soluble REDV ($0\text{--}100 \mu\text{g mL}^{-1}$) followed by flow through REDV-coated microchannels. The results in Fig. 4 show a total suppression of adhesion enhancement relative to experiments where the surface integrins were not blocked at the start of the experiment. If the combination of REDV pre-incubation coupled with shear exposure was able to activate previously-inactive surface integrins, this activation would be manifest as

higher adhesion levels than the baseline levels shown by the shaded and white bars in Fig. 4. The data shown in Fig. 2 and 3 suggest that the postulated mechanism applies to all three EC types examined in the present study.

ECs need to adhere to the extracellular matrix (ECM) in order to survive, proliferate, and migrate.²⁹ This adhesion is mediated by a number of integrins such as $\alpha_4\beta_1$. Furthermore, it is known that the expression level of integrins can vary during key phases of development such as embryogenesis.³⁰ The three EC types examined in our study represent different developmental stages and their integrin expression levels and shear response would therefore be expected to differ. The highly sensitive response of HUVECs to incubation with soluble REDV is consistent with this expectation. Furthermore, in Fig. 3, the greater adhesion (associated with greater integrin surface presentation) of ECFCs relative to HMVECs would be consistent with the progenitor-like character of the former compared to the relatively mature nature of the latter.

The data in Fig. 3 suggest that a characteristic receptor expression profile can be obtained for each sub-type of ECs against which an unknown sample could be compared. The significance of such an approach is the ability to identify the sub-type of EC without the need for antibodies or any kind of labeling. However, a major limitation of this approach is that it would provide clear distinctions only if the unknown sample were homogenous. If the unknown sample contained more than one EC type, the average cell adhesion values would lie between the three EC data series for any given value of soluble REDV concentration and would show significant scatter. In such instances, however, measurements of receptor number could be made using a flow cytometer and fluorescent REDV on a cell-by-cell basis, which could potentially serve as a means to determine relative abundance of multiple EC subpopulations in a heterogeneous suspension without using antibodies.

Conclusions

The significance of the results shown in Fig. 2 and 3 is the ability to clearly differentiate between three EC sub-types that cannot be distinguished in a conventional microfluidic cell adhesion assay (Fig. 1) using a simple label-free technique that relies only on measurements of cell adhesion using an optical microscope. The data shown in Fig. 3, in particular, can be regarded as a set of "fingerprints" for each cell type against which an unknown sample can be compared. This technique, when applied to EC subpopulations, can therefore serve as an effective alternative to flow cytometry-based characterization using multiple surface markers and fluorescent labels. This technique, however, would not be able to resolve the content of heterogeneous suspensions containing more than one type of EC.

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References

- 1 S. Nagrath, L. V. Sequist, S. Maheswaran, D. W. Bell, D. Irimia, L. Ulkus, M. R. Smith, E. L. Kwak, S. Digumarthy, A. Muzikansky, P. Ryan, U. J. Balis, R. G. Tompkins, D. A. Haber and M. Toner, *Nature*, 2007, **450**, 1235–1239.
- 2 S. P. Wankhede, Z. Q. Du, J. M. Berg, M. W. Vaughn, T. Dallas, K. H. Cheng and L. Gollahon, *Biotechnol. Prog.*, 2006, **22**, 1426–1433.
- 3 A. Russom, P. Sethu, D. Irimia, M. N. Mindrinos, S. E. Calvan, G. A. Iris, C. Finnerty, C. Tannahill, A. Abouhamze, J. Wilhelmy, M. C. Lopez, H. V. Baker, D. N. Herndon, S. F. Lowry, R. V. Maier, R. W. Davis, L. L. Moldawer, R. G. Tompkins and M. Toner, *Clin. Chem.*, 2008, **54**, 891–900.
- 4 B. D. Plouffe, T. Kniazeva, J. E. Mayer, S. K. Murthy and V. L. Sales, *FASEB J.*, 2009, **23**, 3309–3314.
- 5 X. H. Cheng, A. Gupta, C. C. Chen, R. G. Tompkins, W. Rodriguez and M. Toner, *Lab Chip*, 2009, **9**, 1357–1364.
- 6 A. A. Adams, P. I. Okagbare, J. Feng, M. L. Hupert, D. Patterson, J. Gottert, R. L. McCarley, D. Nikitopoulos, M. C. Murphy and S. A. Soper, *J. Am. Chem. Soc.*, 2008, **130**, 8633–8641.
- 7 T. Nishimura, J. Miwa, Y. Suzuki and N. Kasagi, *J. Micromech. Microeng.*, 2009, **19**, 125002.
- 8 S. K. Murthy, A. Sin, R. G. Tompkins and M. Toner, *Langmuir*, 2004, **20**, 11649–11655.
- 9 B. D. Plouffe, D. N. Njoka, J. Harris, J. H. Liao, N. K. Horick, M. Radisic and S. K. Murthy, *Langmuir*, 2007, **23**, 5050–5055.
- 10 G. D. Liu, X. Mao, J. A. Phillips, H. Xu, W. H. Tan and L. W. Zeng, *Anal. Chem.*, 2009, **81**, 10013–10018.
- 11 L. S. L. Cheung, X. G. Zheng, A. Stopa, J. C. Baygents, R. Guzman, J. A. Schroeder, R. L. Heimark and Y. Zohar, *Lab Chip*, 2009, **9**, 1721–1731.
- 12 B. D. Plouffe, M. A. Brown, R. K. Iyer, M. Radisic and S. K. Murthy, *Lab Chip*, 2009, **9**, 1507–1510.
- 13 S. K. Murthy and M. Radisic, *Encyclopedia of Microfluidics and Nanofluidics*, ed. D. Li, Springer, 2008.
- 14 J. M. Runnels, P. Zamiri, J. A. Spencer, I. Veilleux, X. B. Wei, A. Bogdanov and C. P. Lin, *Mol. Imaging*, 2006, **5**, 31–40.
- 15 J. M. Josef Neumüller, in *Methods in Molecular Biology*, Humana Press, 1997, pp. 399–425.
- 16 W. J. Zhang, L. Y. Ye, L. Q. Wu, Y. L. Xin, F. Gu, J. X. Niu, Z. H. Yang, G. J. Zhu, G. E. Grau and J. N. Lou, *J. Vasc. Res.*, 2006, **43**, 522–532.
- 17 J. C. Voyta, D. P. Via, C. E. Butterfield and B. R. Zetter, *J. Cell Biol.*, 1984, **99**, 2034–2040.
- 18 F. Bertolini, Y. Shaked, P. Mancuso and R. S. Kerbel, *Nat. Rev. Cancer*, 2006, **6**, 835–845.
- 19 M. Wilhelmi, S. Fischer, R. Leyh, H. Mertsching and A. Haverich, *Thorac. Cardiovasc. Surg.*, 2002, **50**, 230–236.
- 20 B. D. Plouffe, M. Radisic and S. K. Murthy, *Lab Chip*, 2008, **8**, 462–472.
- 21 Y. N. Xia and G. M. Whitesides, *Angew. Chem., Int. Ed.*, 1998, **37**, 550–575.
- 22 S. Usami, H. H. Chen, Y. H. Zhao, S. Chien and R. Skalak, *Ann. Biomed. Eng.*, 1993, **21**, 77–83.
- 23 J. A. Hubbell, S. P. Massia, N. P. Desai and P. D. Drumheller, *Bio-Technology*, 1991, **9**, 568–572.
- 24 L. L. R. Cooper, in *Lab Chip, 2007, Preventing suspension settling during injection, Chips & Tips (Lab on a Chip)*, 21 August 2007, http://www.rsc.org/Publishing/Journals/lc/Chips_and_Tips/suspension_injection.asp.
- 25 S. P. Massia and J. A. Hubbell, *J. Biol. Chem.*, 1992, **267**, 14019–14026.
- 26 E. Tzima, M. A. Del Pozo, W. B. Kiosses, S. A. Mohamed, S. Li, S. Chien and M. A. Schwartz, *EMBO J.*, 2002, **21**, 6791–6800.
- 27 C. Urbich, D. H. Walter, A. M. Zeiher and S. Dimmeler, *Circ. Res.*, 2000, **87**, 683–689.
- 28 E. W. K. Young and C. A. Simmons, *Lab Chip*, 2010, **10**, 143–160.
- 29 G. E. Davis and D. R. Senger, *Circ. Res.*, 2005, **97**, 1093–1107.
- 30 S. M. Albelda and C. A. Buck, *FASEB J.*, 1990, **4**, 2868–2880.

Contributors to the Emerging Investigators issue

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This is the first time that *Lab on a Chip* has put together an issue that features the younger members of the community that are potential rising stars. We often hear the opinions of supervisors and leaders in the field, but rarely do we hear the voices of students, post-docs or young researchers. Therefore we decided to elicit the opinions of these emerging investigators on issues of current concern, such as the killer application, bottlenecks and issues as well as the future for this field. Their responses to the questions listed below are included at the end of each biography and we hope they give an idea of what problems young researchers face and their outlook and aspirations.

(1) *What is your view of the “killer application”?*

(2) *What are the main difficulties you face working in this area of research?*

(3) *What do you think the future holds for this field and these technologies?*

Amar Basu



Amar Basu was born in Rochester, Michigan in 1978. He received BSE and MSE degrees in electrical engineering in 2001 and 2003, an MS degree in biomedical engineering in 2005, and a Ph.D. in electrical engineering in 2008, all with honors from the University of Michigan. Amar has worked for Intel's Advanced Technology group, General Motors, Silicon Graphics, and served as an adjunct faculty member at the University of Michigan from 2007–2008. Currently,

Amar is assistant professor of electrical engineering and biomedical engineering at Wayne State University. His research interests are in developing multiphase fluidic and electronic technologies for high throughput screening in systems biology. Amar was a recipient of the Whitaker Foundation Biomedical Engineering Fellowship, and was voted IEEE Professor of the Year by students 2009. His work as a consultant with Mobius Microsystems and Picocal has resulted in a several US patents.

(1) *I think the killer app. will be high throughput screening for systems biology. The US National Research Council (NRC) emphasizes that next generation solutions to societal challenges in health, energy, food, and environment will be driven by a more comprehensive understanding of complex pathways and interactions in biological organisms. Accordingly, biological research is evolving from reductionist studies of single proteins and genes to system-wide ‘omics’ studies involving thousands of genes, proteins, and environmental conditions. This new model of research clearly requires instrumentation which can perform large-scale screens at a reasonable cost. Microfluidics, particularly droplet-based fluidics, is well positioned to make HTS affordable to researchers by dramatically reducing reagent consumption (the most significant cost of HTS) and capital costs of laboratory automation. Equally important, the ultra-high throughput capability of droplet-based fluidics is leading to promising new screening protocols, such as directed evolution.*

(2) *Interfaces and methods which convert screening libraries between the macro and micro domains are particularly challenging in droplet systems due to the precise volumes and high throughputs needed, as well as the large number of compounds in typical screening libraries. Other challenges include detection in small volumes, interdroplet diffusion, and the difficulty in performing complex fluidic handling (such as washing steps) required for immunological and other heterogeneous assays. Being able to integrate and automate high speed fluidic operations in a user friendly package*

is another engineering challenge which needs to be addressed. Generally speaking, the highly interdisciplinary nature of this research (involving fluidic phenomena, electronic detection/control, chemistry/biochemistry, and biology) makes it enjoyable but also challenging. Finding collaborators willing to invest time in high-risk technologies, and training new students in multiple skill-sets requires patience and earnest effort.

(3) *I think that there is considerable promise in high throughput screening and droplet-based screening in particular. Although there are many practical issues which need to be addressed, the ability to perform screening in pL and fL volumes at unprecedented throughputs has the potential to transform biology in a profound way. For example, we are already starting to see screens in the range of millions which can be used for optimizing and evolving new enzymes, as well as new biological studies at ultra-low volumes. Given the rapid pace of technology development and its growing need in systems biology, I believe that multiphase fluidics will play a central role in the future of lab-on-a-chip technologies.*

Karen C. Cheung



Karen C. Cheung received her Ph.D. degree in bioengineering from the University of California, Berkeley, in 2002. From 2002–2005, she was a postdoctoral researcher at the Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland. She is now an Assistant Professor

at the University of British Columbia, Vancouver, BC, Canada. Her research interests include lab-on-a-chip systems for cell culture and characterization, inkjet printing for tissue engineering, and micro- and nanoscale implantable neural interfaces.

(1) *The killer app. might be successful replacement of entire animal studies using engineered constructs for drug testing.*

(2) *Students working in this area are very open-minded and they welcome the challenges in learning and understanding concepts in these interdisciplinary projects. We need to be able to find more collaborators in medicine and biology who have the time and are willing to work with us in order to really develop the technology for real applications.*

(3) *Although these technologies will bring a lot of benefit to our laboratory diagnostics, a larger impact may be seen in underdeveloped and poor communities. There, lab on a chip can be used to address global health issues. In developed nations, lab on a chip may bring incremental (some) improvement to healthcare. In the rest of the world, cheap, disposable, and rapid lab on a chip analysis could bring much more dramatic improvement to quality of life.*

David T. Eddington



David T. Eddington received a B.S. degree in materials science and engineering from the University of Illinois at Urbana-Champaign and Ph.D. degree in biomedical engineering from the University of Wisconsin-Madison. He joined the Department of Bioengineering at the University of Illinois at Chicago in 2006 after a postdoc in the HST program at Harvard/MIT. His research interests include

developing novel solutions to current unmet experimental and clinical needs through applying simple microfabricated devices.

(1) *For academic research, this would be a device that tests new hypothesis or enables new experimental possibilities that would not be possible with currently available and standardized methods. For a commercial product, this would be a device that someone would want to buy. There are several useful LOC devices currently available that started in academic labs and turned into products such as chemotaxis assays, protein crystallization platforms, and high throughput PCR. The commercial successes of these will enable more to follow. However, even if there is no killer application bringing LOC devices to every biomedical research lab, they will remain as powerful tools enabling new possibilities.*

(2) *The main difficulty is identifying interesting new device designs that are also useful tools that others would benefit from and have not been previously demonstrated. I have many interesting ideas, but only a limited set would be useful.*

(3) *I think LOC devices will continue to offer new solutions for unmet experimental and clinical needs. The field is beginning to transition from proof of concept demonstrations to using these devices to learn something new. Even if few of these become commercial realities, they will continue to offer exciting possibilities for the research community.*

Axel Günther



Axel Günther is an Assistant Professor in the Department of Mechanical and Industrial Engineering with cross-appointment to the Institute of Biomaterials and Biomedical Engineering at

the University of Toronto. He obtained his Ph.D. from the Swiss Federal Institute of Technology (ETH) in Zurich and conducted postdoctoral research at MIT. He has published >25 papers, patented 3 inventions (patent families) and has received the ETH medal (2002), Ontario Early Researcher Award (2009) and I.W. Smith Award of the Canadian Society for Mechanical Engineering (2010). Dr Günther is the Scientific Director of a new Centre for Microfluidic Systems in Chemistry and Biology in Toronto.

(1) *Ultimately successful applications of a technology are often found in quite unexpected and less obvious areas. In the area of biology, I see great opportunities in the comprehensive in vitro investigation of engineered tissues, small intact organs and organisms. In our contribution to this Special Issue, we demonstrate to our knowledge the first scalable approach allowing the structure and function of intact cardiovascular tissues to be assessed in health and disease (e.g., heart failure, diabetes, vasculopathies, tumor biology, and toxicology). The ability to routinely probe small artery function is expected to have wide implications in cardiovascular drug development and, ultimately, personalized medicine. I expect lab-on-a-chip systems to continue in providing new technological solutions that potentially translate to a number of disciplines, including the by now obvious candidates: analytical chemistry, molecular and cell biology, organic chemical synthesis. To complement the creation of new technologies, the scientific community has often to do a better job of translating microfluidic devices into commercially viable solutions. Only that way, microfluidic systems have a chance to become broadly available, mass-produced commodities. This translational aspect is not yet sufficiently addressed, partly because a true translation of the technology and not a mere mass-production of a chip are required. Robust and seamless fluidic world-to-chip interconnects and also hybrid concepts that allow us to combine existing (analytical) technology and lab-on-a-chip systems are of the essence.*

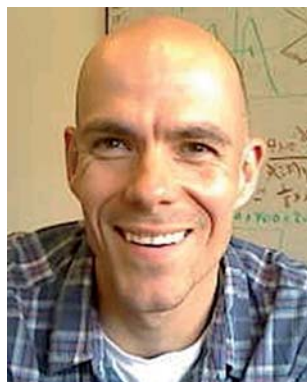
(2) *I thoroughly enjoy working in this field and don't face too many difficulties. I actually think that researchers in our field are quite fortunate, given the diverse*

range of opportunities. A challenge that is common to everyone conducting interdisciplinary science is the necessity of researchers with very different training attacking all (or most) aspects of a problem, not just the aspects closest to their traditional scientific training and experience. A microfluidics researcher working on a cardiovascular question for instance, will need to ultimately think about basic questions in vascular biology, and vice versa. Only such a multifaceted approach will lead to the detailed scientific discussions, sometimes controversial ones, necessary to answer relevant questions, not just device a “new toy”. To reflect this need and comprehensively train future graduate students across disciplines involving nanol/micro-fabrication, cell and vascular biology, analytical and materials chemistry, fluid mechanics and transport phenomena, approximately ten colleagues and I at the University of Toronto and its associated Teaching Hospitals and York University in Canada, Corning, Inc. (Corning, N. Y.) and CMC Microsystems (Kingston, ON) have recently launched the six year graduate training program in Microfluidic Applications and Training in Cardiovascular Health (MATCH).

(3) The future of this field is exceptionally promising, for university researchers as well as for companies. The fact that commercial “killer applications” have yet to be demonstrated currently gives university researchers an edge in securing intellectual property. Companies on the other hand can adapt microfluidic technologies to their existing markets and expertise to serve real commercial needs with new technological solutions. Here are a few bolder options. Soon, we will likely see distributed, iPhone or iPad operated integrated microfluidic platforms that are portable, automated and will allow challenging chemical reactions that are otherwise confined to fume hoods or glove boxes to be routinely carried out. Low cost analytical devices will be widely used to screen water samples for pathogens and to comprehensively assess the interaction between carbon dioxide and sea water to gain an improved understanding of the carbon balance in the world’s oceans. With respect to biological applications, tissue culture and life-cell imaging will be combined and broadly available in intuitive, portable devices as well as integrated

with powerful high-content screening platforms.

Carl Hansen



Carl Hansen is an Assistant Professor in the Department of Physics and Astronomy at the University of British Columbia and the recently established UBC Center for High Throughput Biology. Dr Hansen holds a BSc in Engineering Physics and a PhD in Applied Physics from the California Institute of Technology. He is a Michael Smith Scholar in Biomedical Research, a Canadian Institute for Health Research New Investigator, and holds an affiliate faculty membership at the Institute for Systems Biology in Seattle. The Hansen lab works on the development and application of microfluidic systems for applied and fundamental research in genomics and cell biology. This interdisciplinary work uses methods ranging from microengineering, to single molecule detection, to live-cell microscopy. Specific research questions are focused around the themes of single cell analysis and genomic diagnostics with applications in the fields of stem cell science, cancer biology, microbiology, small RNA, and immunology.

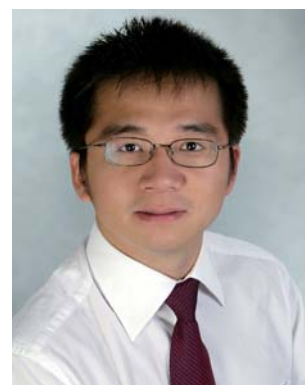
(1) I wouldn’t say there is one obvious “Killer application” in microfluidics. Handling fluids is fundamental and microfluidics will likely have impact in research areas ranging from genetics, to diagnostics, to drug discovery. Ultimately the best applications are the ones that not only benefit both from economy of scale, but also are fundamentally improved in microfluidic implementations. In our work we have identified genomics and cell biology as important areas for development and these are well-suited to the specific technology

platform we use. Other important areas include point of care diagnostics, chemical screening, chemical synthesis, protein engineering, and environmental monitoring. I expect that each of these applications will demand its own flavor of microfluidics.

(2) In our work we have been fortunate to interface with excellent collaborators from the biological sciences. This interaction is critical to working effectively in the field of microfluidics and also sets a high bar for new technologies. I would say that in our research the plumbing is now a solved problem. The main challenges are (i) finding innovative ways to outperform existing methods, (ii) getting control over the biology and the chemistry, and (iii) engineering a system to be reliable and robust.

(3) Microfluidics research will continue to thrive as a field with the emphasis increasingly turned towards applications. Although accelerated adoption of new technologies will be driven by commercialization, the range of applications will likely grow more slowly than demand. This means that customized technologies will be increasingly important and this will require more labs that can work effectively across disciplines. Moving forward I expect that the leaders in the field will be primarily concerned with answering biological questions, using microfluidics as one of several key experimental approaches.

Tony Jun Huang



Tony Jun Huang is an Associate Professor in the Department of Engineering Science and Mechanics at The Pennsylvania State University. He received his Ph.D. degree in Mechanical Engineering from the University of California, Los Angeles (UCLA) in 2005, and

his B.S. and M.S. degrees in Energy and Power Engineering from Xi'an Jiaotong University, China, in 1996 and 1999, respectively. His research is focused on (1) multi-physics of active nanostructures, and (2) multi-physics of microfluidics and lab-on-a-chip systems, including acoustic microfluidics and optofluidics. He has authored or coauthored over 100 technical publications in these fields. His work on lab-on-a-chip has been highlighted in *National Science Foundation* and many public media such as *US News and World Report*, *Yahoo News*, *Live Science*, *Medical News Today*, *Science Daily*, *Wired Science*, *Popular Mechanics*, *Highlights in Chemical Technology*, *Nanotechnology Now*, and *R&D Magazine*. More information about him and his research group can be found at www.esm.psu.edu/huang/.

(1) *There are many important fields (e.g., rapid point-of-care diagnostics) in which lab-on-a-chip systems can be quite useful. Many on-chip technologies developed in recent years have shown superior performance and have great potential to lead to "killer applications". However, I believe the key is whether we can provide simple and robust techniques that work well outside research labs. Fancy techniques that are not reliable or are difficult to employ in the real world just won't cut it. I have heard many complaints from the industry and colleagues that many devices currently developed are actually "Chips-in-a-Lab", rather than "Labs-on-a-Chip".*

(2) *In my opinion, one of the main difficulties in our field is that there is little to no incentive for university laboratories to invest limited research funds to address current challenges in applying our lab-on-a-chip devices to real-world applications. The pressure (especially for funding purposes) is always to come up with "novel" concepts and "novel" designs. Getting funding support to perfect our existing devices (so that it will actually do their job in real-world applications) is difficult.*

(3) *I believe that as researchers, although it is vital to constantly push the envelope of technology and innovation, we need to spend a little bit more time thinking about how to convert some of existing technologies into marketable products. I believe that there are many technological challenges that need to be overcome so as to ensure technologies that work well in the labs will also work well in the real world. Many of these challenges have been*

"conveniently" overlooked in the past; however, this situation has to change before the field of microfluidics and lab-on-a-chip lives up to its potential. Although challenges remain, I am optimistic that with so many talented people working in this field, the lab-on-a-chip community will be able to deliver many useful tools to support applications, innovations, and discoveries and have significant impact in the fields of biology, chemistry, and engineering.

David Juncker



David Juncker was born in Switzerland, in 1973. He studied electronics-physics at the University of Neuchâtel in Switzerland, and spent one year at the National Institute of Metrology in Japan in 1998. He was at the IBM Zurich Research Laboratory under the supervision of Emmanuel Delamar between 1999 and 2004 and received his PhD in 2002 from the University of Neuchâtel. After spending one year as a Post-doc at the ETH Zurich, he joined the Biomedical Engineering Department of McGill University in Montreal as an assistant professor in 2005. He currently holds a Canada Research Chair and directs the Micro and Nanobioengineering lab. His current research interests are capillary-force based microfluidics (including yarn-based devices) for diagnostics, antibody microarrays for biomarker discovery, nanocontact printing and microfluidic probes for microenvironment control of neurons, and lab on a chip systems for cell culture, cell filtering, and model organism manipulation.

(1) *The question of the killer application is one that I have heard repeatedly, and I think it's already there, we just haven't realized it. The LOC killer application existed before the concept of LOC was formulated, namely*

in the form of lateral flow diagnostic systems such as pregnancy tests that abide to the "sample in – answer out" vision of a lab on a chip applied to diagnostics. The question to me is "what can LOC contribute to science and society?" LOC devices will benefit science as an enabler for new research, and society directly by means of better diagnostics; there are already several devices in use for emergency care and they will likely expand from there.

(2) *The interdisciplinary nature of our work, ranging from the development of novel microfabrication processes for making LOC, to yeast cell analysis and to multiplex biomarker validation in breast cancer patients sometimes leaves my brain fuming. But by working together as a team with my students and collaborators, we are able to tackle such complex questions while expanding our knowledge and having a lot of fun at the same time!*

(3) *The range of applications of LOC is staggering as it ranges from basic studies of fluid physics, to the manipulation of live organisms, and so is the range of applications. LOC will be used increasingly as a research tool, although they will probably be more an enabler rather than the end product, as for example in next generation sequencers that all integrate some microfluidic aspects, but are not particularly advertising it. The greatest opportunity for LOC lies in new applications where there is no precedent or alternative. I thus think that personalized medicine, which will require the repeated analyses of multiple molecular markers to monitor disease progression and response to treatment, will be one of the many areas that will drive the emergence of novel LOC-based systems.*

Hirokazu Kaji



Hirokazu Kaji is an Assistant Professor at Department of Bioengineering and

Robotics at the Graduate School of Engineering, Tohoku University, Japan. He received his Ph.D. (2005) in Bioengineering, M.Eng. (2003) in Biomolecular Engineering, and B.Eng. (2001) in Molecular Chemistry and Engineering from Tohoku University. His current interests include surface chemistry, microfluidics, biomaterials, and the application of micro- and nanotechnologies in the fields of cell and tissue engineering.

(1) *The “killer application” for lab on a chip technology has yet to be found from the point of view of biological applications. More research is required to find suitable applications that yield biologically meaningful outcomes in a simple manner. Also, assessment of the value of such applications by biologists will be important.*

(2) *This area is multidisciplinary and has many researchers from various fields. Although communicating with researchers from other fields may be difficult in a short period of time, collaboration with such researchers based on mutual understanding is very exciting.*

(3) *Micro- and nanoscale technologies have provided some insight into fundamental cell biology as well as cell and tissue engineering. This knowledge will be used to direct cell fates as a cell source or to be incorporated into newly designed biomaterials capable of controlling cell behavior. The continued challenges in this area will enable the creation of more physiologically relevant models suitable for fabricating tissues and make drug development and toxicology screening more reliable while decreasing the need for animal testing.*

Ali Khademhosseini



Ali Khademhosseini is a faculty member at Harvard-MIT's Division of HST, Brigham and Women's Hospital and

Harvard University. He has edited 3 books and authored over 100 journal papers, 30 chapters and 17 patent applications. His research efforts have earned him the TR35 Award, the ALA Innovation Award, the early career awards from AIChE, NSF, ONR, EMBS, IAMBE and BMW, as well as the ACS's LaMer and the Unilever awards.

(1) *In my view the “killer application” of lab on a chip devices is still eluding the field. Clearly there have been some commercial successes in various diagnostics and analytical areas, however, there has always been a greater commercial potential than what has been realized so far. If history from other scientific areas, such as tissue engineering, may indicate, the killer applications often arrive with increased maturity of the field. Personally I feel that the killer applications may involve the use of BioMEMs systems for therapeutic applications as biological tissue substitutes or miniaturized implants.*

(2) *The major challenge of the application of the microdevices in tissue engineering has been the inherent limitation of the microfabrication technologies as a two-dimensional platform. We and others have developed a number of approaches to bridge the gap between the 2D microfabrication techniques and 3D microenvironments that are experienced by the cells in the body. However, despite much progress this remains a challenge in the field.*

(3) *The future looks bright for lab on a chip technologies. In particular diagnostic devices and high-throughput screening systems may revolutionize medicine and drug discovery processes. Also, microengineering approaches look highly promising for generating 3D engineered tissues for regenerative medicine and in vitro tissue models.*

Saif A. Khan

Saif A. Khan was born in Mumbai, India, in 1979. He received his Ph.D. in chemical engineering from the Massachusetts Institute of Technology in 2006, where he was an R. T. Haslam Presidential Fellow. In 2006 he joined the National University of Singapore (NUS) as Assistant Professor in chemical and biomolecular engineering. He was appointed Fellow of the Singapore-MIT Alliance (SMA) in chemical and pharmaceutical engineering in 2007. His



current research interests include microfluidics, soft condensed matter physics, colloid science, plasmonic nanomaterials and chemical reaction engineering. His research group at NUS focuses on various aspects of microscale fluid physics and phenomena, with the aim of developing new experimental methods for chemistry and biology that complement or extend existing macroscopic methods.

(1) *We envision rapid, online, sequential separations from minuscule volumes of complex biological mixtures, containing large macromolecules (DNA, RNA, proteins, polysaccharides, etc.), smaller oligomeric molecules (such as micro-RNA) and other components such as salts. This is currently an extremely challenging time and resource intensive task.*

(2) *The most exciting aspect of this area of research, which can also be the most daunting, is its inter-disciplinary nature. Therefore, one of the key challenges is maintaining a constant and vigorous dialogue between experts from different scientific disciplines, and also between us, the ‘method developers’, and potential end-users.*

(3) *The field of ‘compartmentalized’ microfluidics with droplets and bubbles has rapidly expanded in the past few years. Droplet-based chemical and biological assays, essentially miniaturized versions of macroscale experimental protocols, have been well demonstrated and have led to commercial ventures. The near future will see microfluidic methods that go beyond simply miniaturizing established macroscale protocols, offering fundamentally new capabilities that leverage physical and chemical phenomena specific to confined micro- and nanoscale fluid spaces.*

Michelle Khine



Credit: Dave Lauridsen

Michelle Khine is an Assistant Professor of Biomedical Engineering at UC Irvine and scientific founder of Shrink Nanotechnologies (Nasdaq:INKN). She was an Assistant & Founding Professor at UC Merced. Michelle received her PhD under Luke Lee in Bioengineering ('05) from UC Berkeley and UCSF. She co-founded Fluxion Biosciences in graduate school. Michelle was the recipient of the TR35 Award in 2009.

(1) *This technology enables the prototyping of custom and complete microfluidic devices in minutes. This would allow researchers to design on the fly with minimal costs and no infrastructure. Because we can also robustly integrate metallic nanostructures directly in the microfluidic channels for enhanced signal to noise ratio, this technology is particularly applicable for the development of low cost point of care diagnostics.*

(2) *I think whenever you try to do something radically different it is first met with skepticism. So the main challenge has been to prove how effective this technology is.*

(3) *Hopefully adoption by many labs! I sincerely believe that if we make it easier and faster to prototype innovative microfluidic designs, we will help catalyze the translation from research to real-world solutions for point of care diagnostics.*

Catherine Klapperich

Catherine Klapperich joined the faculty of the Boston University College of Engineering in 2003. She was promoted to Associate Professor in 2010. She is the director of the Biomedical Microdevices Laboratory in the Departments of



Biomedical and Mechanical Engineering. She is also a member of the Center for Nanoscience and Nanotechnology at BU. Before coming to Boston, Dr Klapperich was a Visiting Postdoctoral Fellow at Lawrence Berkeley Laboratory in the lab of Dr Carolyn Bertozzi, and was a Senior Research Scientist at Aclara Biosciences in Mountain View, CA. She earned her Ph.D. in Mechanical Engineering in 2000 from the University of California, Berkeley; her M.S. in Engineering Sciences from Harvard University and her B.S. in Materials Science and Engineering at Northwestern.

(1) *I believe that the "killer app" in next generation microfluidics will be in the area of systems biology both as discovery tools and in patient care. The ability to look at large ensembles of single cells that are not synchronized will enable previously unimaginable discoveries in biology. As these technologies mature and new biomarkers for disease are validated, running large numbers of tests on a single patient will become feasible. In the best case scenario, faster and more precise diagnoses and treatments will lower the cost of care.*

(2) *Currently our major challenges lie in the areas of raw materials and standardization. It is very difficult to source quality controlled materials for small scale devices in which one is performing surface sensitive reactions. Standardization of device design (inlets, outlets, materials, etc.) is critical to the future of microfluidic device design.*

(3) *I think there is a lot of critical rethinking of lab on a chip technologies going on around the world right now in both industry and academia. People are really thinking about what is necessary to scale up these technologies to production levels. I've also had a significant increase in my interactions with non-engineers*

interested in deploying these technologies as research tools, so I'm quite hopeful about the future.

J. Christopher Love



J. Christopher Love is an assistant professor in chemical engineering at MIT, an associate member at the Eli and Edythe L. Broad Institute, an associate faculty member at the Ragon Institute of MGH, MIT, and Harvard, and a member of the Koch Institute for Integrative Cancer Research. Dr Love received his Ph.D. in 2004 in physical chemistry at Harvard University. He extended his research into immunology at Harvard Medical School from 2004–2005, and at the Immune Disease Institute from 2005–2007. His research uses microsystems to characterize heterogeneity among single cells. Dr Love was named a Dana Scholar for Human Immunology and a Keck Distinguished Young Scholar in Medical Research in 2009.

(1) *Lab-on-a-chip technologies are beginning to mature commercially for miniaturized bioanalytical assays that replicate large-scale systems and for microscale synthetic chemistry. Validation of these classes of technologies will result from the deliberate demonstration of their usefulness in generating new fundamental discoveries in biology or chemistry that could not be made with existing tools.*

(2) *One difficulty is recruiting personnel with sufficient interdisciplinary training to work fluidly at the boundaries of chemistry, materials science, engineering, and biomedical research. New educational mechanisms that increase knowledge and practical awareness at these interfaces are essential for this developing area of research.*

Shashi Murthy



Shashi Murthy is an Assistant Professor in the Department of Chemical Engineering at Northeastern University in Boston, USA. Prof. Murthy established his laboratory at Northeastern in 2005 following postdoctoral work at the Harvard Medical School and Massachusetts General Hospital under Prof. Mehmet Toner where he designed microfluidic devices to isolate lymphocyte subpopulations from blood. The major thrust areas in his laboratory currently include microfluidic isolation of stem/progenitor cells for regenerative medicine, ocular diagnostics, new materials for adhesion-based cell capture, and understanding cell-level phenomena in the context of microfluidic flow. Prof. Murthy is the recipient of U.S. National Science Foundation's CAREER Award (2008), the agency's most prestigious award for junior faculty members, and the Søren Buus Award for Outstanding Research in Engineering at Northeastern University (2009). He has authored over 30 publications in the areas of microfluidic cell separation and biomaterials. He earned his Ph.D. in Materials Science & Engineering at MIT in 2003 and his B.S. in Chemical Engineering at Johns Hopkins University in 1999.

(1) *I don't think that there will be a distinct "killer application." Rather, I think that the potential is significant for a single product and/or brand based on microfluidic technology to stand out among similar products that may or may not use microfluidic technology. The best analogy that I can think of is the iPod, which already stood out when it was first released many years ago and continues to do so,*

(3) *There are many opportunities for lab-on-a-chip technologies, particularly those for single-cell analysis, in the fields of clinical immunology, disease pathogenesis, and vaccinology. New tools to characterize phenotypic and functional attributes of heterogeneous sets of primary human cells with correlated, dynamic measures are critical for those areas of biomedical research, especially when the volumes of samples of interest are very limited—for example, tissue biopsies. For lab-on-a-chip solutions to best facilitate the study of clinical samples, there is a need to develop and practice methods that conserve the samples available for analysis and collect multiple classes of data from the same cells. Shifting from unidimensional to integrated, multidimensional analytical processes is one of the current challenges facing the field of single-cell analysis.*

Matthew Munson



Matthew Munson received his PhD in bioengineering from the University of Washington in 2004, and completed one post-doctoral year at University of Chicago prior to being awarded a National Research Council post-doctoral fellowship at the National Institute of Standards and Technology, where he now holds the position of Research Bioengineer. His research has focused on exploiting the fundamental physical properties at the microscale to enable development and optimization of analytical tools for the quantification of proteins and the controlled perturbation of protein reaction networks, in both research and clinical applications. This work has spanned several distinct sub-disciplines such as continuous laminar flow, multiphase flow, and electrokinetic

separations, as well as many different device platforms such as silicon, thermopolymer laminates, multilayer PDMS, and hybrid material devices. The views expressed here are his own, and do not reflect the views or policies of NIST or the US government.

(1) *As a scientist, I am interested in any technology or application that can be employed to enable the measurements necessary to test a hypothesis under investigation. As a developer of technology, however, the best technologies are the ones that can be used in the investigation of many different hypotheses. I believe that technology development in the non-commercial setting should lie at the intersection of these philosophies. As such, the killer application in this environment varies between hypotheses and is the tool that most clearly addresses the measurement needs at hand. I have found that microfluidics in general offers an ideal platform for the development of these applications because of the scalability, the deterministic nature of the underlying physics, and the potential for automation.*

(2) *The primary challenge I have faced in this area has been the selection of appropriate problems. The microfluidics community has a great depth of knowledge and understanding of physical phenomena at the microscale. As such, once relevant problems are identified, we have an extensive toolkit that can be applied to their solution. The great opportunity of this challenge is to make connections with scientists in other disciplines who have well defined measurement needs that cannot be addressed by other means. When these connections are made, great science emerges that furthers both the technology developer and basic scientist.*

(3) *I think the future will be dedicated to thoughtful application of the technologies developed within this field to the solution of real research and commercial problems. As we begin to embrace the entirety of the microfluidic toolkit, and abandon the narrow focus that comes with specialization we will be able to solve increasingly complex and increasingly interesting scientific problems. In turn, the application of all available tools to interesting problems will highlight areas where the current toolkit is lacking and will inspire development of new tools that are complementary to the existing ones.*

despite the existence of numerous other products with similar capabilities.

(2) The two main challenges that I face working in this area are skepticism (“why do you need a microfluidic device to do this?”) and high expectations (“when will this device be available in clinics world-wide?”). These challenges may seem contradictory but they can be better understood considering that the former challenge mainly comes from fellow researchers and funding agencies whereas the latter comes from the general public and the media. The first challenge is an extremely important element in our work in this field; simply put, if miniaturization has no clear advantages over conventional approaches, it is simply not worth the effort from an applications perspective. Dealing with high expectations however, particularly in medical applications, is not quite as clear-cut. It is important for us as members of this research community to sustain excitement in the minds of the general public for the potential of this field but at the same time, expectations need to be balanced with the realization that microfluidics is not all that different from other areas in terms of the lag time between discovery and market availability.

(3) I think that this is a very exciting time to be a researcher in this field. The potential for microtechnologies in a wide range of areas has been proven, key scientific and engineering principles have been recognized and formalized, and a healthy level of skepticism exists in evaluating research findings and commercial prospects. All of these factors will contribute to a bright and exciting future for the field. Based on my own expertise, I envision major contributions to the fields of clinical medicine, biology (cell and molecular), and chemistry (synthetic and analytical).

Aydogan Ozcan

Dr Aydogan Ozcan received his Ph.D. degree at Stanford University Electrical Engineering Department. He is currently an Assistant Professor at UCLA, leading the Bio- and Nano-Photonics Laboratory (<http://innovate.ee.ucla.edu/>) at the Electrical Engineering Department. Dr Ozcan holds 17 issued patents and another 8 pending patent applications; and is the author of one book and the co-author of more than 110 peer-reviewed research



articles in major scientific journals and conferences. Dr Ozcan received several awards including NSF CAREER Award, NIH Director’s New Innovator Award, ONR Young Investigator Award, IEEE Photonics Society Young Investigator Award and MIT’s TR35 Award for his seminal contributions to near-field & on-chip imaging, and telemedicine based diagnostics. Dr Ozcan is also the recipient of the National Geographic Emerging Explorer Award, Gates Foundation Grand Challenges Award, Netexplorateur Award, the Wireless Innovation Award given by the Vodafone Americas Foundation, as well as the Okawa Foundation Award.

(1) One of the most important signs of a killer application is that you should be able to explain it to your grandparents in an elevator within less than a minute, and still deeply excite them with the prospects of your work. If this combines with solid research and development validating those prospects, this would indeed be a successful killer application of an idea.

(2) Addressing global health challenges, especially in third-world settings, requires a different mindset. You cannot readily assume that most of the well established technologies that shaped our thinking for decades would be available or would even be functioning in such resource poor settings. As a result, we need to detach ourselves from conventional designs, and rethink the solution for these challenges. This often requires an interdisciplinary effort, which needs both depth and breadth in various fields to provide a well engineered solution.

(3) One of the most impactful things that the consumer electronics market brought to our lives is the cellphone. Benefiting from this revolutionary platform, telemedicine will surely help us to address several global health challenges that we face today; and in

this regard we will see new telemedicine based technologies that aim to make the most out of this digital platform. Hopefully, the end result will make life simpler for everyone, helping to close the gaps between the developed and the developing nations.

Adrian Ozinsky



Adrian Ozinsky is a medical doctor who received a Ph.D. in biochemistry from the University of Cape Town in 1996. After a postdoc in immunology at the University of Washington, he joined the Institute for Systems Biology in 2000, where he became a Faculty member in 2005. His current research focuses on developing tools that can isolate selected cells from patients, and can resolve the behavior of relevant cells from the complex population typically present within each sample. The need to discern responses at the level of individual cells underlies many key challenges in shaping immunity against infections and developing effective vaccine responses, and in probing the steps underlying cancer metastasis. The ISB provides a unique collaborative and interdisciplinary environment to develop new tools, to use the technology to probe immunity and cancer biology, and to collaborate with superb groups at other institutions.

(1) One view is that success should be claimed when microfluidic devices are the default tool that are used on a daily basis, such as how mRNA quality now routinely is assessed with the Agilent bioanalyzer. The vision that inspires me is to aim for a landmark accomplishment, such as a medically-relevant discovery that could not easily have been achieved by other

means. This type of application likely would exploit several of the key advantages of the microfluidics approach, and should combine elements of miniaturization, integration, scaling/parallelization, and automation. We are trying to bring these themes together with a device that is able to measure multiple proteins from each cell of a population of mammalian cells. We anticipate that great insight will follow from measurements that reveal the manner in which individual cells are regulated, and bypass the roadblocks to interpretation imposed by averaged data.

(2) A personal difficulty has been undertaking device development research in a laboratory that previously was focused on biology, and did not have researchers with physical science/engineering training. I was surprised by the relative lack of support by biologist colleagues who preferred to leave device development to others, and advanced their careers by focusing on applying the current generation of available analytical tools rather than developing next-generation tools. I believe that at each moment, science faces critical challenges, and one current challenge lies with purifying relevant cells for analysis and analyzing them directly, while rigorously excluding irrelevant cells. Another challenge within the academic setting is to go beyond the development of a proof-of-concept device, and to be able to develop a device to the point that it readily can be applied by a biologist. Furthermore, academic biologists seem reluctant to credit the incremental successes along the path of development, and express interest only when the final device is fully-operational and has been validated.

(3) While the reality is that few biologists currently utilize microfluidics devices, I think one should be inspired and motivated to develop solutions to critical biological challenges that face researchers and that are not solved with current-generation tools. With the clear recognition of the central role that the cell plays in our conception and understanding of biology, it is not outrageous to anticipate the extraordinary benefits that will result from the widespread application of analytical tools that will provide measurements with single-cell resolution. It is not hard to envision the benefits of moving beyond a dependence on averaged data that suggests what cells might be

doing, and towards single cell data that provides insight into what cells actually are doing.

James M. Spotts



James M. Spotts began his career as a chemist, first at Dartmouth College and then at the California Institute of Technology where earned a doctorate in chemistry in 1998 for laser spectroscopic studies of gas phase metal solvent clusters. He then shifted his research focus to biology, pursuing postdoctoral research in molecular and cellular neuroscience at Children's Hospital Boston and Harvard Medical School. In 2005, he joined to the Institute for Systems Biology as a Senior Research Scientist, where he has focused his efforts on developing microfluidic platforms to perform quantitative measurements from single mammalian cells. Current research projects include the development of devices to perform highly multiplexed protein expression measurements from single cells, to capture and culture single mammalian cells within isolated assay chambers, and to measure single cell transcriptomic profiles.

(1) Biological and biomedical research is entering a phase where a detailed understanding of cellular processes at the single cell level is paramount. A first order "killer application" would directly perform quantitative, highly multiplexed measurements of one cellular variable, either mRNA or microRNA expression, protein expression, or protein post-translational modifications, from each of hundreds to thousands of isolated single cells. Molecular-level snapshots such as these would enable the first direct

comparisons of cellular processes between individual cells, and could reveal correlations in behavior that shed new insight into biological function and responsiveness – information that is inaccessible using standard population-based measurements of cells. A higher order and more distant "killer application" would combine two or more of these single cell measurement capabilities within a single experimental measurement platform. By defining the state of a cell along two or more dimensions, unprecedented detail would be gained about how molecular information is processed and integrated by different molecular agents working in concert within an isolated cellular network.

(2) There are two key challenges that we continuously confront as device developers on one hand, and as research biologists who want to perform routine measurements using these devices on the other. The first challenge is how to bridge the gulf between the often limited functionality of a proof-of-concept device to a scale and format compatible with the routine workflow of the average bench biologist. Merging devices to a standard 96- or 384-well assay format remains inaccessible given the resources of an average academic laboratory, although commercial entities such as Fluidigm have achieved elegant solutions to his challenge. The commercial availability of a standardized assay plate with flexible, defined format onto which custom microfluidic devices could be adapted would be invaluable. The second challenge that we encounter is how to best merge different measurement applications that are often best suited to different materials and formats. The available choices can impose compromises in order to achieve some level of functionality at the expense of performance, or require the adoption of more complicated fabrication procedures and designs that, in turn, impose greater demands on the end user to operate the device.

(3) There is little value to this ongoing background debate whether microfluidic technologies have or have not had a significant impact to date. The bottom line is that the scale, sensitivity, sample numbers, and degree of measurement multiplexing necessary for biomedical researchers to explore new frontiers of biological and biomedical inquiry will require micro/nanofluidic technologies. As agents who can guide this transition, we need to

maintain focus not only on developing devices that directly address the key roadblocks to these groundbreaking applications, but also on how to best translate these proof-of-principle technologies into commercial products that will encourage broad adoption by the biomedical community. Achieving this, impact will invariably follow.

Todd Squires



Todd Squires earned a B.S. in Physics and B.A. in Russian literature at UCLA, then spent a year as a Churchill Scholar at Cambridge University. He earned his Ph.D. in physics at Harvard University, focusing on problems in colloidal hydrodynamics and electrokinetics. He then spent three years as a Lee A. DuBridge Prize Postdoctoral Fellow and National Science Foundation Mathematical Sciences Postdoctoral Fellow at the California Institute of Technology, where he continued theoretical work in electrokinetics and microfluidics, and initiated new studies of nonlinear microrheology. Squires has been on the chemical engineering faculty at the University of California Santa Barbara since 2005. He continues to investigate a range of topics in micro-scale fluid mechanics and transport, both experimentally and theoretically. Honors include the NSF CAREER Award, the Beckman Young Investigator, the Camille and Henry Dreyfus Teacher-Scholar award, the 2009 Francois Frenkiel Award, the 2010 Allan P. Colburn Memorial Lecturer, and the 2010 Pierre-Gilles de Gennes Prize.

(1) For me as a scientist, the “killer application” is truly the lab on a chip itself.

The clean room can serve as an exquisite “machine shop” to construct experimental laboratories that can probe fundamental scientific questions in a way that would be otherwise impossible. More broadly, I feel there are many “killer apps” for microfluidics, ranging from cheap, fast, disposable medical diagnostic chips (enabled by the small sample volumes and favorable physics/chemistry) to micro-reactor/fabrication chips for the synthesis of designer materials. Our lab has been investigating electrokinetic effects, largely to develop intuitively clear (but quantitatively accurate) pictures to understand the core physico-chemical effects that arise in these systems. At the same time, however, we are pursuing strategies that would employ induced-charge electrokinetic effects to establish high fluid pressures with low, AC voltages – with the ultimate goal of enabling small electrokinetic pumps that could drive fluids in arbitrarily complex chips in a way that would be portable or even implantable.

(2) The difficulties we face are many – electrokinetic effects are, at their core, interfacial effects. As such, they depend sensitively on the chemical equilibria established between the surface and the electrolyte. One’s ignorance of the precise state of the surface can limit one’s ability to predict – and thus rationally design – electrokinetic devices. The work described here makes quantitative predictions for the induced-charge electrokinetic flows over a precisely-defined surface, and finds quantitative agreement with experiments. Using “controllably contaminated” surfaces, however, gives flows that are 10–100 times slower than one would expect over “clean” metal surfaces. Understanding why these flows are slow does not change the fact that they are. With what we’ve learned here, we’re now working to explore and design material systems that will maximize ICEK flows, with the hope of developing surfaces that will give robust, strong flows in a range of conditions.

(3) As for the future, several outstanding questions remain concerning electrokinetics, where existing theories make predictions that are wrong on even a qualitative level. Such discrepancies reflect gaps in our core understanding, which I suspect will be resolved within the next decade. Again, microfabrication allows one to precisely define systems to

probe key questions in a way unthinkable decades ago. More broadly, I’ve been fascinated by the growing level of sophistication the community has shown in manipulating ion transport – whether for ICEK, concentration polarization and pre-concentration, water desalination, directed self-assembly of colloids and nanoparticles, and electric double-layer capacitors (‘super-capacitors’) for energy storage. It seems that interesting, fundamental scientific questions as well as compelling applications, will persist for the near future.

Shoji Takeuchi



Shoji Takeuchi received the B.E., M.E., and Dr Eng. degrees in mechanical engineering from the University of Tokyo, Tokyo, Japan, in 1995, 1997, and 2000, respectively. He is currently an Associate Professor in the Center for International Research on Micronano Mechatronics (CIRMM), Institute of Industrial Science (IIS), University of Tokyo. His current interests include membrane protein chips, bottom-up tissue engineering and bio-hybrid MEMS. He received several awards including the award for Advanced Research from the Japan Society of Medical Electronics and Biological Engineering in 2001, Young Scientists’ Prize, the Commendation for Science and Technology by the Minister of Education, Culture, Sports, Science and Technology in 2008, and the JSPS prize from the Japan Society for the Promotion of Science in 2010.

(1) Our group has proposed a meander-shaped dynamic microarray that can transport, immobilize and make an array of samples efficiently with a small amount of sample volume since all of the introduced

beads can be trapped at each site sequentially. We believe our device presented here as paired sample array can be applied to observe molecular diffusion and analyze cell–cell interactions between the two samples; these analyses are very important in the pharmacokinetics studies and tissue engineering and so on.

(2) We had some trouble trapping small samples (smaller than 2 μm), and elastic samples such as single cells, liposomes or micro droplets; they tend to pass through the narrow channels after trapping. I think that, as the next challenge, we need to add some mechanisms in the fluidic channels to overcome the above problems such as micro pillars, valves and electrodes and so on.

(3) In the near future, we will try to improve the dynamic microarray by adding new functions such as: (i) to make an ordered array of heterogeneous samples, (ii) to release all samples at once for reuse, (iii) to increase the density of sample array by modifying the design of the channels, and (iv) to trap a variety of samples such as single cells, protein-coated microbeads, liposomes and spheroids.

Wei Wang



Wei Wang received his Ph.D. degree at the Aerospace School, Tsinghua University, China, majoring in Thermal Engineering, in 2005. After that, he joined the MEMS research center in Peking University (China) as a postdoctoral researcher. From 2007, Dr Wei Wang started his research group on micro/nanofluidics in Peking University (<http://ime.pku.edu.cn/~microfluidics/>). Presently, Dr Wei Wang is an associate professor in Institute of Microelectronics, Peking University and the vice director of MEMS research center in Peking

University. Dr Wei Wang's research interests include polymer micro/nanofabrication and micro/nanofluidics. So far, Dr Wei Wang has published more than 20 peer-reviewed journal articles on related topics.

(1) For me, it's hard to say there are any killer apps in micro/nanofluidics, as we are still on the way to succeed in real commercial applications. But, I would love to take what Prof. G. Whitesides did on the PDMS based soft-lithography and what Prof. A. Manz did on the continuously-flow (bio) reactor as killer applications in the micro/nanofluidics society, as they really changed our minds on how to do micro/nanofluidics and what micro/nanofluidics can do.

(2) As a researcher with a fluid mechanics and microelectromechanical system (MEMS) background, I'm facing difficulties in communication with researchers in the fields of biology or medicine, not the problem of personal communication ability, but the difficulties in establishing 'bridges', rethinking disciplinary borders, and discovering modes of collaboration among people whose 'meanings' and 'processes' are very different, as said by Ronald Schleifer in *Stanford Humanities Review*, 1995, 4(1).

(3) No doubt, micro/nanofluidics holds a bright future, especially when people pay more and more attention to their health, and the micro/nanofluidics technologies have been widely acknowledged as worthy potential solutions for points of care, local health monitoring, disease diagnosis, or even clinical treatments. In my opinion, the main issue which may affect the future of micro/nanofluidics in the above fields is the price/performance ratio of the product. We do need to find out a way to make micro/nanofluidics devices with a reasonable price/performance ratio. To achieve this target, I really think we should learn more from the existing successful technologies, such as integrated circuit (IC), and some microelectromechanical systems (MEMS) applications.

Justin Williams

Justin Williams is an Associate Professor of Biomedical Engineering at the University of Wisconsin-Madison. He obtained his Masters and PhD in Bioengineering from Arizona State University in 2001. He followed with postdoctoral fellowships in Neurosurgery at the University of



Wisconsin and in Neuroengineering at the University of Michigan. He joined the faculty at the University of Wisconsin in 2003 as an Assistant Professor. His research program is centered around the development of implantable microtechnology for treating neurological disease and microfluidic platforms for studying basic neurobiology.

(1) I think an area where LOC applications are poised to make significant progress is in the field of basic biology. An application that could simultaneously explore a myriad of factors, and their interactions, that influence a cell's micro-environment, growth and development would be especially powerful to the biological community. Ideally, this technology would also be robust, scalable and amenable to automation, which are some of the reasons why it remains an illusive challenge to the LOC community.

(2) One of the difficulties we face is in trying to get researchers in other fields to adopt new technologies. There are a number of issues we face in this regard, but in particular, validation of new microtechnologies against existing more standardized techniques can be challenging. For example, there are many different reasons why cells may grow differently in microfluidic environments; some are insightful, others may just be artifacts. It can often be difficult to tease out these differences, and can be a particular stumbling block for new investigators in this field.

(3) The field continues to expand at a rate that can make it difficult to keep up with at times. As fast as it is expanding though, there is clearly still room for continued growth. In the future I hope that microtechnology is going to become ubiquitous in many current fields and will start to emerge into areas that haven't even been considered yet.

Intimidating yet Inspiring: Emerging Investigators special issue

DOI: 10.1039/c0lc90043e



From AEH: Intimidating challenges are routinely (and fearlessly) tackled by our field – this is especially true of our new investigators. In 2009, my co-chair and I at the Gordon Research Conference (GRC) on the Physics and Chemistry of Microfluidics introduced a new session dedicated to “emerging investigators”. The session was spectacular – I was inspired by the presenters’ rigorous content and unabashed creativity, as well as the attendees’ relentless inquiries. I walked away with insight into the problems that these emerging investigators identified as being important, which gave

When Harp Minhas, editor of *Lab on a Chip*, invited us to guest-edit this special Emerging Investigators issue as part of its 10th Anniversary celebrations, we jumped at the opportunity. The generation of scientists and engineers featured in this issue is the first to emerge as independent researchers with expertise targeted directly at our discipline (in contrast to the pioneers of microfluidics, who boldly migrated from other fields). As such, this generation is extraordinarily versatile, and is known for combining diverse tools and approaches – for example, 3D optical ablation with microreactors and mixing (see Hansen’s work, DOI: 10.1039/c004051g), or polymer engineering with 3D-cell culture (see Khademhosseini’s work, DOI: 10.1039/c004732e) – to carry

From ARW: “Wow, that’s an intimidating group.” I muttered these words about the audience just before stepping to the podium to give my first talk at a scientific conference – the Micro Total Analysis Systems (μ TAS) meeting in Enschede, The Netherlands in May, 2000. Despite some nerves, I made it through the talk, and after a degree, a postdoc, and a few years as a professor, I wind up here, with the same thought: “wow, that’s an intim-

me opportunity to reflect on my own work. This special issue of *Lab on a Chip* aims to do the same for a broad and diverse audience. The range of topics included here has brought into sharp focus the intimidating breadth and difficulty of the challenges tackled by emerging investigators in our field. Perhaps most importantly, this issue paints a picture of the problems that the new generation of scientists in our field identifies as being important – and the broad impact their contributions are making across a wide range of disciplines.

out ever-more-complex and useful science. Moreover, this generation is moulding our field in significant ways, moving it from superficial overlap with challenges in chemistry, biology, medicine, and the environment, to the development of actual solutions to real-world problems – see, for example, Karen Cheung’s method for anticancer drug testing (DOI: 10.1039/c004590j) and Aydogan Ozcan’s tool for detecting waterborne parasites (DOI: 10.1039/c004829a). The innovation, creativity, and practicality featured in the papers in this issue of *Lab on a Chip* should make us all optimistic about the future of our field and the positive impact we are having on myriad others.

This generation of researchers is appropriately championed by the Royal

idating group.” But this time, these words are directed towards the authors who contributed to this special issue of *Lab on a Chip*. From Axel Günther’s work growing human arteries on a chip (DOI: 10.1039/c004675b) to Tony Huang’s liquid lenses for on-chip optical analysis systems (DOI: 10.1039/c005071g), this issue features the best of the best in the field of microfluidics and related technologies.



Society of Chemistry (RSC), publisher of *Lab on a Chip*. The RSC fosters the development of young researchers in this field, through poster prizes at conferences (including μ TAS, the GRC, and other important forums), the Pioneers of Miniaturisation Prize (http://www.rsc.org/Publishing/Journals/lc/miniaturisation_prize_2010.asp), the free “Chips and Tips” web feature (http://www.rsc.org/publishing/journals/lc/chips_and_tips/index.asp), showcasing LOC videos on YouTube (<http://www.youtube.com/user/labonachipVideos>) and a flickr presence (<http://www.flickr.com/photos/labonachip/>), a highly active and deeply engaged editorial staff, and more. We are honoured that the tradition continues with this special issue

of *Lab on a Chip*. As you flip through the articles, be sure to enjoy the author biographies and the personal views that we solicited from each of the contributing authors (*i.e.*, answers to questions such as “What interests you most about microfluidics and related technologies?” and “What do you think the future holds for this field?”).

So yes, we are a little intimidated – but more importantly, we are inspired, excited,

and enthusiastic about the maturation of our field. Almost daily we are reminded of the growing importance of collective knowledge and tool sets from unrelated areas, spanning clinical medicine to neuroscience to energy sciences to molecular biology and beyond. So join us in enjoying this special issue: we think that you too will be eager to see where this generation of emerging investigators is taking us.

Aaron Wheeler

Canada Research Chair of Bio-analytical Chemistry
University of Toronto

Amy E. Herr

Assistant Professor, Department of Bioengineering
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Aaron Wheeler completed his Ph.D. in Chemistry in 2003, working with Dick Zare at Stanford University. He then spent two years as an NIH postdoctoral fellow at UCLA, working with Robin Garrell (Chemistry and Biochemistry), with close ties to CJ Kim (Mechanical Engineering) and Joe Loo (Biological Chemistry). Since 2005, Wheeler has been the Canada Research Chair of Bio-analytical Chemistry at the University of Toronto, with a primary appointment in the Chemistry Department, and cross-appointments at the Institute for Biomaterials and Bioengineering (IBBME), and the Banting and Best Department of Medical Research (BBDMR). In Toronto, Wheeler is fortunate to work with a creative, prolific research group, whose success has led to international recognition including a Sloan Fellowship and an Eli Lilly and Company Young Investigator Award. Wheeler's research interests range broadly from proteomics to cell-based assays to clinical analysis, with a central theme focused on the development of practical and useful microfluidic tools.

Amy E. Herr received a BS degree in Engineering & Applied Science from the California Institute of Technology in 1997 and MS (1999) and PhD (2002) degrees from Stanford University in Mechanical Engineering as a US National Science Foundation Graduate Research Fellow, advised by Professors Thomas Kenny and Juan Santiago. From 2002–2007, Prof. Herr was a technical staff member at Sandia National Laboratories. In 2007, she began her appointment as an Assistant Professor of Bioengineering at the University of California, Berkeley and holds a joint appointment as a Faculty Scientist at the Lawrence Berkeley National Laboratory. She has served as Chair (2009) and Vice-chair (2007) of the Gordon Research Conference (GRC) on the Physics & Chemistry of Microfluidics, as well as on international conference technical program committees including μ TAS since 2008, Transducers, and IEEE Sensors. She has been honored to be named: a 2010 Alfred P. Sloan Research Fellow in chemistry, a 2009 Defense Advanced Research Projects Agency (DARPA) Young Faculty Awardee, a 2009 Hellman Family Faculty Awardee, a 2008 Regents' Junior Faculty Fellow, as well as a 2007 Outstanding Mentor from Sandia. Her research interests center on advancing quantitation in life sciences and clinical problems, particularly through the study and application of electrokinetic phenomena in multi-stage microanalytical technology.