Published in final edited form as:

Lab Chip. 2010 January 21; 10(2): 235. doi:10.1039/b914874d.

Spatially resolved shear distribution in microfluidic chip for studying force transduction mechanisms in cells[†]

Jianbin Wang^b, Jinseok Heo^{a,b}, and Susan Z. Hua^{a,b}

^a Department of Physiology and Biophysics, SUNY-Buffalo, Buffalo, NY, 14260, USA

^b Department of Mechanical and Aerospace Engineering, SUNY-Buffalo, Buffalo, NY, 14260, USA

Abstract

Fluid shear stress has profound effects on cell physiology. Here we present a versatile microfluidic method capable of generating variable magnitudes, gradients, and different modes of shear flow, to study sensory and force transduction mechanisms in cells. The chip allows cell culture under spatially resolved shear flow conditions as well as study of cell response to shear flow in real-time. Using this chip, we studied the effects of chronic shear stress on cellular functions of Madin-Darby Canine Kidney (MDCK), renal epithelial cells. We show that shear stress causes reorganization of actin cytoskeleton, which suppresses flow-induced Ca^{2+} response.

Introduction

The shear stress introduced by flow regulates many cellular functions, including proliferation, migration, transport, and apoptosis.¹ For example, the epithelial cells in the kidney tubule are subject to the urine flow that modulates ion transport and kidney physiology.^{2,3} Vascular endothelial cells are constantly exposed to hemodynamic forces caused by the flow of blood that affects cell remodeling and other cell functions.⁴ There is increasing interest in studying flow sensory and force transduction mechanisms that convert the mechanical force into biochemical signals, and further alter cell physiological functions and phenotypes.

A flow chamber consisting of two parallel plates with a spacer is commonly used to study the effects of shear stress on cells. Such an apparatus produces a uniform flow field, with shear stress controlled by change in the inlet flow rate.⁵ The flow chamber can also be defined by a gasket with tapered geometry to generate shear stress gradient.⁶ However, in physiological or pathological conditions, cells are often subjected to shear stresses with a varying degree of complexity, which modifies the internal organization and physiological functions of the cells.⁷ Using microfabrication technology, small features with various designs can be integrated in the microfluidic chamber, enabling precise control of the local microenvironments, as shown in this paper. Additionally, a microfabricated pre-bonded chip device requires only minuscule amounts of cell culture media or reagents, providing convenience for long-term cell culture under a flow condition. A diversity of microfluidic biochips has been developed to generate a concentration gradient of biochemicals⁸ or continuous perfusion⁹ to manipulate the microenvironment during cell growth. These chips were used to study cell adhesion,¹⁰ growth and morphology,^{11,12} and viability.¹³ In this paper, we present a simple microfluidic chip that

[†]Electronic supplementary information (ESI) available: Diagram of device assembly for optical/fluorescence microscopy, movies to show Ca^{2+} increase in MDCK cells and graph to show Ca^{2+} response of cells from different regions in the same chamber. See DOI: 10.1039/b914874d

Correspondence to: Susan Z. Hua.

generates a wide range and modes of shear stresses within a perfusion chamber, enabling one to examine the effect of shear stresses on cell growth and cell functions in a high-throughput manner while other culture conditions remain the same.

To demonstrate the efficacy of the chip, we have studied Madin-Darby Canine Kidney (MDCK), renal epithelial cells. Like endothelial cells, renal epithelial cells are constantly exposed to varying shear flow. Cells respond to increases in shear stress by rapidly increasing Ca^{2+} influx possibly through stress-sensitive ion channels and subsequent Ca^{2+} -induced release of Ca^{2+} from intracellular stores.¹⁴ The Ca^{2+} uptake activates a cascade of signal pathways that regulates ion transport, reorganization of actin cytoskeleton, and cell volume regulation.¹⁵ In a feedback scheme, the modification of cytoskeleton structure may alter downstream cellular functions and physiology. The widespread interest and study of stress transmission and the role of cytoskeletal reorganization in shear-induced Ca^{2+} influx of renal epithelial cells are raising new questions for inquiry.^{15,16} Using our microfluidic device, we show that chronic exposure of shear stress induces reorganization of actin filaments in renal epithelial cells (MDCK) progressively. The disruption of actin fibers caused by shear stress diminishes the intracellular Ca^{2+} uptake in response to subsequent changes in a fluid flow, indicating that the cytoskeleton is closely associated with mechanosensitive channels that mediate Ca^{2+} entry.

Materials and methods

Microfluidic device design and construction

The microfluidic chip consists of a micro-sized barrier in the path of the fluid flow and is designed to generate a wide range and modes of shear stresses within the flow chamber. Fig. 1A shows a SEM image of a section of the microfluidic chip. As shown in Fig. 1A, an oblique barrier 60 μm wide, 1500 μm long, was located in a flow chamber having dimensions of 500 μm wide, 15 mm long (full channel not shown), and 100 μm in chamber height. The distribution of shear stresses in the vicinity of the barrier region was designed to provide three different flow conditions: (1) uniform shear stress (outside the oblique barrier region); (2) stable fluid flow with linear shear gradient (below and above the oblique barrier); and (3) non-uniform flow field (at the tip of the barrier).

A finite element model was developed to simulate fluid flow and distribution of shear stresses in the chamber using commercial CFD software, ANSYS CFX. The steady-state Navier–Stokes equation was solved to determine the flow field. The solution of the flow field was assumed to be converged when the dimensionless residuals are less than 10^{-4} . The distribution of wall shear stress in the barrier region is shown in Fig. 1B. The wall shear stress along the red dashed line in Fig. 1B is plotted in Fig. 1C, showing a shear gradient. The shear stress across the channel in the barrier region (along the yellow dashed line in Fig. 1B) is shown in the inset in Fig. 1C.

We used Hough Transform to analyze the orientation of actin filament within cells. Using Matlab R2008b (Mathworks Inc.), standard Hough transformation was applied to the images of selected single cells and the angles and relative position of fibers were calculated.

The microfluidic chamber was constructed on a cover glass as a substrate using a PDMS film patterned with the geometry of microfluidic channels and the barrier (Fig. 1A). Briefly, the PDMS film was casted against SU-8 photoresist master fabricated on a silicon wafer. Holes were punched in the PDMS to create inlet and outlet ports for delivering solution. The whole chip can fit in a 35 mm cell culture dish. For fluorescence imaging, the microfluidic chip was placed in a homemade assembly as shown in the ESI.[†]

Cell culture in microfluidic device

Madin-Darby Canine Kidney (MDCK) cells (ATTC) were grown in Dulbecco's modified Eagle medium (DMEM) containing 10% fetal bovine serum and 1% penicillin and streptomycin. After the microfluidic chip was perfused with the cell culture media for 24 hrs, culture media containing suspended cells were introduced into the chamber. The microfluidic chip was then placed in the incubator at 37 °C for ~1 hr to allow the cells to adhere to the bottom of the channel. To culture cells under static conditions, media in the channel was changed gently every 12 hrs to sustain the cell growth. To culture cells under a shear flow, the cells were allowed to grow to ~80% confluence, and were exposed to shear flow controlled by a peristaltic pump.

Staining actin-filament in a microfluidic chip

Cells were rinsed by introducing phosphate-buffered saline (PBS, 10 mM, pH 7.4) solution into the chip for 10 min. To fix the cells, 4% formaldehyde solution in PBS was filled in the channel for 10 min at room temperature, then PBS buffer was perfused for 10 min to rinse the cells. Cells were permeabilized with 0.5% Triton X-100 in PBS for 10 min and then rinsed with PBS buffer for another 10 min. Then, to minimize nonspecific adsorption, cells were incubated with 1% bovine serum albumin (BSA) in PBS buffer for 20–30 min. To stain actin filaments, cells were incubated with 330 nM of fluorescein-phalloidin (Invitrogen, CA) for 30 min, followed by rinsing with PBS buffer for 10 min. Replacing PBS buffer with antifading reagents (Proglong Gold antifading reagent, Invitrogen, CA) helped reduce photo-bleaching during fluorescence image acquisition. Fluorescence micrographs were collected with inverted epi-fluorescence microscope (Axiovert 200M, Zeiss, Germany) equipped with CCD camera (AxioCam MRm, Zeiss, Germany) and FITC filter set (Ex: 470 ± 40 nm; dichroic filter: 495 nm; Em: 525 ± 50 nm). A 63× oil-immersion objective lens was used. For fluorescence imaging, the microfluidic chip was placed in a homemade assembly (see ESI[†]).

Cytosolic Ca²⁺ measurements

To load Ca²⁺ sensitive fluorescence dye, the chamber was perfused slowly with isotonic solution for a few minutes to wash the cells, and then filled with isotonic solution containing 5 μM Fluo-4 AM (Invitrogen, Carlsbad, CA). After the incubation of the chip at 37 °C for 30 min, the cells were rinsed and incubated at room temperature for another 15 min. Fluorescence micrographs were recorded using the same Zeiss fluorescence imaging system as above. Time-lapse experiments were recorded using AxioVision software (Zeiss).

Solutions and reagents

Isotonic solutions consisted of 75 mM NaCl, 5 mM KCl, 1 mM CaCl₂, 2 mM MgCl₂, and 10 mM HEPES titrated to pH 7.4. Osmolarity was adjusted to 320 mOsm with mannitol and measured with an osmometer (Model 303, Advanced Instrument).

Results

MDCK cells were cultured in the microfluidic chamber while being subjected to various flow rates ranging from 2.7 μL/min to 30 μL/min, corresponding to a wall shear stress of 0.54 dyn/cm² to 6 dyn/cm². Reynolds number ranges from 0.45 to 5 in the straight part of the channel. The level of shear stresses is within the range of physiological conditions.¹⁴ Fig. 2 shows representative optical micrographs of the control cells cultured in a static condition and cells

[†]Electronic supplementary information (ESI) available: Diagram of device assembly for optical/fluorescence microscopy, movies to show Ca²⁺ increase in MDCK cells and graph to show Ca²⁺ response of cells from different regions in the same chamber. See DOI: 10.1039/b914874d

exposed to a constant flow rate of 5.5 $\mu\text{L}/\text{min}$ (1.1 dyn/cm^2) for a period of 24 hrs, respectively. Epithelial cells subjected to shear stress did not show a significant change in cell shape, unlike endothelial cells that align themselves along flow direction.

To evaluate the effect of chronic shear stress on the cytoskeleton structures, we analyzed the development of actin cytoskeleton under various flow conditions. Abundant actin fibers were found to be uniformly distributed in the control cells cultured in a static condition (Fig. 3A). This observation is in agreement with the previously reported results.¹⁷ Laminar flow of 5.5 $\mu\text{L}/\text{min}$ (1.1 dyn/cm^2) for 2.5, 6, and 18 hrs induced a progressive reorganization of actin cytoskeleton as shown in Figs. 3B–D, respectively. Clots of tangled actin stress fibers were formed close to cell junctions or nuclei when cells were subjected to the shear flow for 2.5 hrs (Fig. 3B). After 6 hrs of shear flow the actin filaments accumulated around the edge of the cells, but diminished in the cytosol (Fig. 3C). An 18 hr exposure of the cells to shear flow caused further aggregation of peripheral actin cytoskeleton, eliminating most of actin fibers from the cytosol (Fig. 3D). Using Hough transformation¹⁸ we calculated the orientation dependence of the actin fibers on fluid shear stress.^{19,20} Single cell images from each picture of Fig. 3A–D were selected and the orientation of actin fibers within the selected cells was calculated, and is shown in Fig. 3E–H, respectively. The results clearly demonstrate that actin fibers are well aligned along certain directions in the cells grown in static condition ($\sim 35^\circ$ angle in Fig. 3E), but randomly aligned with no preferred orientation in the cells subjected to shear flow of 5.5 $\mu\text{L}/\text{min}$ for 18 hrs (Fig. 3H). The extent of reorganization of actin cytoskeleton depended on the level of shear stress and exposure times. For example, under low shear stress level, such as a flow rate of 2.7 $\mu\text{L}/\text{min}$ (0.54 dyn/cm^2), only few cells showed tangled actin fibers even after 18 hrs of exposure to the shear stress. In contrast, cells subjected to a shear flow of 30 $\mu\text{L}/\text{min}$ (6 dyn/cm^2) exhibited a complete disruption of cytoskeleton within 6 hrs (not shown). A similar cytoskeletal reorganization induced by flow was observed in proximal tubule cells, another kidney tubule cell, that was associated with the formation of tight junctions and adherent junctions.^{16,17}

Since our microfluidic device can generate a shear stress gradient, it can be conveniently used to observe the effect of a broad range of shear stresses on cells in a single experiment. After cells were subjected to an inlet flow rate of 2.7 $\mu\text{L}/\text{min}$ (0.54 dyn/cm^2) for 2.5 hrs, we examined the cells in two locations along the wedge-shaped area below the barrier. Cells located at the broad and narrow sections of the wedged area experienced a shear stress of $\sim 0.5 \text{ dyn}/\text{cm}^2$ and 3.0 dyn/cm^2 , respectively. While partial cytoskeleton tangling was observed from cells in the lower shear stress region (Fig. 4A), more lateral cytoskeleton accumulation was observed in the cells grown in the narrow region (Fig. 4B).

An acute increase in the fluid flow rate causes an increase in intracellular Ca^{2+} concentration in most kidney epithelial cells including MDCK.¹⁴ Reorganization of cytoskeleton due to long term exposure to shear stress may obstruct the mechanosensor transduction mechanisms, and therefore, impede Ca^{2+} response when subjected to an acute flow rate. To examine the response to an acute increase in flow rate of cells that were previously exposed to shear flow, we measured time dependent responses of intracellular Ca^{2+} . MDCK cells were cultured in a shear flow of 5.5 $\mu\text{L}/\text{min}$ for 3 hrs, and then stimulated with an acute change in flow rate. Typical fluorescence images of MDCK cells before and after the acute increase in the flow rate from 0.3 $\mu\text{L}/\text{min}$ to 20 $\mu\text{L}/\text{min}$ are shown in Fig. 5A and B, respectively. The time course of relative fluorescence intensity that reflects the change in intracellular Ca^{2+} concentration in response to the change in the flow rate is shown in Fig. 5C (also see Movies in ESI[†]). Cells were previously subjected to a flow rate of 5.5 $\mu\text{L}/\text{min}$ for 0, 3, 6, and 18 hrs, respectively. The acute change of shear flow caused substantial increase in intracellular Ca^{2+} of control cells (Fig. 5C). Long-term exposure of cells to the shear stress reduced the intracellular Ca^{2+} response to further

change in flow rate. As the cells experienced the shear stress for longer times, intracellular Ca^{2+} responses to an acute change in the shear flow became weaker.

To further elucidate the advantages of the chip, we have measured the time course of Ca^{2+} response of MDCK cells to an acute flow change from two regions in the same chamber, the narrow region above the barrier and the broad region below the barrier. The cells were pre-exposed to an inlet flow rate of 5.5 $\mu\text{L}/\text{min}$ for 6 hrs. Weaker Ca^{2+} increase was observed from the cells grown in the narrow region, indicating a higher degree of reorganization of cytoskeleton due to higher shear stress in the narrow region (see ESI[†]). These results are consistent with the data obtained by directly changing the flow rate (Fig. 5C).

Discussion

Flow stimulated intracellular Ca^{2+} increase is widely observed in epithelial cells, and the sensors are postulated to be either located on the apical membrane, such as primary cilia, microvilli, stretch-activated ion channels, or on the basolateral plane, such as integrins.¹⁵ In MDCK cells, it has been reported that bending of primary cilia in MDCK cells is responsible for the intracellular Ca^{2+} increase.²¹ The ciliary bending activates membrane proteins polycystin 1 and 2 (PC1 and PC2),²² where PC1 senses the bending of the cilium, whereas PC2 appears to function as a stretch-activated Ca^{2+} -permeant channel, causing Ca^{2+} entry.²³ Although we have not found a direct evidence of integrins as a flow sensor in MDCK cells, it has been shown that a fluid shear stress at cell apical surface could be transmitted to the cell's basal focal adhesions domain *via* actin cytoskeleton, thus mediating the stretch-activated channels to facilitate Ca^{2+} uptake.²⁴ Many studies have suggested that shear sensing by epithelial cells occurs at multiple places, and they share similar sets of second messengers, such as Ca^{2+} concentration, to alter cell functions.¹⁵

Our results show that chronic shear flow causes reorganization of actin cytoskeleton in MDCK cells. This is in agreement with previous works in other renal epithelial cells.¹⁷ The flow-induced Ca^{2+} response was diminished when cells were pre-exposed to shear flow and actin filaments were significantly reorganized (Fig. 5), suggesting the loss of flow sensor or an impeded signal transduction from the flow sensor to ion channels that permit Ca^{2+} influx. The relatively low fluid shear stress (about 10-fold lower than shear stress applied to endothelial cells) cannot damage cilia sensor, because primary cilia can be grown under a shear stress level similar to ours.¹⁶ Since basic structure of primary cilia is microtubule, shear force sensed by sensor proteins, such as PC1, can be distributed to other local domains of cells *via* cytoskeleton. It was shown that cytoskeleton structure has a profound effect on stress transmission and distribution on cell membrane, thereby, regulating Ca^{2+} influx *via* stretch-activated ion channels, such as PC2, in renal epithelial cells.²⁴ In fact, the modification of actin cytoskeleton structure using drugs, such as cytochalasin B or D, also abolished the flow-induced Ca^{2+} influx in many other epithelial cells.²⁴ Therefore, chronic exposure of MDCK cells to fluid shear stress may affect the force transduction pathways *via* cytoskeleton reorganization other than disable the mechanosensors in the cells. In a separate experiment we have shown that a flow-induced Ca^{2+} entry was mediated by mechanosensitive channels, because the Ca^{2+} response was inhibited by GsMTx4, a blocker specific to stretch-activated channels (data not shown).²⁵ Thus, we conclude that flow-induced reorganization of actin cytoskeleton could affect the mechanotransduction mechanism that regulates the activity of mechanosensitive sensitive ion channels, which is a pathway for Ca^{2+} entry.

Conclusions

We have presented a microfluidic device that can be used to generate complex flow patterns for culturing cells in a perfusion chamber. The chamber allows full access to fluorescence

microscopy that can be used to monitor cell response to flow or other stimuli in real time. Since physiological flow can be highly modified during various physiological or pathological conditions, the flow chamber can be used to mimic physiological conditions in order to gain an understanding of the pathophysiology of various renal or vascular diseases.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by National Institute of Health DK77302 and National Science Foundation NSF CMMI-0825707. We would like to thank Dr. Frederick Sachs for helpful discussions.

References

1. Tzima E, Irani-Tehrani M, Kiosses WB, Dejana E, Schultz DA, Engelhardt B, Cao G, DeLisser H, Schwartz MA. *Nature* 2005;437:426–431. [PubMed: 16163360]
2. Woo K, Dutta AK, Patel V, Kresge C, Feranchak AP. *J Physiol* 2008;586:2779–2798. [PubMed: 18388137]
3. Apodaca G. *Am J Physiol Renal Physiol* 2002;282:F179–190. [PubMed: 11788431]
4. Li YS, Haga JH, Chien S. *J Biomech* 2005;38:1949–1971. [PubMed: 16084198]
5. Lawrence MB, Springer TA. *Cell* 1991;65:859–873. [PubMed: 1710173]
6. Rossi M, Lindken R, Hierck BP, Westerweel J. *Lab Chip* 2009;9:1403–1411. [PubMed: 19417907]
7. Liu W, Murcia NS, Duan Y, Weinbaum S, Yoder BK, Schwiebert E, Satlin LM. *Am J Physiol Renal Physiol* 2005;289:F978–988. [PubMed: 15972389]
8. Shamloo A, Ma N, Poo MM, Sohn LL, Heilshorn SC. *Lab Chip* 2008;8:1292–1299. [PubMed: 18651071]
9. Korin N, Bransky A, Dinnar U, Levenberg S. *Lab Chip* 2007;7:611–617. [PubMed: 17476380]
10. Lu H, Koo LY, Wang WM, Lauffenburger DA, Griffith LG, Jensen KF. *Anal Chem* 2004;76:5257–5264. [PubMed: 15362881]
11. Song JW, Gu W, Futai N, Warner KA, Nor JE, Takayama S. *Anal Chem* 2005;77:3993–3999. [PubMed: 15987102]
12. Tanaka Y, Sato K, Yamato M, Okano T, Kitamori T. *J Chromatogr, A* 2006;1111:233–237. [PubMed: 16569583]
13. Hallow DM, Seeger RA, Kamaev PP, Prado GR, LaPlaca MC, Prausnitz MR. *Biotechnol Bioeng* 2008;99:846–854. [PubMed: 17879304]
14. Liu W, Xu S, Woda C, Kim P, Weinbaum S, Satlin LM. *Am J Physiol Renal Physiol* 2003;285:F998–F1012. [PubMed: 12837680]
15. Quinlan MR, Docherty NG, Watson RW, Fitzpatrick JM. *Am J Physiol Cell Physiol* 2008;295:C1–F11.
16. Duan Y, Gotoh N, Yan Q, Du Z, Weinstein AM, Wang T, Weinbaum S. *Proc Natl Acad Sci U S A* 2008;105:11418–11423. [PubMed: 18685100]
17. Essig M, Terzi F, Burtin M, Friedlander G. *Am J Physiol Renal Physiol* 2001;281:F751–762. [PubMed: 11553522]
18. Ballard DH. *Pattern Recognit* 1981;13:111–122.
19. Kurner J, Frangakis AS, Baumeister W. *Science* 2005;307:436–438. [PubMed: 15662018]
20. Chandran PL, Barocas VH. *J Biomech Eng* 2004;126:152–166. [PubMed: 15179845]
21. Praetorius HA, Spring KR. *J Membr Biol* 2001;184:71–79. [PubMed: 11687880]
22. Nauli SM, Alenghat FJ, Luo Y, Williams E, Vassilev P, Li X, Elia AE, Lu W, Brown EM, Quinn SJ, Ingber DE, Zhou J. *Nat Genet* 2003;33:129–137. [PubMed: 12514735]
23. Gonzalez-Perrett S, Kim K, Ibarra C, Damiano AE, Zotta E, Batelli M, Harris PC, Reisin IL, Arnaout MA, Cantiello HF. *Proc Natl Acad Sci U S A* 2001;98:1182–1187. [PubMed: 11252306]

24. Alenghat FJ, Nauli SM, Kolb R, Zhou J, Ingber DE. Exp Cell Res 2004;301:23–30. [PubMed: 15501441]
25. Suchyna TM, Tape SE, Koeppe RE 2nd, Andersen OS, Sachs F, Gottlieb PA. Nature 2004;430:235–240. [PubMed: 15241420]

Fig. 1.

A microfluidic device to study the effect of shear stress on cells. (A) SEM image of casted PDMS film containing a section of the microfluidic chamber. (B) Simulation of wall shear stress distribution in the microfluidic chamber with an inlet flow rate of 5 μL/min. A uniform shear stress is generated in the channel away from the oblique barrier to fluid flow, and a shear gradient is generated between the barrier and the walls. (C) Wall shear stress along the dashed line (red) in Fig. 1B, showing a shear gradient. Inset in Fig. 1C shows wall shear stress distribution across the channel along the dashed line (yellow) in Fig. 1B.

Fig. 2.
(A) MDCK cells grown in the fluidic channel under static condition for 3 days. (B) Cells grown in constant flow rate of 2.7 $\mu\text{L}/\text{min}$ for 24 hours after static growth of 2 days. Scale bar represents 50 μm .

Fig. 3. Effect of fluid shear stress on the development of actin cytoskeleton of MDCK cells. (A) Typical fluorescence image of actin cytoskeleton in cells cultured in static conditions. (B)–(D) Reorganization of actin cytoskeleton of MDCK cells subjected to shear flow of 5.5 $\mu\text{L}/\text{min}$ for 2.5, 6, and 18 hrs, respectively. The images were taken in the uniform flow region, away from the oblique barrier. (E)–(H) Hough transformation of selected single cells from (A)–(D), respectively, showing orientation dependence of actin fibers on fluid shear stress.

Fig. 4.

Effect of shear stress on rearrangement of actin cytoskeleton observed from two locations in the same chamber - in the broader region between oblique barrier and the wall (A) and the narrow region between the oblique barrier and the wall (B). The shear stress was estimated at 0.5 dyn/cm^2 and 3.0 dyn/cm^2 at an inlet flow rate of $2.7 \text{ } \mu\text{L/min}$.

Fig. 5.

Flow induced Ca^{2+} increase in MDCK cells cultured under various flow conditions. (A) and (B) Fluorescence images of MDCK cells in microfluidic chamber before and after a change in flow rate from 0.3 $\mu\text{L}/\text{min}$ to 20 $\mu\text{L}/\text{min}$. The cells were pre-exposed to fluid shear stress of 5.5 $\mu\text{L}/\text{min}$ for 3 hrs in the same chamber. Ca^{2+} increase was observed in ~60% of the cells in various degrees; circles indicate typical cells selected in Fig. 5C. (C) Time resolved Ca^{2+} increase in response to an acute change in flow rate for cells cultured in a flow of 5.5 $\mu\text{L}/\text{min}$ for 0 (black), 3 (red), 6 (green), and 18 hrs (blue). Pre-exposure to fluid shear stress diminished the intracellular Ca^{2+} response to flow change. The error bars show the standard deviation of 12 individual cells.