Supplementary Information:

Engineering of polarized tubular structures in a microfluidic device to study calcium phosphate stone formation

Zengjiang Wei[a,b], Prince K. Amponsah[c], Mariyam Al-Shatti[c], Zhihong Nie[b,*], Bidhan C. Bandyopadhyay[c,*]

[a]Research Institute of Materials Science, South China University of Technology, Guangzhou 510640, China

[b]Dr. Zhihong Nie, Assistant Professor, Department of Chemistry and Biochemistry, University of Maryland, College Park, MD 20742, USA, E-mail: znie@umd.edu.

[c]Dr. Bidhan C. Bandyopadhyay, Principle Investigator, Calcium Signaling Laboratory, DVA Medical Center, 50 Irving Street, NW, Washington, DC 20422, USA E-mail: bidhan.bandyopadhyay@va.gov.

*Corresponding authors

Materials and Chemicals

Tetraethoxysilane (TEOS), methyltrimethoxysilane (MTES), HCl (37 wt%), ethanol, $CaCl_2$, Na_3PO_4 and Fibonectin were purchased from Sigma-Aldrich. Poly(dimethylsiloxane) (PDMS) (Sylgard 184 Silicone Elastomer Kit) was purchased from Dow Corning, Midland, MI. Anti-Z01 (mouse; 1:200 dilution) and Anti-Na^+, K^+-ATPase (rabbit; 1:100) antibodies were purchased respectively from Invitrogen and Sigma chemical. All chemicals were used as received. Water used in this experiment was purified by deionization and filtration with a Millipore (MA, USA).

Fabrication of microchannels with circular cross-section

Preparation PDMS channel. The polydimethylsiloxane (PDMS) microfluidic device was prepared using a typical soft lithography method.[1] The procedures are described briefly as follows. First, 18 g Sylgard 184 Silicone elastomer oligomer and 2g cross-linker were mixed together by an agitator. The mixture was degased to remove bubbles by placing it in a vacuum desiccator and evacuating the chamber. The PDMS precursor was then poured onto a silicon masters in a petri dish, and cured in an oven at 65^{o}C overnight. The cured PDMS with groves was released from the mode and bonded onto another piece of PDMS substrate using an Oxygen Plasma processing.

Glass coating. The PDMS microfluidic channels were coated with a layer of glass using sol-gel approach developed by Weitz *et al.*.[2] Briefly, TEOS (2mL), MTES (2mL), Ethanol (2mL), and Water (2mL, pH=4.5) were fully mixed and sonicated for 5 minutes. The mixture was placed in an oven at 65 ^{o}C overnight. Before glass coating, the PDMS microfluidic channel was placed on a hot plate for 5 mins at 80 ^{o}C. The precursor mixture was then injected into the microchannel using a syringe connected with a plastic tube. This process was repeated for about 10 times to achieve targeted thickness of glass coating. During the

process, the microfluidic device was maintained on the hot plate at 80 ^{o}C. Finally the microchannel was rinsed wtih ethanol and checked under a microscope (Figure SI1).

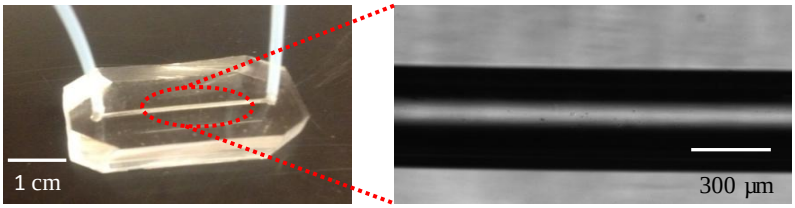

Figure SI1. A photograph of a microfluidic device with microchannels coated with a layer of glass and optical microscopic image of the glass coated microchannel.

Cell Culture and Transfection

SMIE and HK2 cells were cultured in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% fetal calf serum, 2mM glutamine, 1% penicillin/streptomycin at 37 ℃ in 5% CO_2. SIME cells were transiently transfected with 1 µg of required plasmids (GFP) using Lipofectamine reagent 2000. All these reagents were purchased from Invitrogen.

Injection and culture of cells inside microchannels

Cells (~1x10^{6} cells/mL) were directly injected into the channel using a syringe pump. The device was placed in an incubator for 10 min to allow cells to settle. The devices were rotated by 90^{o} to reseed the cells again in the microchannel. Afterwards, the device was placed in an incubator for 10 min again. To avoid cell detachment from the walls, the flow rate of liquids should be less than 0.1 mL/h to minimize the shear stresses on attached cells. This step was repeated twice to cover the entire surface of the circular channels with cells. During the reseeding processes, after the last cell suspension was injected, the microchannels were placed upright into an incubator for 4 h. Afterwards, 500 mL of culture media was continuously injected into each channel to feed attached cells, and to remove any unbound cells.

Immunofluorescence and confocal imaging

The cells cultured in the channel were rinsed with phosphate buffer saline (PBS) pH 7.4 and then fixed with 3% paraformaldehyde solution in PBS, for overnight at 4^{o}C. Afterwards cells were rinsed with PBS, and treated with 100 mM of glycine in PBS for 20 min. Cells were then washed and permeabilized with methanol at -20°C for 5 min. Following incubation with a blocking solution containing 5% donkey serum and 0.5% BSA in PBS (PBS-BSA) for 20 min, the cells were incubated with primary antibodies for 1 h at room temperature, washed with PBS-BSA and probed with the required FITC or rhodamine-conjugated secondary antibody (Jackson Immuno Research, West Grove, PA). The channels containing the cells

were mounted with antifade reagent (Electron microscopy sciences, Hatfield, PA). Fluorescence images were taken using a confocal laser scanning microscope (Ziess 710) attached to an inverted confocal microscope microscope. Details of the images are indicated in the Figure legend. Staining of Z01 and NaK were performed as described earlier. [3]

Raman confocal imaging in MF systems

Raman measurements were carried out using a Horiba LabRAM confocal Raman microscope. A microfluidic device was placed under a Raman microscope equipped with automation stages, and renal fluids were introduced into the microchannel using syringe pumps. The flow rate of the fluids was adjusted in the range of 1.0×10^{-4} to 10 mL/hr, in order to simulate the fluidic shear in renal tubules. After selection of a focusing point, the composition of species flowing or precipitated in microchannels was monitored by collecting Raman spectra.

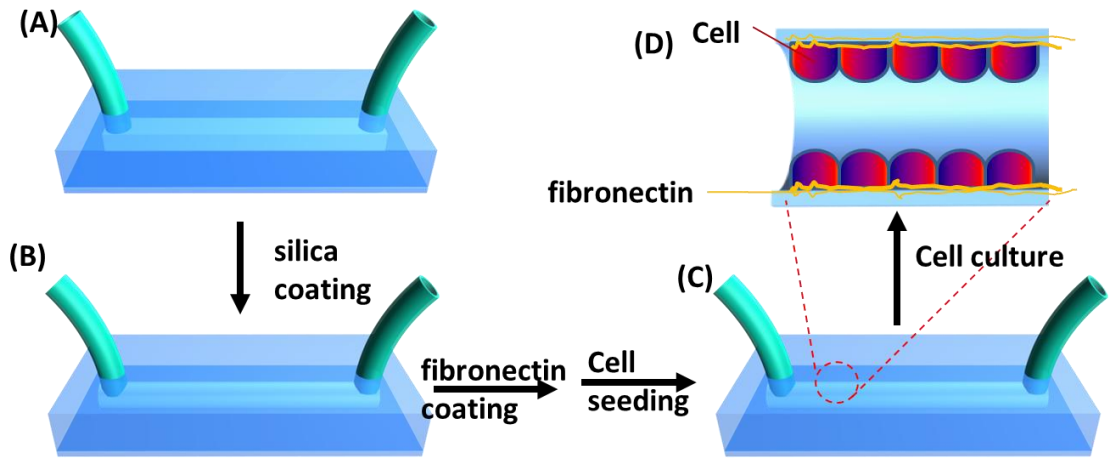

Figure SI2. Fabrication of a microfluidic device and cell culture in the device.

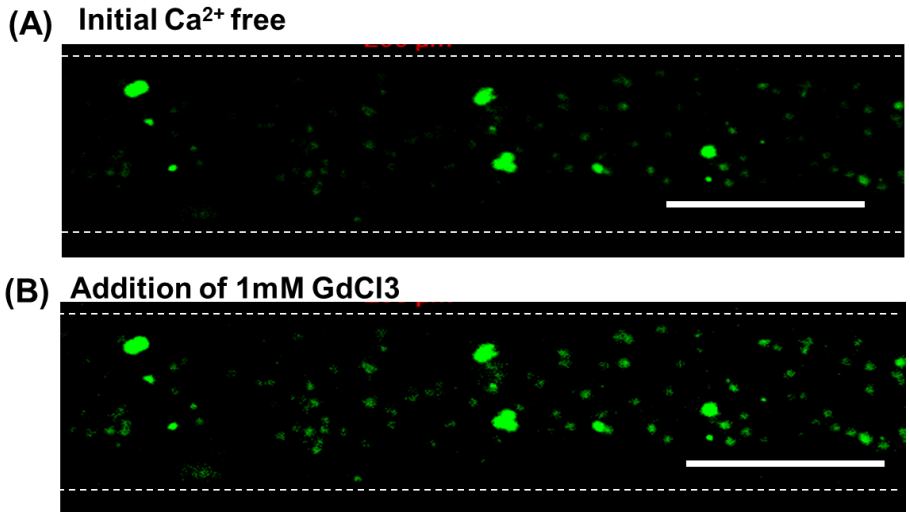

Figure SI3. Intracellular Ca^{2+} profile of renal proximal tubular cells flowing HBSS with (A) Ca^{2+} free and (B) 1 mM $GdCl_3$, into the proximal tubules inside MFs. Scale bars are 200 μm.

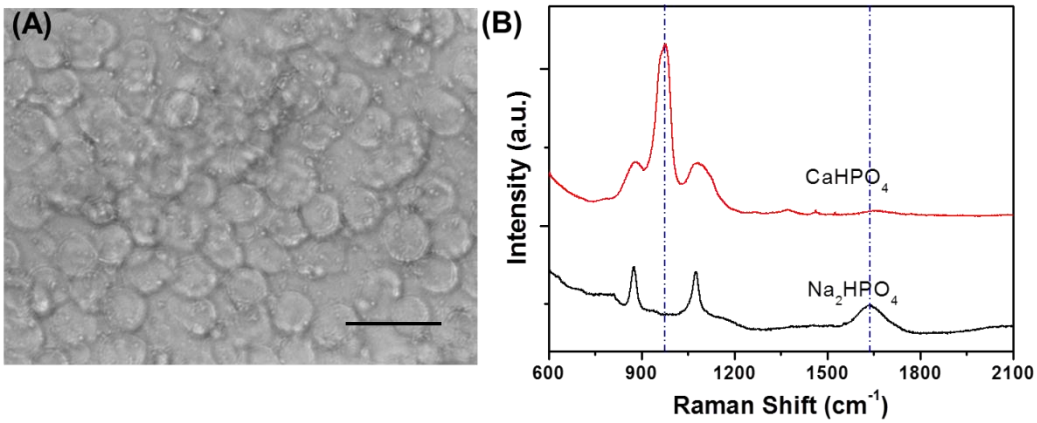

Figure SI4. A. Optical microscopy image of proximal renal tubules deposited with $CaHPO_4$ crystals on the cell walls within microfluidic channels. The spherical white spots are the $CaHPO_4$ deposits. B. Raman spectra of HPO_4^{2-} in solution and in $CaHPO_4$ precipitates, showing the feasibility of monitoring stone formation in MFs. The Raman spectra were obtained by scanning x-y plane at the middle of z-axis of the microchannel using a confocal Raman microscopy. Scale bar is 50 μm.

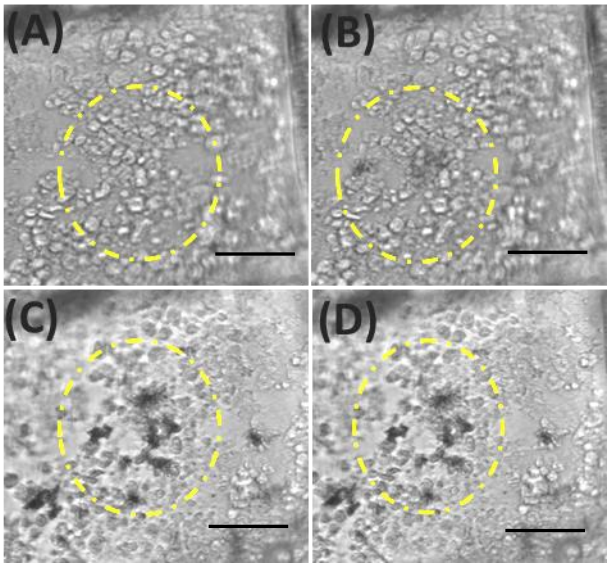

Figure SI5. Optical microscopy image of proximal renal tubules deposited with calcium oxalate crystals on the cell walls within microfluidic channels at different times after flowing precursors: (A) 5 mins; (B) 10 mins, (C) 15 mins, (D) 20 mins. The black clusters are calcium oxalate stones. Scale bars are 200 μm.

References:

1. Xia, Y.; Whitesides, G. M. *Angew. Chem.* **1998**, 110, 568-594.
2. Abate, A. R.; Lee D.; Do, T.; Holtze, C.; Weitz, D. A. *Lab Chip* **2008**, 8, 516-518.
3. Bandyopadhyay, B.C.; Swaim, W.D.; Liu, B.; Redman, R.S.; Patterson, R.L.; Ambudkar, I.S. *J. Biol. Chem.* **2005**, 280, 12908-12916.