DEVELOPMENT OF METHOD FOR SIMULTANEOUS MEASUREMENT OF VISCOSITY AND SURFACE TENSION FORCE IN BIO-MIMETIC EXTENDED-NANO SPACE

Lixiao Li, Yutaka Kazoe, Kazuma Mawatari, Yasuhiko Sugii and Takehiko Kitamori

School of Engineering, The University of Tokyo, Japan

ABSTRACT

Understanding fluid and interfacial properties in inter/intracellular space is crucial for biological system. Here, an *in vitro* method to simultaneously measure the viscosity and wetting property was developed using capillary filling controlled by MPa external pressure in bio-mimetic extended-nano space (10^1-10^3 nm) which mimicked inter/intracellular space (10^1-10^3 nm). Bio-mimetic extended-nanoconfinement effect on water properties was evaluated. It suggested that specificity of two-dimensionally nanoconfined channel, in which the viscosity showing much higher values than bulk, while wetting property is independent on bio-mimetic extended-nanoconfinement. This study will offer a deeper understanding of biological fluid and also contribute to biological system design.

KEYWORDS

Bio-mimetic extended-nano space, viscosity, surface tension force, nanofluid, capillary flow

INTRODUCTION

Cells communication plays significant role in biological system, like signaling and regulation. It was reported that cells communicated through inter/intracellular space of 10^1-10^3 nm such as synapse and tunneling nanotube. [1, 2] The communication is achieved by transferring ions, metabolites, and small signaling molecules via inter/intracellular space. Therefore, clarifying the liquid properties in inter/intracellular space is crucial. In previous work, specific liquid properties, like higher viscosity and higher proton mobility in intercellular space have been suggested *in vivo* [3]. However, it is difficult for *in vivo* study to control the space size, focus on specific molecules and exclude the effect of cell and macromolecule, thereby detailed analysis has not been achieved which strongly limited the investigation of cellular communication mechanism in biological system.

As micro/nanotechnologies have been rapidly developed, miniaturized system for biology and analytical chemistry are shifting from microspace to nanospace. Our group focused on 10^1-10^3 nm space, called extended-nano space, which is a transitional region from single molecules to condensed phase. Since size of the space and fluid flow can be accurately controlled, the previous work revealed specific liquid properties such as higher viscosity, lower permittivity and higher proton mobility. Based on these results, we proposed a model of proton transfer phase where water molecules are loosely coupled within the wall of 50 nm. [4] Since extended-nano space have the similarity to inter/intracellular space in size and specific liquid properties, recently, we successfully developed bio-mimetic extended-nano space by modifying lipid bilayers in fused-silica nanochannel and also found higher proton mobility. [5] However, it is lack of the method for analyzing fluid and interfacial properties in bio-mimetic extended-nano space which limits further understanding of cells communicational mechanism by molecular diffusion, convection and interaction with cell membrane. Therefore, in this study, a simultaneous method to measure the viscosity and wetting property in bio-mimetic extended-nano space was developed.

CONCEPT

Figure 1 illustrates a schematic of *in vitro* simultaneous measurement of fluid and interfacial properties in bio-mimetic extended-nano space. Bio-mimetic extended-nano space is created by lipid bilayers modification to fused-silica nanochannel. To simultaneously evaluate fluidic and interfacial properties in bio-mimetic extended-nano space, a method based on measuring capillary filling speed was developed, since capillary flow is a dominant phenomenon in nanospace. Capillary filling is dependent on both fluidic and interfacial properties, however, to investigate the fluidic property, most of previous studies alternatively have taken the effect of wetting dynamics into account by evaluating contact angle in the bulk scale, the simultaneous measurement of fluidic and interfacial properties in nanospace has never been realized. In present study, an external pressure P_{ex} with MPa order is applied to the liquid in the nanochannel to

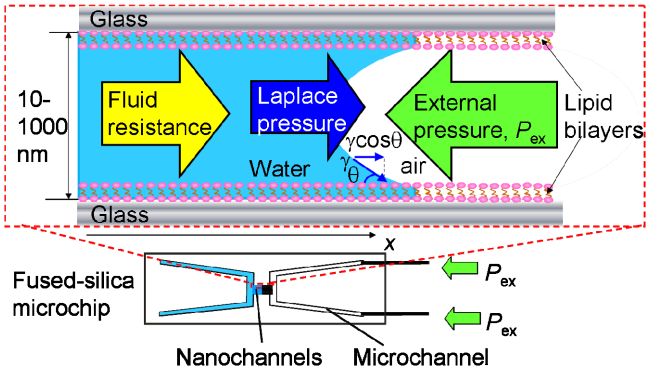

Figure 1. Schematic of *in vitro* simultaneous measurement of viscosity and wetting property in bio-mimetic extended-nano space.

978-0-9798064-5-2/μTAS 2012/$20©12CBMS-0001

control the capillary flow, since Laplace pressure in nanospace is MPa order. In addition, because lipid bilayers will denature when the temperature is over 40 degree, control of receding capillary filling is considered to be used. The pressure balance from Laplace pressure, external pressure and fluidic resistance of the capillary filling in the steady state is expressed as follows:

$$\text{Square channel (square cross section):}\quad \frac{\Delta x^2}{\Delta t} = \frac{4.5 D_h^{\,2}}{64\mu} P_{ex} - \frac{4.5\gamma D_h}{16\mu}\cos\theta \qquad (1)$$

$$\text{Plate channel (slit cross section):}\quad \frac{\Delta x^2}{\Delta t} = \frac{D^2}{6\mu} P_{ex} - \frac{D\gamma\cos\theta}{3\mu} \qquad (2)$$

where $\Delta x = x(t) - x_0(t_0)$ is the meniscus displacement from a reference position x_0 and $\Delta t = t - t_0$ is the time difference, γ is the surface tension and θ is the dynamic contact angle. Eqs 1 and 2 show that a ratio between the square of meniscus displacement and the time difference, $\Delta x^2/\Delta t$, linearly varies with the external pressure P_{ex}. Therefore, the viscosity μ and the surface tension force defined as a force parallel to the channel derived from the surface tension, $\gamma\cos\theta$, can be obtained from a slope and intercept of a fitting line between $\Delta x^2/\Delta t$ and P_{ex}.

EXPERIMENTAL

(1) Nanofabrication and lipid bilayers modification

Nanochannels were fabricated on the synthetic silica glass substrate by the fabrication scheme presented in the previous work. [4] The size of nanochannel was accurately measured by scanning electron microscope (SEM) for width and atomic force microscope (AFM) for depth before bonding. Nanochannels and microchannels were designed to minimize hydrodynamic effect of microchannel on nanochannel less than 1%.

The nanochannel was modified by dioleoylphosphatidylcholine (DOPC) to form lipid bilayers to create bio-mimetic extended-nano channel as presented by previous work. [5] Briefly, dioleoylphosphatidylcholine (DOPC) was mixed with Texas Red-DHPE with ratio of 99:1 in CCl_3. After removing CCl_3, the mixture was added into 1 mM NaCl solution and turbid vesicle solution was formed. The turbid vesicle solution was frozen in liquid nitrogen to make single vesicle. After filtration by the Mini-Extruder, single vesicle solution was introduced into nanochannel by syringe pump with flow rate of 50 μL/h. Vesicles were extended and adsorbed on the channel surface. Afterwards the plus unmodified vesicles were removed by water rinsing.

(2) Measurement system

As shown in Figure 1, MPa external pressure was supplied by high pressure system developed by our group to control receding capillary filling motion. The filling process was simultaneously recorded by inversed microscope equipped with high speed camera (1000 frames/s) in inverted bright-field. To neglect pressure drop of gas, the liquid length in the nanochannel should be sufficient making fluid resistance much larger than gas resistance.

RESULTS AND DISCUSSION

Viscosity and wetting property were simultaneously investigated by control of receding capillary filling with MPa order external pressure. The filling image is shown in Figure 2 selecting from the filling video recorded by microscope and high speed camera. It was showed that by utilizing the MPa order external pressure, receding capillary flow can be successfully controlled and simultaneously measurement method for liquid viscosity and wetting property was established.

Figure 3(a) shows measured viscosities of water as function of the representative channel size in bio-mimetic extended-nano space. For square channel with two-dimensional nanoconfinement, with size deceasing, the viscosity increased and amounted up to almost 4 times of bulk value in around 400 nm square bio-mimetic nanochannel. In our previous work, higher proton mobility in bio-mimetic extended-nano space was reported and a loosely coupled water molecules by hydrogen bond in the vicinity of wall was suggested. The higher viscosity in square bio-mimetic nanochannel suggested that a specific water layer is induced in the square bio-mimetic nanochannels. On the other hand, in plate bio-mimetic nanochannel with one-dimensional nanoconfinement, the viscosity is the same as the bulk value and that result suggested that the specific water layer appeared just in two-dimensional bio-mimetic nanoconfinement. In biological system, the tunneling nanotube intercellular space is like two-dimensional nanoconfinement, while synapse possessed one-dimensioanlly nanoconfined space like plate nanochannel. The study result revealed that the specific fluid property in tunneling nanotube and not in synapse. Figure 3b shows the

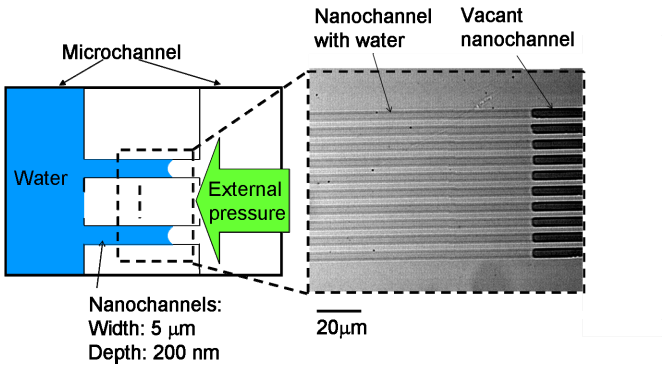

Figure 2. Instaneous image of receding capillary flow in lipid bilayers modified extended-nano channel.

surface tension force γcosθ as function of the channel size. Obvious dependency of the wetting property on the channel size and confinement dimension is not observed, in contrast to the viscosity. From a viewpoint of theory, it is well known that the dynamic wetting is described by two models, the hydrodynamic models strongly related to the fluid property and the molecular kinetic theory determined by interactions between several layers of liquid molecules and the surface. Since the significant difference of viscosity between square and plate nanochannels as shown in Figure 4a is not reflected to the surface tension force, the wetting dynamics in extended nanospace is considered to be governed by the molecular kinetic theory. Therefore, the results suggest that the wetting property is dependent on spaces much smaller than extended nanospace, as proposed by the molecular-kinetic theory considering interactions between the surface and several-layered liquid molecules, which have similar scale to the adsorbed water layer.

CONCLUSION AND PERSPECTIVE

The method for *in vitro* simultaneous analysis of the fluid and interfacial properties for inter/intracellular space was successfully established. Water viscosity and surface tension force in extended-nano space was investigated with varying space size and dimension. The results indicated that the intercellular space geometry has effect on the water viscosity and wetting property was regulated by the space size smaller than bio-mimetic extended-nano space. This method will play significant role in evaluation of biological fluid behavior in inter/intracellualr space and have contribution to exploration of bio-nanofluid dynamics and development of novel biological system.

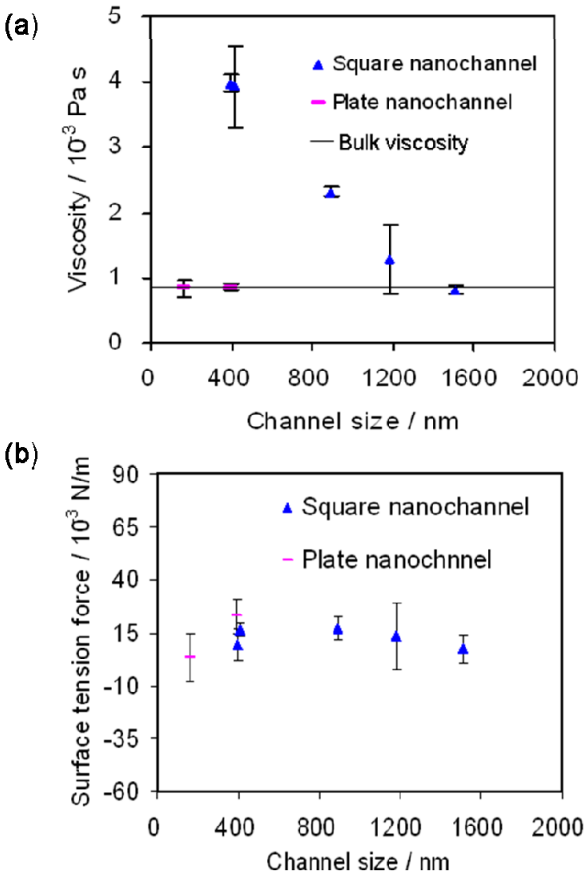

Figure 3. Bio-mimetic extended-nano geometric effect on water viscosity and wetting property.

ACKNOWLEDGEMENTS

The authors acknowledge the financial support of the Core to core program and Grant-in-Aid for Specially Promoted Research of Japan Society for the Promotion of Science (JSPS).

REFERENCES

[1] "*In vivo* Diffusion Analysis with Quantum Dots and Dextrans Predicts the Width of Brain Extracellualr Space," Thorne, R. G. and C. Nicholson, PNAS, **103**, 5567 (2006).
[2] "Nanobubular Highways for Intercellular Organelle Transport," A. Rustom, R. Saffrich, I. Markovi, P. Walther, and H. H. Gerdes, Science, **303**, 1007 (2004).
[3] "Water as an Active Constituent in Cell Biology," P Ball, Chem. Rev., **108**, 74 (2008).
[4] "Integrated extended-nano chemical systems on a chip," T. Tsukahara, K. Mawatari, and T. Kitamori, Chem. Soc. Rev., **39**, 1000 (2010).
[5] "Surface Modification of Lipid Bilayers in Extended-nano Space for Making Artificial Intercellular Structures," H. Emon, K. Mawatari, T. Tsukahara, and T. Kitamori, Proc. microTAS., 1501 (2009).

CONTACT

Takehiko Kitamori, tel: +81-3-58417231 or kitamori@icl.t.u-tokyo.ac.jp