IMPACT OF OSMOSIS ON MICRO-DROPLETS - A NEW ROUTE TO NOVEL SENSORS

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ABSTRACT

Micro-droplets generated by microfluidic techniques provide a powerful system to monitor chemical reactions and biological systems in picoliter volumes over time. In particular the analysis of reactions inside the droplets is of high importance for various technological applications. Such applications usually require specific labels and detection systems, which are e.g. based on fluorescence labeling. Here we report on a novel approach that exploits the osmosis-driven change of droplet size as a quantitative and label-free detection method. To evaluate the potential of droplets as sensor-systems, we quantify the impact of osmosis on the droplet size in various static and dynamic systems.

KEYWORDS: Osmosis, Droplet, Microfluidics, Sensor, Label-Free, Metabolic Activity

INTRODUCTION

A striking example of the capabilities of microfluidics is the formation and application of picoliter-droplets. Monodisperse aqueous droplets dispersed in oil can be easily fabricated with rates of up to 10 kHz. Furthermore, they can be sorted and fused using simple channel designs and electrical fields [1]. Picoliter-droplets are especially useful for biological assays as the individual micro-vessels can be used as reactors containing small numbers of molecules or cells [2, 3]. Moreover, the monodispersity of the droplets together with the possibility to create and monitor huge numbers of droplets allow for quantitative and statistical analyses [4]. Finally, the small size of the droplets size greatly reduces the volumes of reagents, and thus expense, for screening libraries containing millions of compounds [5]. These capabilities make the application of droplet-based microfluidic methods suitable for various fields of fundamental research, as well as for high-throughput applications in bio-technological and pharmaceutical industry.

However, since common microfluidic devices and methods still require additional external and macroscopic elements for detection, analysis and processing, the full potential of miniaturization is usually not exploited. To expand the degree of miniaturization and thus benefit from its advantages, new approaches are required which allow for the integration of the various processes at the micron scale. We here present a novel ansatz which uses osmosis to apply droplets as a label-free sensor-system: Depending on the composition of the droplet content, osmosis leads to a change in the droplet's volume. This change of size can be used as a novel marker for reactions inside the droplets without the need for a specific label.

We report on a first quantitative analysis of the osmosis-driven size change of droplets, applying static and dynamic systems of micro-droplets: Static systems are composed of droplets containing a fixed number of solutes, which is constant over time. In contrast, dynamic systems comprise droplets containing an active entity, which changes the composition over time by increasing or decreasing the number of solutes inside the droplet.

THEORY

Monodisperse droplets can be stable in size for several days or weeks [3] as the so-called Ostwald Ripening, an emulsion destabilizing process, proceeds very slowly and other emulsion instabilities can be avoided by experimental design. However, when droplets differ in their composition regarding the concentration of solutes inside the droplets, osmosis becomes a crucial mechanism changing the size of the droplets. In particular, this mechanism has to be considered when droplets contain active entities, e.g. enzymes or cells, which are capable to change the composition of the encapsulated solution via chemical reactions.

Driven by osmotic pressure differences between the droplets a pressure compensating net flow of water into droplets of higher osmolarity arises. These droplets grow in size, whereas the droplets of lower osmolarity shrink (Figure 1). Since digestion or synthesis of molecules alters the amounts of substances and thus the osmotic pressure, the droplet size change is directly correlated to the reactions inside the droplet.

This change in droplet size was previously observed by Schmitz et al. [6] while incubating monodisperse droplets, some of them containing yeast cells. After several minutes shrinkage of yeast-containing droplets occurred. More recently the same system was used by Joennson et al. to

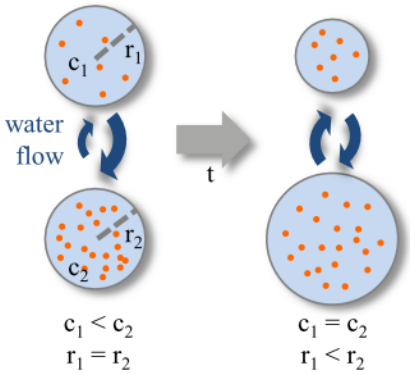

Figure 1: Following the concentration gradient, water flows from droplets containing higher solute concentrations to those containing lower ones. Eventually, a dynamic equilibrium is reached, where droplet sizes are stable.

demonstrate a method for size separation of microdroplets [7]. However, as yet a thorough analysis and quantification of this effect has not been presented.

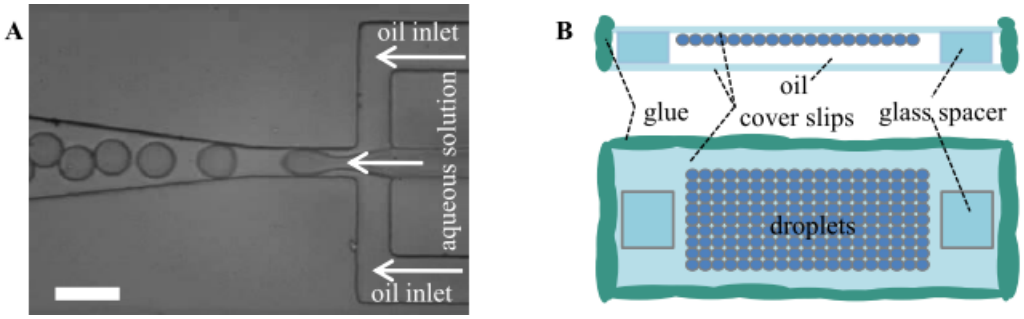

Figure 2: (A) Water-in-oil droplets with diameters ranging from 20 to 60 microns are generated by a flow-focussing geometry, a so-called nozzle. Scale bar: 40 µm. (B) Schematic of an oil-filled observation chamber with droplets. Dimensions of cover slips: 24 x 60 mm, inner height: 120-140 µm.

EXPERIMENTAL

For our analysis, monodisperse microdroplets are generated with a nozzle-like structure (see Figure 2A) made of PDMS (Polydimethylsiloxane, Sylgard 184, Dow Corning) by means of softlithography as described elsewhere [6]. We use a perfluorocarbon oil (FC-40, Acros Organics) as continuous phase and various aqueous solutions as dispersed phase. Furthermore, a surfactant (Krytox-PEG600-Diblock) was added to the oil-phase to stabilize the droplets and prevent adsorption of solutes to the oil-water-interface. Surface tensions are measured with the help of an OCAH 230 tensiometer (DataPhysics Instruments GmbH). Aqueous and oil phase are put in 1 ml syringes (Omnifix-F, Braun, with Neolus 0,4x20 mm needle, Terumo) mounted on precision syringe pumps (Aladdin-1000, World Precision Instruments) and connected with polyethylene-tubings (Portex Fine Bore, inner diameter 0.28 mm, Smiths Medical International Ltd.) to the inlets of the PDMS device. Two different types of droplets are produced for both, the static and dynamic systems: The static systems comprise droplets with different solute concentrations (YPD medium or bromphenol blue) which are generated by two separate nozzles and differ in size. The size difference allows for an easy discrimination of the droplet species. The dynamic systems are composed of droplets with and without yeast cells in addition to YPD growth medium. Both droplet types are produced by one nozzle with the yeast suspension as aqueous phase, randomly distributing the yeast cells in single equal sized droplets. The osmolarities of the aqueous phases are measured with an osmometer (Osmomat 030, Gonotec GmbH).

The droplets are collected in a pipette tip plugged in the outlet of the PDMS device. For incubation and observation they are put in an observation chamber (Figure 2B) filled with oil, which is sealed with gas-tight glue (Twinsil, Picodent GmbH). Due to buoyancy the aqueous droplets form a monolayer at the chamber ceiling. To obtain hydrophobic surfaces, the glass slices of the observation chamber were previously incubated for 1 min with Ombrello (Moton Automotive Germany) and thoroughly washed with ultra pure water. The observation chamber is put on a ZEISS Axiovert 200 microscope equipped with an ORCA-ER camera (Hamamatsu). For image acquisition Wasabi (Hamamatsu) is used. Image processing and analysis, including the measurement of the droplets' radii and the calculation of the solute concentration in droplets after a certain time, is done by self-written Matlab (The MathWorks, Inc.) routines.

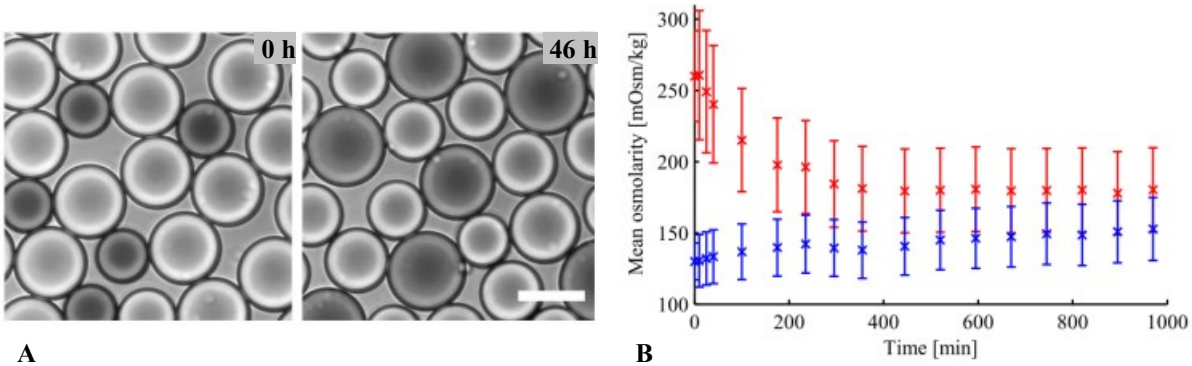

Figure 3: (A) Size change of an ensemble of pure bromphenol blue droplets (dark) mixed with 1:10 diluted ones (light) within 46 hours. Scale bar: 30 µm. (B) Development of the mean osmolarity in droplets containing 260 mOsm/kg (red) and 130 mOsm/kg (1:2 dilution, blue) YPD growth medium at t = 0. Concentrations for t > 0 are calculated using the droplets' volume changes.

RESULTS AND DISCUSSION

With our experimental set-up, even polydisperse droplet ensembles of equal composition and with radii ranging from 10 to 60 µm are stable for at least several days and up to several weeks. This stability in size implies that the Laplace pressure differences between the droplets are not sufficient to induce a significant flow of water from smaller to bigger droplets at the time scale of few days.

However, in static systems comprised of droplets containing two different initial concentrations of bromphenol blue (e.g. pure (60 mOsm/kg) and 1:10 diluted ones) or YPD (e.g. pure (260 mOsm/kg) and 1:2 diluted ones), significant size changes of droplets occur within the first hours (see Figure 3A). These are caused by the osmotic pressure differences, which at the beginning are one to two orders of magnitude greater than the typical Laplace pressure differences of less than 2 kPa. As shown in Figure 3B, osmolarity differences amongst the droplet species are compensated by a net flow of water, which results in the change of droplet sizes until an equilibrium state is reached after roughly 16 hours.

In the dynamic system consisting of droplets with and without yeast cells we also observe significant size changes of droplets until an equilibrium state is reached: droplets containing active yeast cells shrink, whereas empty droplets grow in size (see Figure 4A). Furthermore, droplets initially comprising more than one yeast cell shrink faster than droplets with only one encapsulated yeast cell (see Figure 4B). Both can be explained by the metabolic activity of the yeast cells, which proliferate as long as glucose and nutrients are available and thereby lower the net concentration of solutes, resulting in an water efflux into droplets containing no cells. Since all nutrients are metabolized after a certain time and therefore all yeast containing droplets exhibit about equal solute concentrations, the final droplet volume is independent of the initial cell number in a droplet.

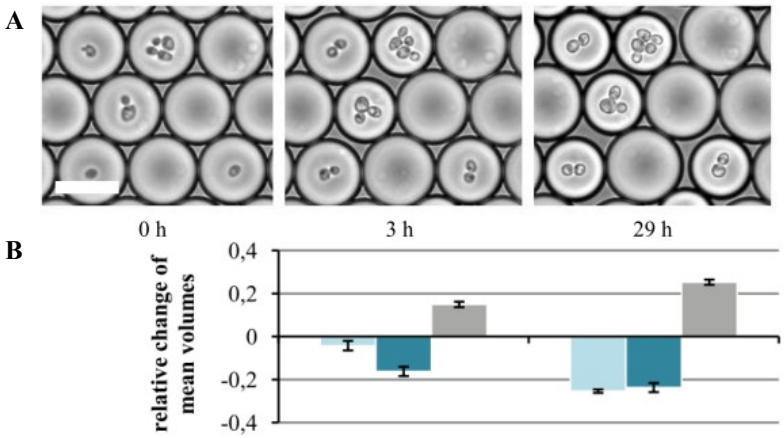

Figure 4: (A) Droplet sizes at 0, 3 and 29 hours. Scale bar: 30 µm. (B) Changes of mean volumes of droplets initially containing no (grey), one (pale blue) and more than one (dark blue) yeast cells, normalized with the mean droplet volume at t = 0.

CONCLUSION

Osmosis-driven size change of droplets grants immediate access to changes in concentration, particularly those caused by reactions, without the need for a specific marker. Its quantitative analysis allows for the development of novel types of label-free assays, in which the droplets themselves are used as a novel sensor system.

ACKNOWLEDGEMENTS

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