APPLYING MICRODROPLETS AS SENSORS

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ABSTRACT

We here report on label-free cytotoxicity screenings in picoliter-sized droplets, applying our recently described method for label-free detection of chemical reactions inside of microdroplets [1]. Our method is based on the osmosis-driven size change of droplets, linking concentration changes of solutes inside a droplet to the droplet's size. Furthermore, we present a modification of the sensor system which accelerates the rate of the droplets' volume change and simplifies the investigation of growth dynamics.

KEYWORDS: Droplet, label-free, Sensor, Osmosis, Diffusion, Reservoir, Gel

INTRODUCTION

Digital microfluidics offers various benefits for biological assays: individual droplets comprising small amounts of cells serve as micro-reactors which only require a small amount of reagents and allow for high throughput systems with thousands of experiments performed in parallel. However, the reliance on markers for analyzing the droplets' contents raises technological difficulties (e.g. performing washing steps in droplets) and limits the range of applications. Therefore, label-free methods for the analysis of droplets are needed. Recently, we presented a method which allows for droplets themselves being used as sensors and doesn't require any labels [1].

Our system exploits the osmosis-driven size change of droplets containing differing amounts of solutes: 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 by an active entity, e.g. cells, alters the amounts of substances and thus the osmotic pressure, the droplet's size change is directly correlated to the reactions inside the droplet. Therefore, chemical reactions inside the droplets can be followed without the use of any labels.

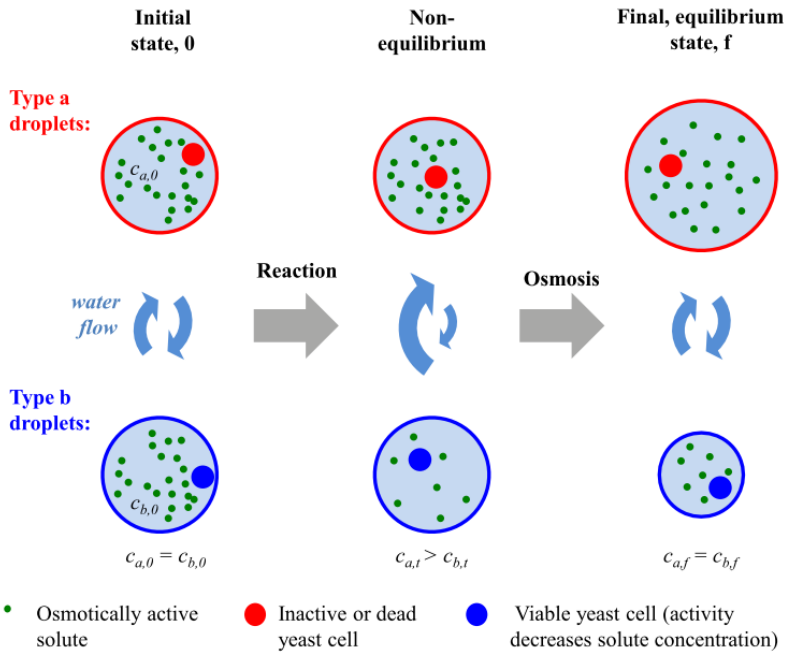

Figure 1 *Schematic of the basic principle of the droplet sensors. The slightly water-permeable carrier oil serves as a semipermeable membrane.*

The size change of yeast filled droplets was first observed by Schmitz et al. while incubating monodisperse droplets, some of them containing yeast cells [2]. After several minutes, shrinkage of yeast containing droplets occurred. The same system was then used by Joensson et al. to demonstrate a method for size separation of microdroplets [3]. More recently, we presented the first proof of osmosis being the underlying mechanism of the droplets' size change [4]. We quantified the osmosis-driven size change both experimentally and theoretically [1], using droplets containing various fixed amounts of saccharose or yeast cells in growth medium. Furthermore, we showed that the shrinkage of droplets containing viable yeast cells is due to their metabolism lowering the osmolarity. Most recently, Boitard et al. focused on the metabolism of the yeast causing the size changes, and showed that the sensor method can also be applied to detect activity of bacteria and enzymatic systems [5].

We here report on the first application of the droplet sensor technique to cytotoxicity screenings and furthermore present an optimization of the sensor system.

CYTOTOXICITY SCREENINGS

For the cytotoxicity assays presented here, monodisperse microdroplets are generated with a nozzle-like structure made of PDMS (Polydimethylsiloxane) by means of softlithography. We use a perfluorocarbon oil with 20 mM of Krytox PEG600 surfactant as continuous phase and yeast cell suspensions (saccharomyces cerevisiae in YPD growth medium) with or without the toxin Glutaraldehyde (GA) as dispersed phase. The droplets are put in an incubation and observation chamber which is sealed with gas-tight glue. The methods used are more precisely described in [1]. As a control experiment shows, any amounts of encapsulated living cells lead to droplet shrinkage (Figure 2). Figure 3 shows examples for the two types of assays performed: Either the toxin is washed out prior to droplet production, which allows for an easy quantification of the toxicity (ideally there's only one cell encapsulated per droplet), or it remains inside of the droplets to investigate time-dependent effects. In case the toxin was washed out and some cells were still alive after incubation, shrinkage of droplets occurred. With the toxin inside, none of the droplets showed shrinkage, even at very low toxin-concentrations which in bulk medium rendered less than 10% of the cells nonviable after 4 hours. For investigation of possible explanations, experiments with other cytotoxic substances are planned.

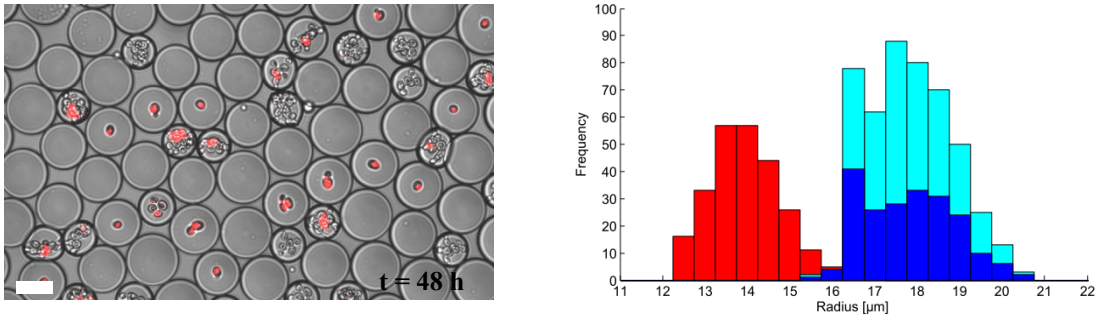

Figure 2 *Control experiment: initially monodisperse droplets comprising yeast cells after 48 h incubation. Dead cells are labeled with propidium iodide (red in micrograph). Droplets containing viable yeast cells (red bars in histogram) can be separated from empty droplets (blue) and droplets containing exclusively dead cells (pale blue). (Scale: 30 µm)*

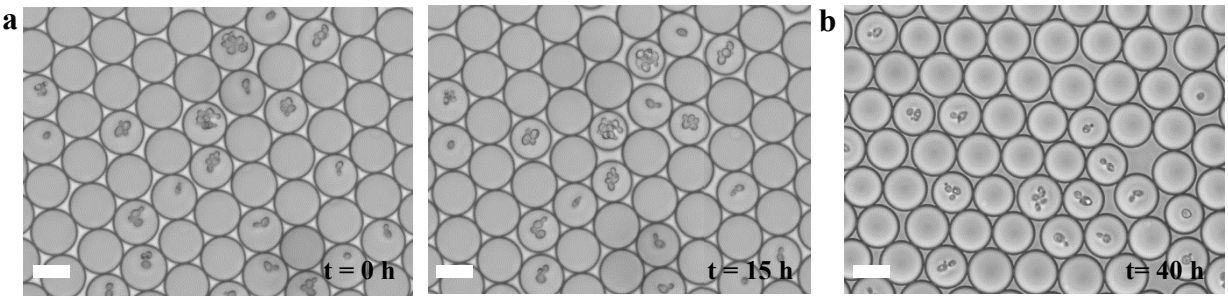

Figure 3 *(a) Size changes of droplets filled with yeast, which was incubated for 5 min in 0.5% GA prior to droplet production. (b) Droplets filled with yeast and 0.01% GA remain stable in size, because the permanent presence of the toxin renders the yeast cells nonviable. (All scales: 30 µm)*

OPTIMIZING THE SENSOR SYSTEM

To accelerate the size change of the droplets and render the dynamics of the volume change of each droplet comparable, we modified the sensor system by adding an agarose gel of predefined osmolarity to the observation chamber. The gel is situated at the top of the incubation chamber so that all droplets are in direct contact (Figure 4a), minimizing the diffusion pathways. In addition, since the volume of the gel is several orders of magnitude bigger than the volumes of all droplets combined, the osmolarity of the gel is assumed to be constant over time. Both lead to a faster volume change of the droplets compared to the basic system without reservoir. A further advantage of this kind of dedicated, well-defined reservoir is that the size change of one droplet is (in first approximation) independent of the neighboring droplets and therefore the dynamics of the droplets' volume changes are comparable.

The set-up of the incubation chamber is similar to those described in [1], except an agarose gel with 1 to 5 mm height is added to the chamber. The gel is prepared by mixing 5 % w/v agarose with a 500 mM saccharose solution (or whatever solution yields the desired osmolarity), heating the mixture in a microwave at 700 W until it is bubble-free, and pouring it in a PDMS ring of the desired height on a silanized glass slide. Then a second glass slide is pressed on top and the mixture is allowed to gel for 30 minutes at room temperature. Afterwards the gel is peeled off and put on top of a glass slice with some glass spacers and the droplet emulsion. Finally, the chamber is closed with a second glass slice and sealed with gas tight glue.

First experiments show that isoosmolar droplets remain stable in size for at least 24 hours (Figure 5 b), whereas droplets differing from the agarose gel's osmolarity change their size according to theoretical predictions (Figure 5 c). For example, using a 500 mM saccharose agarose gel and 100 mM saccharose droplets with an initial mean radius of

$r_i = 22.5 \pm 0.5$ µm results in a final mean radius $r_f = 13.0 \pm 0.5$ µm after 30 hours of incubation. Assuming $V_f = 1/5 * V_i$ holds in equilibrium state, one obtains a final radius $r_f = r_i / 5^{1/3} = 13.2$ µm, which is in very good agreement with the experiment.

Compared to multi-layer PDMS approaches [6], the agarose gel set-up is easier to construct and also allows a variety of molecules other than water to diffuse through the gel. For further optimization of the sensor system in terms of water exchange rates, the effect of various oil and surfactant types could be investigated.

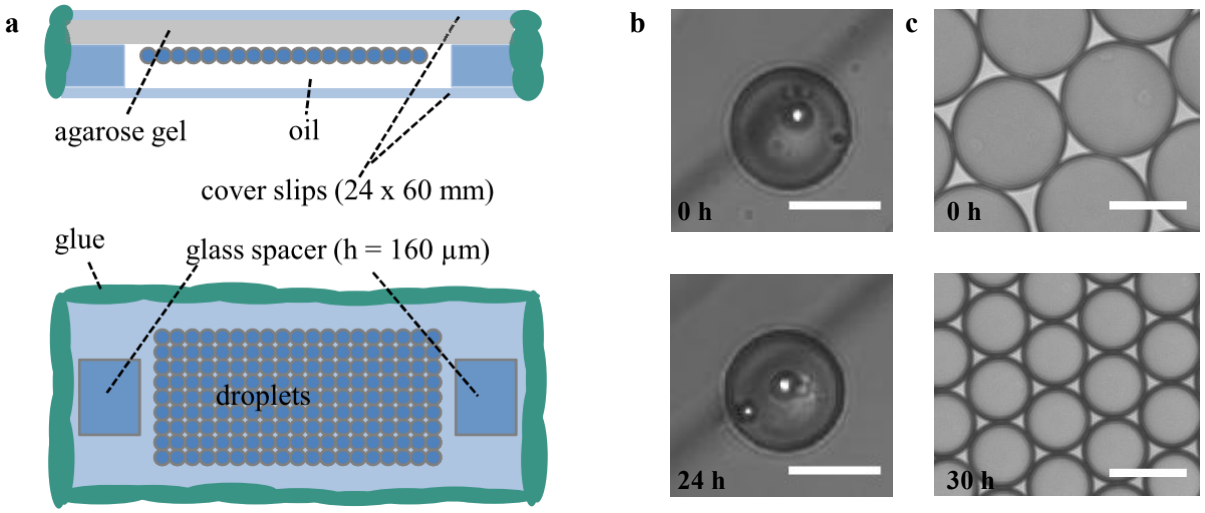

Figure 4 *(a) Schematic of the observation chamber containing an agarose gel. (b) 500 mM saccharose droplets in contact to a 500 mM saccharose saturated agarose gel at t = 0 (top) and after 24 hours (bottom). No size change is visible. (c) Shrinkage of 100 mM saccharose droplets in contact to a 500 mM agarose gel after 30 hours. (All scales: 20 µm).*

OUTLOOK

Applying the droplet sensor system to label-free cytotoxicity screenings and live-dead-assays yields easy to interpret quantitative outputs: thousands of experiments with binary outcome (droplet shrinks or doesn't) performed in parallel allow for a statistical interpretation of the results. The generality of this approach which only requires a normal light microscope for data analysis renders this method interesting for a broad scope of applications, e.g. investigating antimicrobial resistances. By microfluidic size separation of the droplets, even on-chip analysis is feasible. Furthermore, the principle of using droplets as sensors isn't limited to osmosis-driven size changes. For example, one may think about using functionalized surfactants, which either crosslink upon binding of a substrate and thereby alter the droplet's elasticity or raise or lower the surface tension of a droplet, causing coalescence or splitting of droplets. Both approaches link biochemical reactions inside a droplet to a physical parameter of the droplet, namely the elasticity or just plain existence, and therefore also facilitate on-chip analysis.

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