# DROPLET STABILITY BY USING DSURF FLUOROSURFACTANT

## INTRODUCTION

Biomillenia's microbiome-on-a-chip technology maintains a much higher diversity of living microbes in millions of parallel culture vessels than with standard microbiology methods. It uses its proprietary droplet microfluidics platform to create bacterial cell banks, evaluate microorganisms for desired phenotypes and to find new routes to prevent and treat dysbiosis. Dysbiosis of the human microbiome is a research area of increasing interest, especially in cancer immunotherapy[1,2], skin diseases[3,4], and in general in inflammatory diseases[5,6]. Droplet-microfluidics with its miniaturized culture vessels and its inherent high throughout was proven to be an excellent tool for microbiome research. Most of the microfluidic droplet techniques require biocompatible surfactants to keep the droplets stable. By forming a monolayer at the water-oil interphase the amphiphilic molecules prevent droplet coalescence even when droplets are in direct contact with each other. However, the droplet stability can be affected by growth of bacteria in droplets. Especially complex bacterial communities extracted from natural samples can pose a risk to the emulsion quality, due to various secreted metabolites, as well as different growth rates and morphologies exhibited by the various species. Additionally, some bacterial species derived from natural samples are particularly sensitive to the growth micro-environment. Microfluidic surfactants might be one factor to be considered when establishing the micro-environment for the growth of natural bacteria. Hence, the optimal surfactant is providing droplet stability also during long-term incubation while being biocompatible for a wide spectrum of bacterial species.

Here, the performance of three commonly used surfactants are compared at three different concentrations. The droplet stability over time and the droplet occupation rates were determined by encapsulating a microbial community derived from human skin.

Biomillenia

**Biomillenia is a team of experts in microfluidics, microbiology, molecular biology and bioinformatics who are passionate about harnessing smart microbes to restore the body's healthy balance and help prevent lifestyle diseases.**

## MATERIAL AND METHODS

**Reagents**
Novec HFE7500 (3M) containing 5%, 2% or 0.5% (w/w) concentration of Competitor 1 surfactant, Competitor 2 surfactant, or dSurf (Fluigent), respectively.

**Microbe sample**
A microbial sample collected from skin of healthy volunteers was used for droplet generation. A general microbial skin sample, as opposed to a single strain like *E. coli*, was used here because a bacterial community consisting of many species better represents the experimental condition for microbiological research. The microbial skin sample was prepared according to Biomillenia's proprietary standard sample preparation workflow and was suspended in a standard medium for skin microbes containing 0.5% (v/v) TWEEN80. TWEEN80 is a lipophilic molecule in the aqueous phase that interfers with the droplet stabilizing characteristics of the surfactants.

**Droplet generation**
The droplets were prepared on Biomillenia's proprietary microfluidic platform with PDMS chips. The two liquid phases were controlled during droplet generation by the use of high precision pressure pumps (Fluigent, Flow EZ). Droplets were generated at frequencies of 7-10 kHz. The droplet volume was set to 20 pL. Droplets were collected and incubated in custom made vessels at 37 °C allowing fot the storage of the droplets without exposing them to a direct gas interface.

**Droplet observation**
From the droplet collection vessel, a small number of droplets were sampled to check for droplet stability and bacterial occupation at 0, 1, 3 and 7 days of incubation. For imaging by microscopy, droplets were spread onto a monolayer surrounded by the respective oil-surfactant combination.

## RESULTS

**Droplet generation**
During droplet generation we found that all three tested surfactants at all concentrations provided an immediate stabilization of aqueous droplets. For all tested surfactants similar pressure settings for the continuous oil phase and the aqueous phase could be used throughout the droplet generation process, resulting in droplet generation frequencies of 7-10 kHz and monodispersed droplet populations with an average volume of ~20 pL (Fig. 1).

We did not observe any droplet coalescence in the exit channel of the chip, in which droplets were having direct contact to each other.

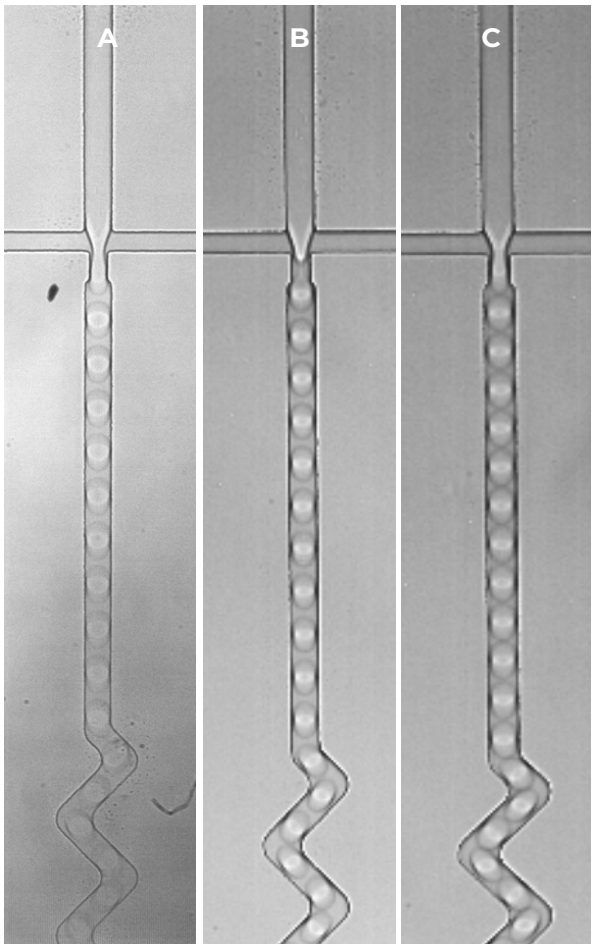


**Figure 1.** Droplet generation with dSURF (A), Competitor 1 (B) and Competor 2 (C). Immediate droplet stability during droplet generation was observed at the flow focusing unit for all tested concentrations of 0.5% of reagent

**Droplet stability and bacteria occupancy**
The collected droplets were spread in Biomillenia's proprietary observation chip for best imaging of microbial occupation in droplets after 0, 1, 3 and 7 days incubation at 37 °C. As shown in Fig. 2, a monolayer of droplets surrounded by the respective oil-surfactant combination was imaged microscopically.

For each tested surfactant the concentration of 0.5% sufficient to ensure the stability of droplets over long term periods. The extent of droplet coalescence is highest for Competitor 1 after one day while for dSURF and Competitor 2 the instability becomes visible only later and to a lesser extent. Surfactant concentrations of 2% and higher result in stable populations over 7 days at 37 °C for dSURF and Competitor 2 while for Competitor 1 a concentration of 5% is required to reach similar results. (Fig. 2).

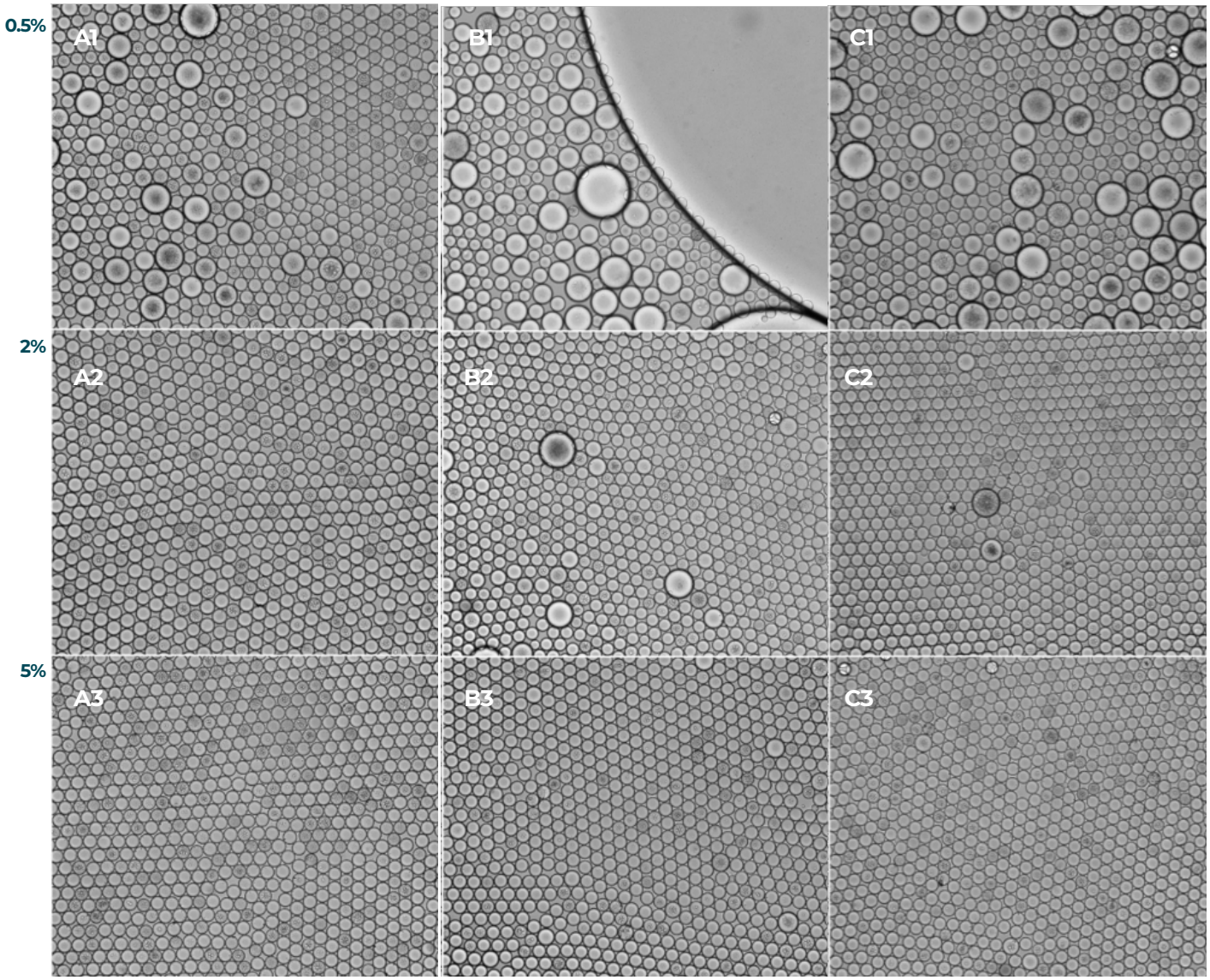


**Figure 2.** Images of droplets with dSURF–0.5% (A1), dSURF–2% (A2), dSURF–5% (A3), with Competitor 1–0.5% (B1), Competitor 1–2% (B2), Competitor 1–5% (B3), and with Competitor 2–0.5% (C1), Competitor 2–2% (C2), Competitor 2–5% (C3) after 7 days incubation.

For droplets of the expected size, we determined the occupation with microbial cultures by image analysis after at least one day of incubation for the droplet images shown above. Droplets containing more than three cells were categorized as occupied. We analysed at least 1000 droplets in total for each droplet population. Since the aqueous cell suspension was prepared under the same conditions for each droplet population a similar droplet occupation was expected. The emulsion failure for Competitor 1 at the concentration of 0.5% prevented the determination of the occupation rate.

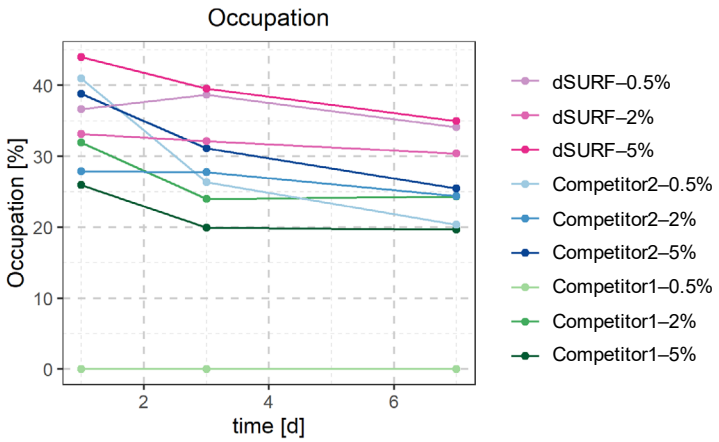


**Figure 3.** Droplet occupation frequency over time. The occupation was determined by image analysis from microscopy images of droplets at each sampling time point. The emulsion failure for Competitor 1 at the concentration of 0.5 % prevented the determination of the occupation rate.

# CONCLUSION

The stability of microfluidic emulsions strongly depends on the content of droplets and its interplay with the surfactant used. Hydrophobic compounds in the aqueous phase, along with microbial growth of various species present far from ideal conditions for microfluidic droplets, but exemplify well the experimental challenges encountered when microfluidic techniques are truly applied in microbiology. Hence, surfactants are needed that help to accommodate those complex sample characteristics.

Within the described experimental setting dSURF performed best among the tested surfactants in terms of maitaining droplet stability and biocompatibility. The required surfactant concentration for keeping the droplets with microbial growth stable over long term incubation at 37 °C was 2% (w/w) for each of them, compared to 5% (w/w) necessary for Competitor 1. Using lower surfactant concentrations is not only of advantage for financial reasons but is also a way to prevent inter-droplet transport of molecules from the aqueous phase.[7]

In addition to improved droplet stability, a trend in the droplet occupation rate with bacterial cultures is also detectable. As the aqueous phase for each droplet population was prepared with the same amount of cells, a similar occupation rate can be expected assuming no differences in biocompatibility for the three used surfactants. dSURF seems to have a higher occupation rate which could be indicative of better biocompatibility.

In conclusion, dSURF is a new surfactant for droplet microfluidics in the market which performs very well compared to widely established microfluidic surfactants and is highly suitable for complex biological applications.

## REFERENCES

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[2] Cancer Letters, volume 447, page 41–47 (2019)
[3] Nature Reviews Microbiology, volume 16, page 143–155 (2018)
[4] Microbiome, volume 7, article number 95 (2019)
[5] Nature Reviews Microbiology, volume 17, page 497–511 (2019)
[6] Cell, volume 178, page 1041–1056, (2019)
[7] Nature Communications, volume 7, article number 10392 (2016)

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# APPENDIX

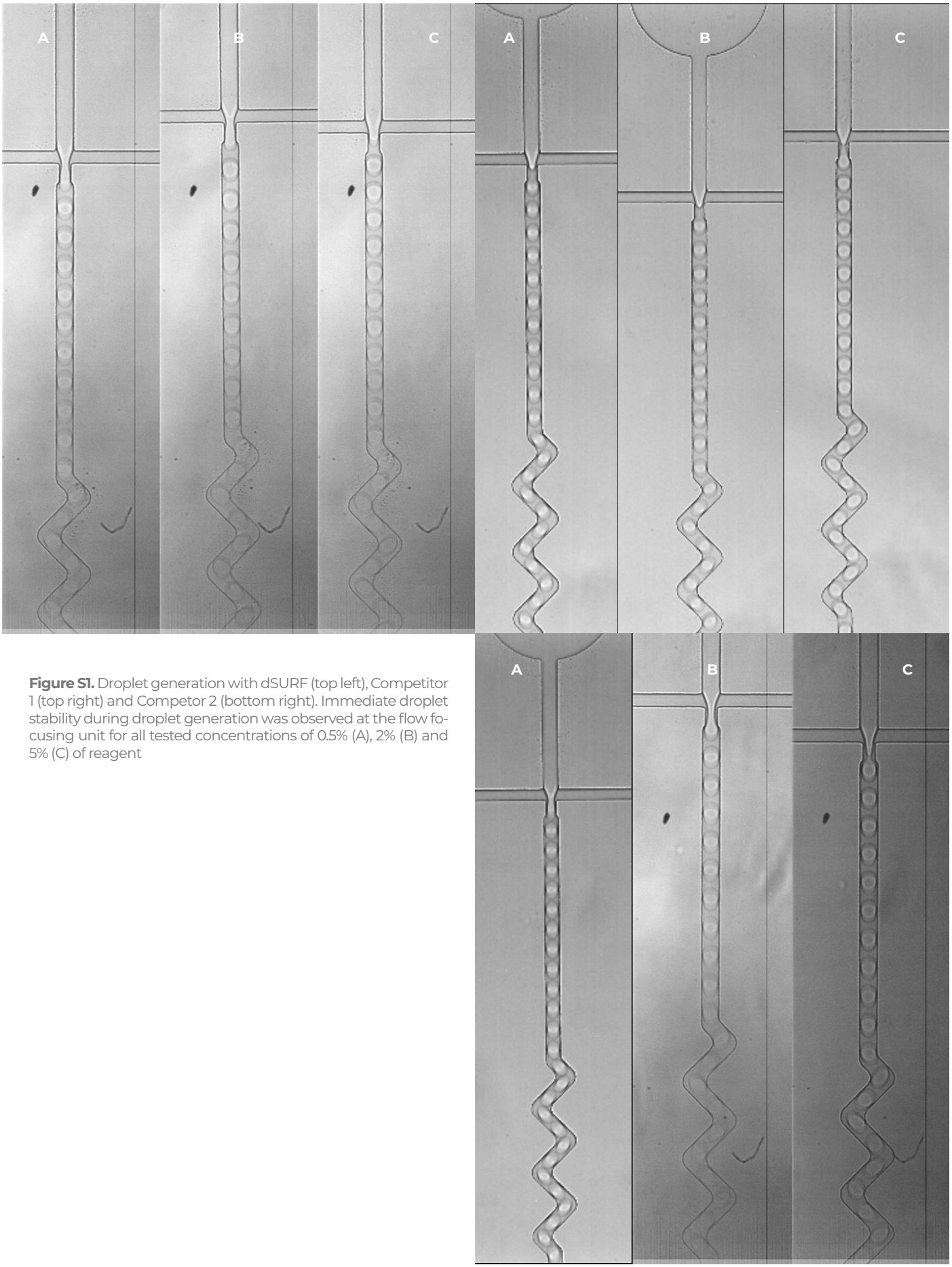


**Figure S1.** Droplet generation with dSURF (top left), Competitor 1 (top right) and Competor 2 (bottom right). Immediate droplet stability during droplet generation was observed at the flow focusing unit for all tested concentrations of 0.5% (A), 2% (B) and 5% (C) of reagent

# APPENDIX

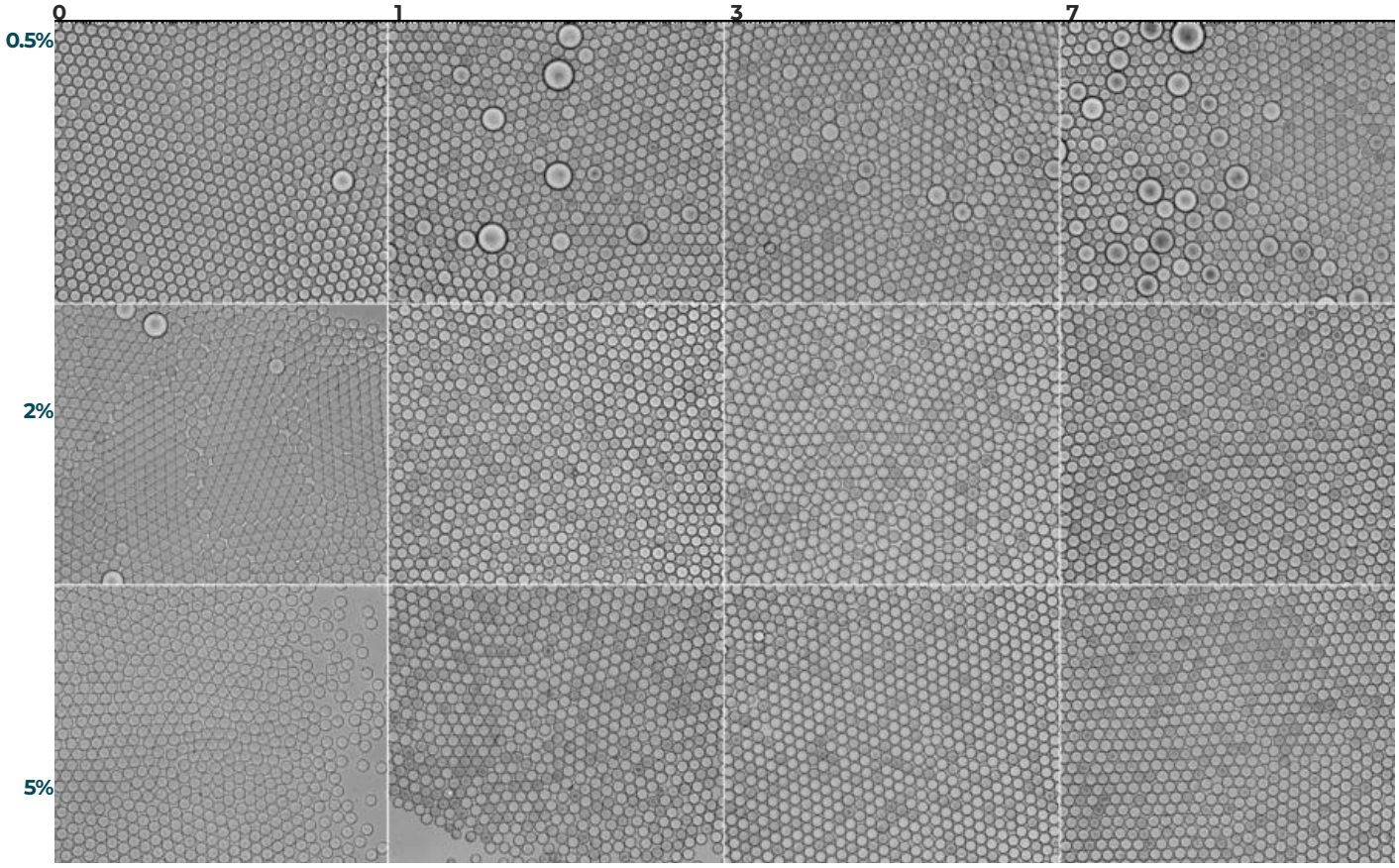


**Figure S2-A.** Droplet images for dSURF (A) for all three concentrations after the indicated number of days of incubation

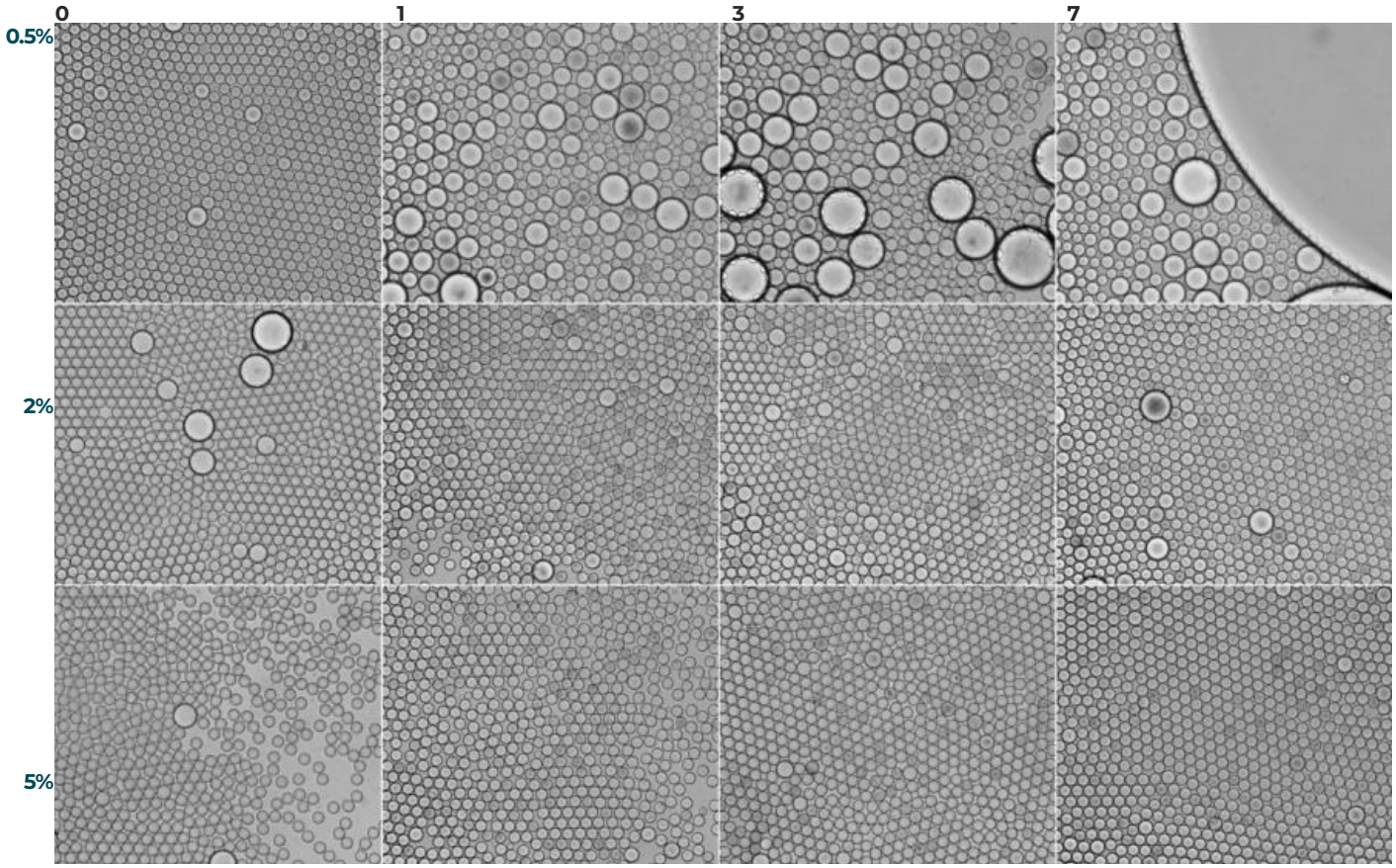


**Figure S2-B.** Droplet images for Competitor 1 for all three concentrations after the indicated number of days of incubation

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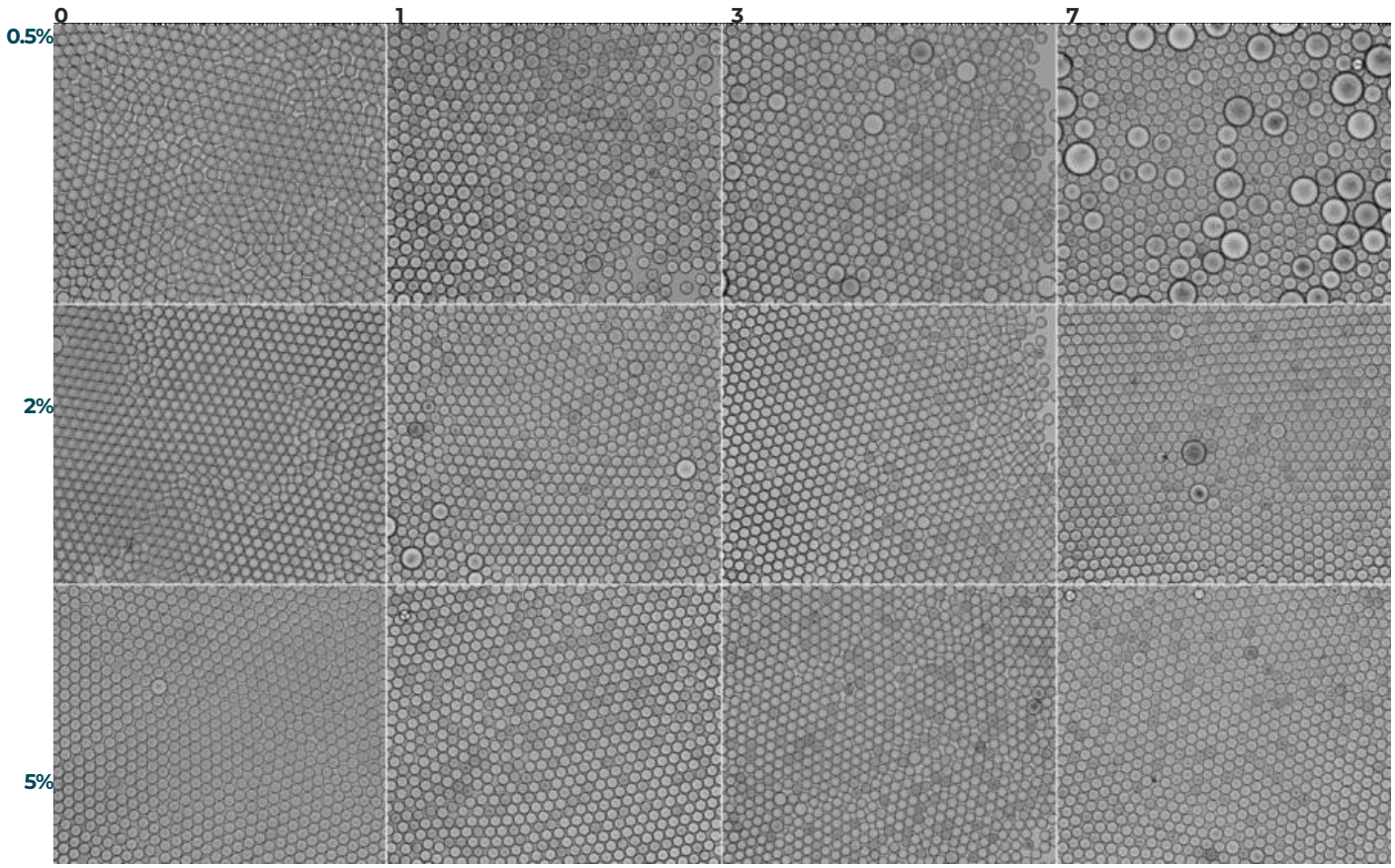


**Figure S2-C.** Droplet images for Competitor 2 for all three concentrations after the indicated number of days of incubation