Droplet-based synthetic biology: chemotaxis and interface with biology

Silvia Holler[1] and Martin Michael Hanczyc[1,2]

[1]Laboratory for Artificial Biology, Department of Cellular, Computational and Integrative Biology (CIBIO)
University of Trento, 38123, Trento, Italy
[2]Chemical and Biological Engineering, University of New Mexico, MSC01 1120, Albuquerque, NM 87131-0001, USA
silvia.holler@unitn.it

Abstract

Liquid droplets possess some life-like behaviors and have been the subject of artificial life studies. Life-like behaviors such as fission, fusion and movement can be artificially recreated exploiting highly simplified chemical systems. Recently we showed that droplet-based chemotactic systems can be interfaced with biological systems (1). We developed a chemotactic droplet able to move light cargos such as hydrogel alginate capsules embedded with living cells as a transporter. We transported efficiently and in a sterile way a few types of bacteria and yeast, and we are now modifying our protocols to transport efficiently human cell lines. We recently discovered that some eukaryotic cell lines release surfactants when placed in our artificial transport system, thereby reinforcing the interface between the artificial and living systems. This is an example of not only how the interface between artificial life and biological life could be designed but how the one system can augment the other. In this case the living system produces the surfactants that the droplet needs for cargo transport and the artificial system provides the transport for the otherwise sessile mammalian cells.

Introduction

Protocell systems are examples of bottom-up synthetic biology. A leading property of cells and living organisms is the ability to move. Motile protocells can be created using simple chemical systems: for example, droplet of oil in water or droplet of water in oil. Lively droplets of water in oil were first described by Otto Bütschli in 1892 (2). He used alkaline water droplets in olive oil to initiate a saponification reaction. This simple protocell system recreated an entity that moved and seemed to behave like an amoeba. Since then many researchers have been developing oil droplet systems as models of living systems (3), (4). For example, the research of the group of Hagan Bayley in Oxford created 3D customized patterns of water droplets in oil with stable lipid bilayers forming the droplet-droplet interfaces as mimics of living tissues (5). In addition these networks of droplets with integrated porins can show current transmission. Each droplet in this system can be complemented with cell-free expression systems controlled by light activating protein expression (6). In this way they demonstrated that life-like behaviours such as current transmission and protein expression can be activated even in water-in-oil droplets.

We mainly focused our work on chemotactic 1-decanol motile droplets. Chemotaxis is defined as a stimulated migration towards an increasing (or decreasing) chemical gradient, and 1-decanol droplets, formed in an aqueous medium containing decanoate at high pH, show chemotaxis when a chemical gradient is placed in the external aqueous environment. Droplets using such chemical gradients are able to solve 3d mazes, displaying a rudimentary artificial intelligence. This kind of movement can be compared to already well-described system of eukaryotic chemotaxis. For example, *Dictyostelium amoebae* migrates along an increasing concentration of cyclic adenosine-3′,5′-monophosphate (cAMP) (7). Cejkova et al. showed in 2014 1-decanol chemotaxis towards a salt source (8). This system works even in mazes (9) and can be exploited to transport non living (9) objects. There is a challenge and benefit to begin to interface living and artificial systems to exploit potential synergies, increase robustness or increase the functionalities of both systems. We then attempted to interface the purely artificial decanol droplet system with living cells.

We therefore show how to interface the purely artificial decanol droplet system with living cells, preserving the function of both systems.

Protocell-cell transport system

We developed our artificial chemotactic system to make it compatible with natural living systems by creating a partially hydrophobic alginate capsule as a protective unit that can be precisely embedded in a droplet, transported along chemical gradients and deposited. This system was able to transport *Escherichia coli, Bacillus subtilis and Saccharomyces cerevisiae*. Both bacteria survived the transport. However, yeast survived but not in a consistent and repeatable way. The droplet containing a capsule with live cargo could be manipulated with salt gradients several times with the capsule remaining stably attached to the droplet. In addition, several capsules can be stably fixed to a single decanol droplet. For a video of this system, see:

https://www.youtube.com/watch?v=zCB2bPhFoCI. We afterwards conceived the idea to develop this system to transport mammalian eukaryotic cells. To do this we needed to evolve the droplet chemotaxis system under conditions more conducive to physiological environments. We decreased the pH from 12 to 7 and tried to transport A549 cells inside our alginate capsules. We found that A549 cells can be encapsulated in alginate hydrogels and survive. When in capsules incubated in growth medium DMEM, the cells survive and secrete into their environment some compounds that lower the surface tension and act as surfactants. The water phase in which capsules are incubated shows, if analyzed using pendant drop method with a tensiometer, a reduction in surface tension (60-55 mN/m) if compared to water (72 mN/m) and can be used in our artificial system as chemotactic water phase. Some of the molecules secreted by the cells modulate the surface tension of the alginate capsule. This surface modification allows the normally hydrophilic hydrogel capsule to associate efficiently and for an extended time with the hydrophobic 1-decanol droplets (up to 1 hour in the case of cell culture water phase mixed 1:1 with water). The secretion of surfactants is probably due to the mucus secreting phenotype transition of A549 cells. This transition leads to surfactant release in the water phase in which the capsules are incubated. This surfactant secretion is shown only when A549 are in capsules and this demonstrates that the integration of the biological system (A549) with the artificial one (capsules) can be exploited to increase the functionalities of the system. In addition, this association is selective for live cells as dead or non-proliferating cells do not produce the required amount of surfactant. The capsule containing live cells can then be transported using chemical gradient to a specific location and dropped though the addition of a water phase with concentrated surfactant.

We show that chemotactic droplet systems can interface with biological systems and transport live cells in petri dishes, but other scenarios are possible. Active droplets containing cells could be applied in smaller environments such as microfluidic chips, leading to the implementation of next generation technologies for cell screening (e.g. live vs dead). Chemotaxis systems and alginate capsules are inexpensive and easy to manipulate and could be applied more widely. For example, alginate capsules could be exploited to delivery bacteria or enzymes for improved bioremediation (10). Droplets determine the transport to locations not accessible by human hands and capsules could protect bacteria from harsh environmental conditions (11). This same approach could be used for environmental planning, to test possible bacterial/enzymatic/chemical treatments, transported by capsules and droplets, on systems with reduced scale. Therefore we expect a certain degree of societal impact through the ongoing development of this artificial life technology.

Acknowledgements

This work was financially supported in part by the European Commission FP7 Future and Emerging Technologies Proactive 611640 (EVOBLISS) and by the European Union's Horizon 2020 research and innovation program under grant agreement 824060 (ACDC).

References

Holler, S. Porcelli, C. Ieropoulos, I. A. and Hanczyc, M. M. (2018) Transport of Live Cells Under Sterile Conditions Using a Chemotactic Droplet. Sci Rep 8:8408

Bütschli, O. (1892) Untersuchungen über microscopische Schaume und das Protoplasma. Leipzig.

Armstrong, R. and Hanczyc, M.M. (2013) Btschli dynamic droplet system. Artif Life. Summer-Fall 19(3-4) 331-46

Caschera, F. Rasmussen, S. and Hanczyc, M.M. (2013) An Oil Droplet Division-Fusion Cycle ChemPlusChem 78 52?54

Bayley, H. Cronin, B. Heron, A. Holden, M.A. Hwang, W. Syeda, R. Thompson, J. and Wallace, M. (2008) Droplet interface bilayers Mol Biosyst. 4(12) 1191?1208

Booth, J. M. Restrepo Schild, V. Box, S. J. and Bayley, H. (2017) Light-patterning of synthetic tissues with single droplet resolution. Sci. Rep. 7, 9315

Ševčíková, H. Čejková, J. Krausová, L. Přibyl, M. Štěpánek, F. and Marek, M. (2010) A new traveling wave phenomenon of *Dictyostelium* in the presence of cAMP. Physica D: Nonlinear Phenomena 239 879-888

Čejková, J. Novak, M. Štěpánek, F. and Hanczyc, M.M. (2014) Dynamics of chemotactic droplets in salt concentration gradients. Langmuir 30 11937-11944

Cejkova, J. *et al.* (2016) Chemotaxis and chemokinesis of living and non-living objects. Springer, Advances in Unconventional Computing 256-260

Pieper, D. H. and Reineke, W. (2010) Engineering bacteria for bioremediation. Curr. Opin. Biotechnol. 11(3) 263-270

Islam, M. A. Cheol-Heui, Y. Yun-Jaie, C. and Chong-Su C. (2010) Microencapsulation of Live Probiotic Bacteria. J. Microbiol. Biotechnol. 20(10) 1367-1377