Bio-printed hydrogel micro-droplets for culturing and analysis of microbial communities

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Microbes are found everywhere. Their communities shape local environments, changing the composition and structure of soils, water, air and even living hosts. The production, secretion, and consumption of chemicals by these consortia are essential to their coordination and survival as a community and have also been harnessed for centuries to serve as the foundation for the industrial production of commodity chemicals. While many methods exist to identify the composition of a microbial community, the role of specific species within the community, and how they impact the chemical profile of the local environment is not well characterized. Techniques that allow rapid screening of chemical production by individual members or select consortia would be valuable for both fundamental studies of microbial activity, as well as the optimization of commercial chemical production efforts. Combined with the ability to manipulate the spatial patterning of community members, the ability to monitor chemical production and view the response of neighboring species could be used in fundamental studies of microbial communication or in the creation of complex spatially segregated bioprocessing pipelines.

In this work, we have developed a high throughput method for printing cell-laden hydrogel droplets and assembling microbial communities for biological studies. Microbes are encapsulated into alginate hydrogel micro-droplets by dispensing cells suspended in an alginate solution into a $CaCl_2$ solution under centrifugal force. The cell-encapsulated hydrogel micro-droplets are then mixed with low melt agarose under sterile conditions. The mixture is loaded into a bio-printer and printed at 25 to 37 degrees Celsius, depending on the concentration and the type of agarose used. A variety of microbial cells have been used to build microbial communities of one or more species, including *E. coli* cells expressing GFP, an engineered 'sender-receiver' *E.coli* system, and yeast cells.

We have successfully demonstrated the use of hydrogel micro-droplets to encapsulate and position microbial cells in desired patterns. The sizes of the hydrogel micro-droplets range from 100 to 400 μm in diameter. Droplet size can be tuned by varying the centrifugation speed and modulated by varying the concentration and viscosity of the alginate solutions. We have printed the cell-containing micro-droplets in patterns of squares and arrays of dots. The cells distributed via this method remain viable and form micro-colonies inside the micro-droplets. We have demonstrated that this system can be used to spatially segregate cells while permitting chemical crosstalk using a sender-receiver *E.coli* system in which the sender cells produce a soluble signal (acyl-homoserine lactone) that induces GFP fluorescence when taken up by the receiver cells. The

chemical signals produced in the microbial communities can be examined optically (fluorescence microscopy) or analytically (Raman spectroscopy).

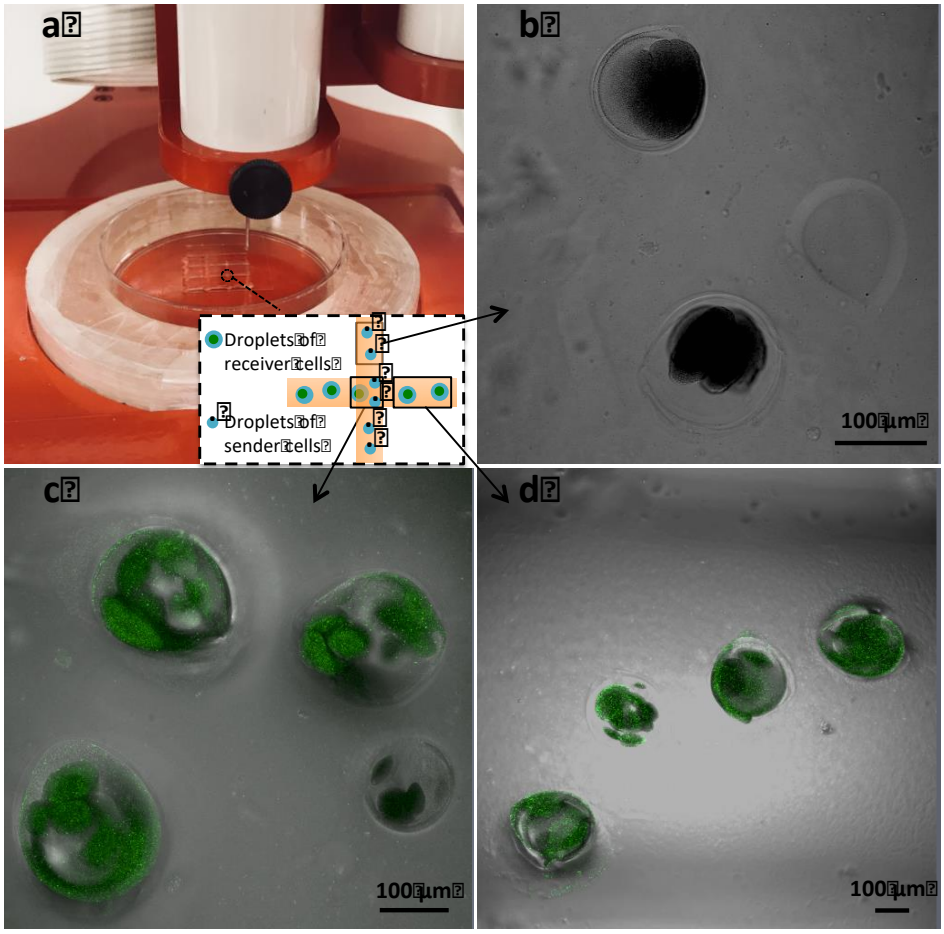

Figure 1: Intercellular communication between cells encapsulated in different hydrogel droplets through chemical signaling. (a) A photo of the bioprinting setup and the printed grid of sender-receiver *E.coli* species. The zoom-in box shows a scheme of the grid at the cross-junction of the printed horizontal and vertical lines. The vertical lines contain hydrogel droplets with sender cells, and the horizontal lines contain hydrogel droplets with receiver cells. The receiver cells are engineered to only emit fluorescent signals when activated by the chemical signals from the sender cells. Fluorescent microscopic images in the section of (b) vertical lines, (c) cross-junction, (d) horizontal lines are shown.