Monodispersed double emulsions as programmable microenvironment for cellular studies

Ying Zhang, Hon Fai Chan, Yi-Ping Ho, Ya-Ling Chiu, Lingchong You, Kam W. Leong
Biomedical Engineering Department, Duke University

Statement of Purpose: Miniaturized bioreactors have emerged to be important tools for high-throughput biochemical and cell analysis. It improves the cost-effectiveness and sensitivity of high-throughput studies by reducing sample consumption and enhancing signal response due to the small sample volume. Microfluidics mediated emulsion formation, in particular, provides rapid, scalable and reliable synthesis of monodispersed droplets, which could be used as discrete bioreactors in the size ranging from femtoliter to picoliters. However, due to solvent compatibility issues, water in oil (W/O) single emulsion only allows for short term assays. Water-in-oil-in-water (W/O/W) double emulsions, which is compatible with aqueous biological systems, are less studied due to the difficulty in their generation and poor stability. In this study, we explore the potential application of W/O/W double emulsion droplets for cell cultivation, genetic activation and study of more complicated biological events such as bacteria quorum-sensing, and high throughput generation of human mesenchymal stem cell (hMSC) spheroid.

Methods:

Microfluidic chips were fabricated with a standard soft lithographic protocol using poly (dimethyl) siloxane (PDMS). To form stable double emulsion droplets in hydrophobic PDMS channels, the chips have to be patterned with differential wettability. To simplify the process, we used two-chip setup: W/O droplets formed in a hydrophobic chip was fed into a second chip which had been treated hydrophilic to form W/O/W double emulsions (Fig. 1a). Fluorinatedcarbon oil was chosen as the organic phase for its biocompatibility and high oxygen retention. We encapsulated *E.coli* bearing simple and complex genetic circuit and observed their growth and gene expression inside the double emulsion droplets. For the spheroid study, hMSC in culture medium or in alginate solution were also encapsulated.

Results: *Bacteria study:* chemical diffusion mediated gene activation

Monodispersed DE with tunable sizes were formed from a two-chip setup. By encapsulating various molecules with DE droplets, we demonstrated the oil layer was selectively permeable to small chemicals. To show the potential application of double emulsion as a perturbable microenvironment for cellular study, we used inducible gene expression system as a model. *E. coli* cells (MC4100Z1 cells) carrying a green fluorescence protein (GFP) gene controlled by a TetR-regulated promoter were encapsulated. GFP expression was inducible by a small chemical, anhydrotetracycline (aTC), which was only given in the external solution surrounding DE droplets. GFP expression became detectable 1 h after aTC addition, indicating fast transport of the molecule (Fig.1b). Nutrient transport, though happened much slower, was evident from flow cytometry study when bacteria containing DE were suspended in PBS and PBS plus glucose.

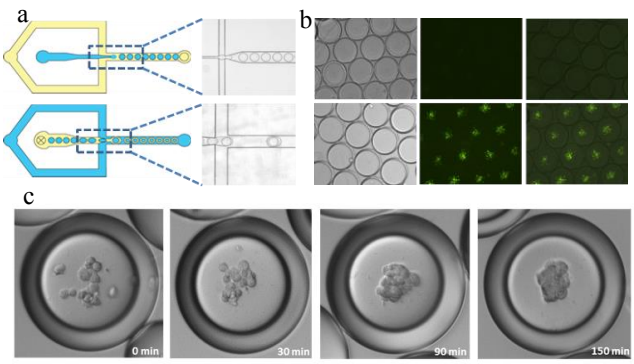

Figure 1. DE formation and cell encapsulation. a) DE formation. b) aTC induced GFP expression by *E.coli* cultured in DE. c) Rapid hMSC spheroid formation in DE.

Mammalian cell study: Rapid hMSC spheroid formation in double emulsion

To explore the potential use of DE for mammalian cell study and tissue engineering, we encapsulated hMSC inside the droplets. Due to the strong hydrophobicity of the oil shell, hMSC grew near the core of the droplets, and formed aggregated 3D spheroid. This process took approximately 150 mins (Fig. 3c), which was faster than other reported methods. We speculate this was due to the micro-scale confinement that promoted cell-cell interaction. The percentage of spheroid formation in the droplets was nearly 100%. Compact spheroids could be retrieved after six hours post-encapsulation with the aid of a droplet releasing agent. The release efficiency was above 95% and high cell viability was maintained during the process. Staining of extracellular matrix (ECM) proteins, collagen 1 and laminin indicated complex ECM network formation, mimicking the situation *in vivo*. When alginate-RGD was used as support matrix for hMSC spheroid formation in DE droplets, we observed increased α5β1 integrin expression. Moreover, Alizarin red staining for calcium ions showed drastic increase in calcium deposition, suggesting osteogenic differentiation.

Conclusions: We have demonstrated efficient production of well-controlled DE using a modular two-chip design. The selectively-permeable oil barrier creates a discrete microenvironment whose aqueous core could be well defined and precisely modulated for cell cultivation. DE also serves as bioreactor for rapid cell aggregation. The inner phase could be replaced with hydrogels to present microenvironmental cues. The proposed platform obviates the need for labor-intensive fabrication of stem cell spheroids and replating of spheroids into matrix scaffold.

References:

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