Lipid Bilayer-Coated Polydimethylsiloxane Microfluidics for Gene Delivery

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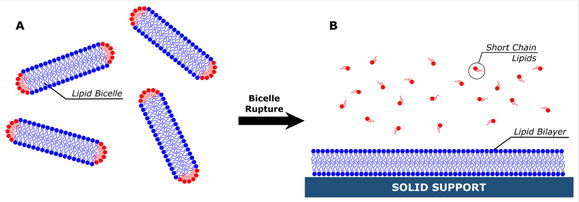
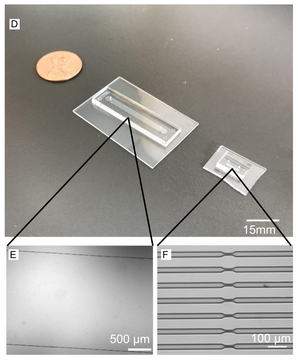
Introduction

- Gene-editing tools are leading to a new era of personalized medicine, including gene therapy for genetic blood disorders and CAR-T cell therapies for blood cancers.
- Because of the limitations of lentivirus-based gene delivery, there is strong demand for scalable technologies to edit blood cell genomes with high efficiency and speed.
- Constrictive channel microfluidic systems have been shown to deliver biomolecules using rapid cell squeezing to achieve membrane disruption.
- A remaining challenge with constrictive channel microfluidic systems is clogging.
- We report that using PDMS constrictive channels leads to dramatically less cell clogging and device failure. Further, coating channels using lipid bicelles reduces nonspecific protein adsorption and cell adhesion.

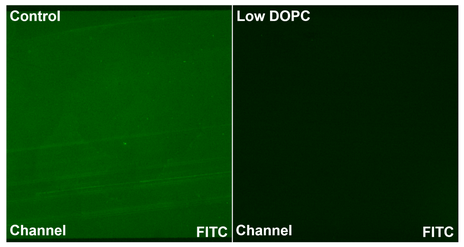
Methods

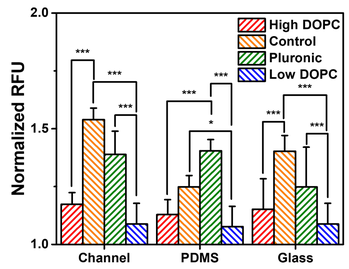


Bilayer formation. Bicelles are formed from short chain and long chain phospholipids (A). Bicelle rupture creates a lipid bilayer (B) that prevents clogging as cells pass through a constrictive channel (C). We manufactured constrictive channels (D, F) as well as larger demonstration channels (D, E).

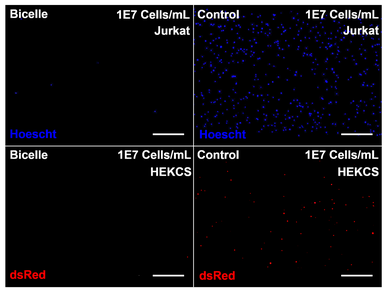


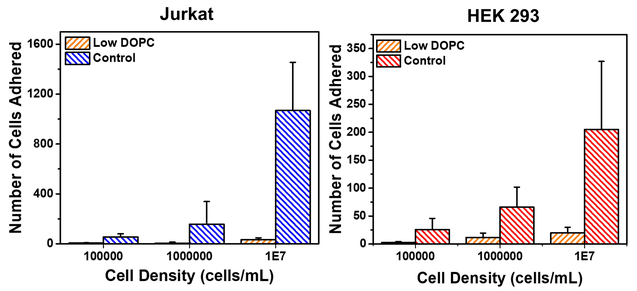
Results



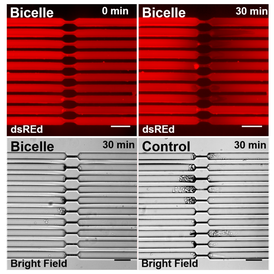


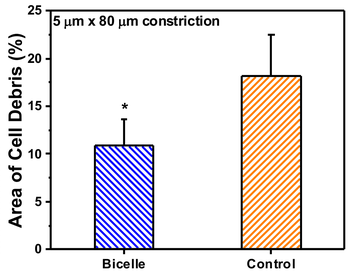
Bicelle-treated channels demonstrate reduced protein adsorption compared with untreated controls. FITC-conjugated bovine serum albumin was flowed through bicelle-treated and control channels. After washing, fluorescence was measured from the entire channel, the PDMS component alone, and the glass component alone.



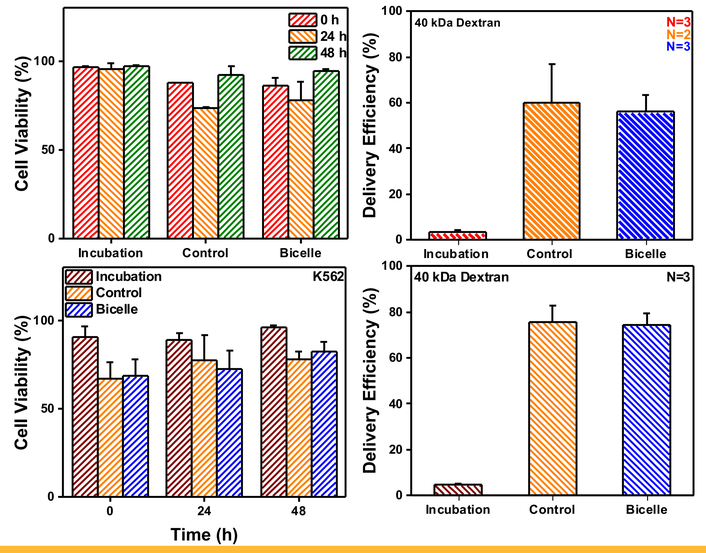


Bicelle treatment reduces cell adhesion. Fluorescent Jurkat and HEK cells were flowed through bicelle-treated and control channels followed by washing and fluorescence microscopy.





Bicelle-treated constrictive channels build up less cell debris than uncoated channels. Jurkat cells were flowed through bicelle-treated and untreated 5µm constricted channels for 30 min. Cell debris was imaged using phase contrast microscopy and quantified by area using ImageJ. Imaging of the lipid bilayer itself was achieved by spiking with dsRed-conjugated long chain phospholipids and showed moderate loss of bilayer after device usage.



Bicelle treatment does not increase intracellular delivery of 40 kDa dextran or increase cell viability. Jurkat (top panels) and K562 cells (bottom panels) were flowed through constrictive channels with FITC-conjugated 40 kDa dextran. Cell viability was measured at 0h, 24h, and 48h using Trypan blue. Dextran delivery was measured at 0h using flow cytometry and compared with cells treated with dextran without flowing through constrictive channels (incubation).

Discussion

- While bicelle treatment reduced protein adsorption, cell adhesion, and cell debris buildup, it did not affect the ultimate effectiveness of the devices as measured by delivery efficiency, cell viability, or cells recovered.
- Bicelle treatment likely would help to prevent cell clogging and device failure, but our PDMS/glass constrictive channels did not fail despite high flow rates, high cell densities, and long run times.
- Even without bicelle treatment, our devices demonstrated much greater resistance to clogging and failure than other devices reported in the literature, with similar delivery efficiencies.
- Bicelle treatment may prevent clogging and device failure when our constrictive channels are used with cell types other than Jurkat and K562.
- With further development, our constrictive channel microfluidic devices could meet the demand for a scalable, high-speed, high-efficiency tool to deliver nucleic acids and proteins to blood cells.

Future Work

- We plan to evaluate cell clogging and device failure with HEK 293 cells flowing through constrictive channels with and without bicelle treatment.
- We plan to evaluate the kinetics of bilayer formation from bicelles using timelapse fluorescence microscopy of ds-Red-conjugated bicelles in addition to fluorescence recovery after photobleaching.
- We plan to evaluate our device's efficiency at delivering protein and nucleic acids to Jurkat cells using GFP plasmid, FITC-conjugated BSA, and fluorescently-tagged Cas9 protein.