Miniaturized high-throughput cell screening of transfection enhancers using droplet microarrays

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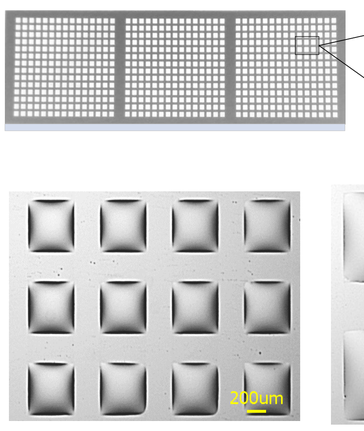
Introduction

DNA delivery, especially via the non-viral route, has been demonstrated as a powerful research tool for biological research and clinical therapies [1]. However, many transfection reagents have relatively low transfection efficiency, especially on suspension blood cells such as Jurkat. In this project we hypothesize that by treating Jurkat and CHO-K1 cells with small molecules it is possible to improve transfection efficiency in a safe, efficient and controllable way. In order to identify such transfection enhancer molecules, thousands of structurally diverse molecules are being tested with cells. Current high-throughput screening (HTS) technologies based on microtiter plates cannot be used in such screenings due to prohibitively high costs associated with large volumes required.

Method



Microscope images of 500 μm and 1000 μm droplet microarrays (DMA)

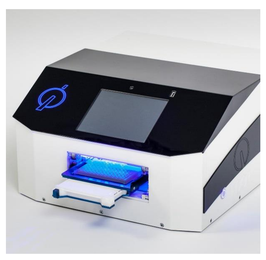
Hydrophilic spots	Superhydrophobic borders	Spots per array
1000 μm	500 μm	196×3=588
500 μm	250 μm	729×3=2187

Our group has recently developed a droplet microarray (DMA) platform based on the superhydrophobic-hydrophilic surfaces, which can be applied to high-throughput screenings of live cells [2-3].

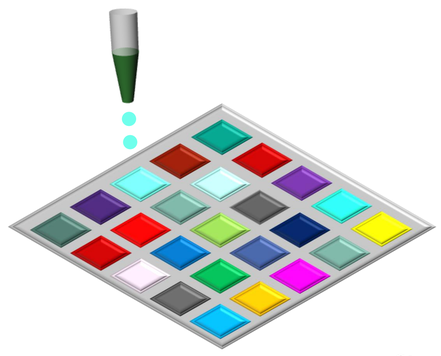
Step one

sciFLEXARRAYER S11, a fully automated non-contact dispenser for printing drugs.

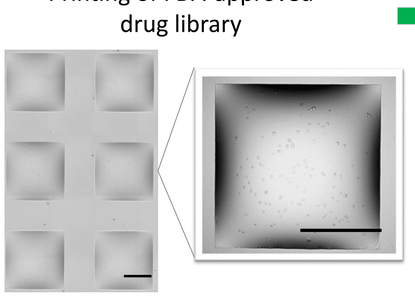
Step two



I-DOT One Non-Contact Dispenser for printing transfection mixture and cells.

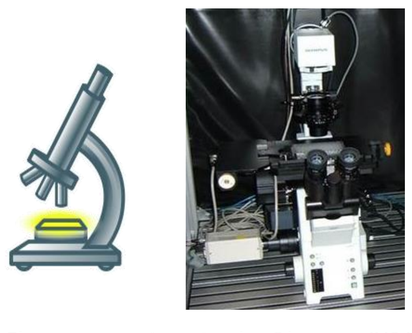


Printing of FDA-approved drug library

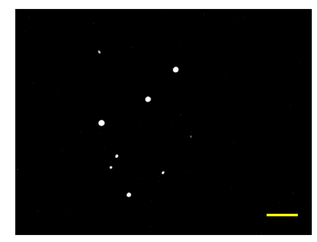


Microscope images of printing of transfection mixture and cells

Step three



Fluorescence imaging by Olympus IX81 motorized inverted research microscope after cells were incubated for 24 h.

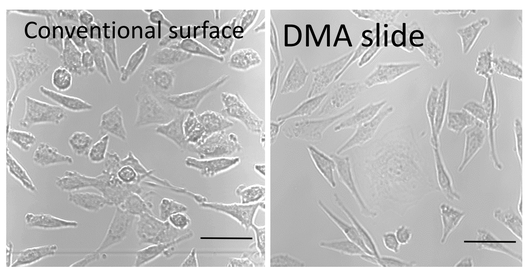


Transfection was quantified by GFP positive cell numbers.

Results

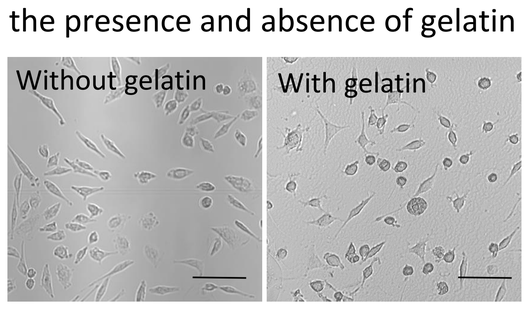
Cell culture on DMA slides

(I) Cell morphology on conventional surface and our DMA slides



Scale bar: 50 μm

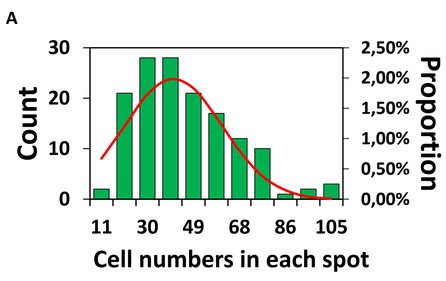
(II) Cell morphology on DMA slides in the presence and absence of gelatin



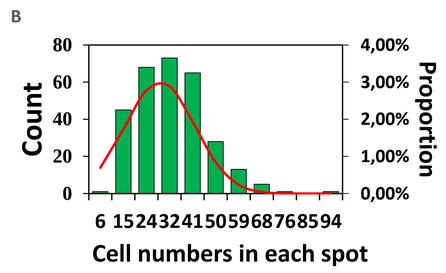
Scale bar: 100 μm

Cell number distribution

(III) Cell number distribution on DMA slide after I-DOT printing



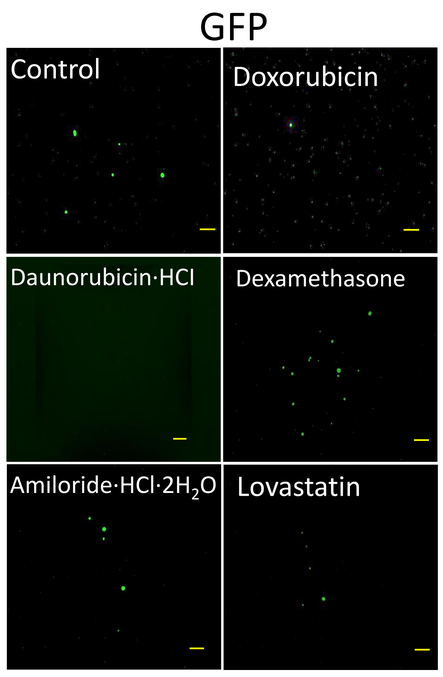
Jurkat cell number distribution



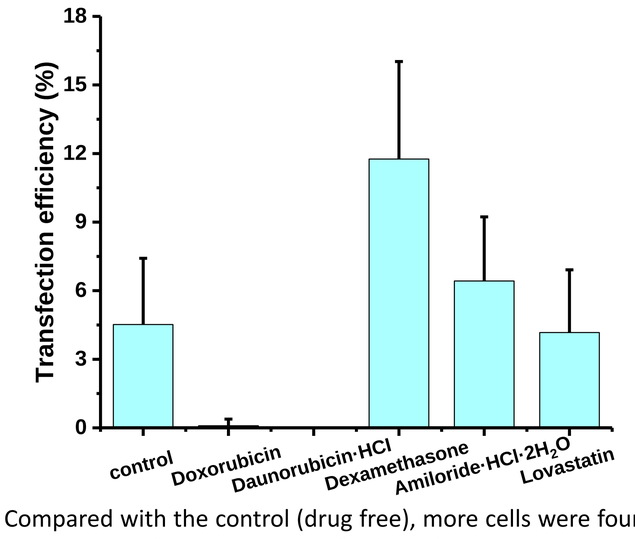
CHO-K1 cell number distribution

Transfection

(IV) One step transfection with five FDA-approved drugs



Scale bar: 100 μm



Compared with the control (drug free), more cells were found to express GFP after on step transfection with Dexamethasone and Amiloride·HCl·2H_2O for 24 h, accompanied with 11.8% transfection efficiency of Dexamethasone and 6.4% expressed GFP of Amiloride·HCl·2H_2O group, respectively. Doxorubicin, Daunorubicin·HCl and lovastatin had no enhancement effect on gene transfusion.

Conclusion

Our DMA slides have shown as a controllable and inexpensive approach to do gene transfection compared with conventional platform microplates. Influence of five FDA-approved drugs on transfection has already been investigated and HTS with the whole FDA-approved drug library is in progress now.

References

1 Luo, D., & Saltzman, W. M. (2000). Synthetic DNA delivery systems. *Nature biotechnology*, 18(1), 33.

2 Popova, A. A., Schillo, S. M., Demir, K., Ueda, E., Nesterov-Mueller, A., & Levkin, P. A. (2015). Droplet-Array (DA) Sandwich Chip: A Versatile Platform for High-Throughput Cell Screening Based on Superhydrophobic–Superhydrophilic Micropatterning. *Advanced Materials*, 27(35), 5217-5222.

3 Ueda, E., Geyer, F. L., Nedashkivska, V., & Levkin, P. A. (2012). DropletMicroarray: facile formation of arrays of microdroplets and hydrogel micropads for cell screening applications. *Lab on a Chip*, 12(24), 5218-5224.