Automatic Single Cell Printing for Tissue Engineering and Stem Cell Research

A. Gross, J. Schöndube, S. Rubenwolf, A. Yusof, G. Roth, R. Zengerle and P. Koltay
University of Freiburg - IMTEK, Department of Microsystems Engineering, Laboratory for MEMS Applications
Andre.Gross@imtek.de

Introduction

We present a novel method for automatic non-contact printing living single cells currently being developed within the PASCA project (www.pasca.eu). A microfluidic dispenser [1] with optical feedback prints single cells confined in free flying droplets of only hundreds of picoliter volume onto arbitrary substrates. Single yeast, cancer (HeLa) and stem cells (fibroblasts, keratinocytes) have been successfully printed with this method at viabilities of up to 95 %. Apart from applications in research and diagnostics the technology can also be used for tissue engineering. To show the feasibility an extra cellular matrix comprised of collagen and alginate has been printed.

Methods

Main components of the single cell manipulator (SCM) are a three-axes lab-robot, a camera and a transparent dispenser-chip to generate the droplets. The dispenser-chip is fabricated from silicon and glass. The camera observes the cells inside the chip and decides whether a single cell will be expelled within the next droplet. Cell solution (10^5 cells/ml) is supplied to a reservoir and droplets of 100 pl are printed at arbitrary positions on various substrates.

Results

Optical observation can be operated at 100 Hz and object recognition allows selection of individual cells with 89 % success rate. However, depending on cell density in solution single cell printing frequencies can be much lower. Single yeast (*S. cerevisiae)* and HeLa cells were printed with viabilities of 95 % and 75 %, respectively. Also viability of printed fibroblasts and keratinocytes has been confirmed. Printing of collagen and alginate droplets has been achieved with 300 µm resolution. Also multi-layered composite structures of alginate, collagen and cells were printed.

Conclusion

The SCM method has been shown to print viable single cells of several common cell lines. In future multi-layered collagen/alginate structures could, in combination with cell printing, allow for creating three-dimensional cell tissues.

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References

[1] P. Koltay, R. Steger, G. Birkle, H. Huang, H. Sandmaier and R. Zengerle. “Microdispenser array for highly parallel and accurate liquid handling”. *SPIE’s MICRO/MEMS, (2001)*.

[2] B. R. Ringeisen et al. “Jet-based methods to print living cells”. *Biotechnol. J. 1, 930–948, (2006)*

[3] W. C. Wilson Jr. and T. Boland. “Cell and Organ Printing 1: Protein and Cell Printers”. *The Anatomical Record Part A 272A:491–496, (2003)*

[4] C. M. Othon, X. Wu, J. J. Anders and B. R. Ringeisen. “Single-cell printing to form three-dimensional lines of olfactory ensheathing cells”. *Biomed.Mater. 3, 034101 (6pp), (2008)*

[5] W. Streule et al. “PipeJet: a simple disposable dispenser for the nano- and microliter range”. *Journal of the Association for Laboratory Automation, Volume 9, Issue 5, Pages 300-306, (2004)*