Integrated Instrument for Printing Single Cells and Hydrogel Matrices for Future Tissue Engineering


* Laboratory for MEMS Applications, Department of Microsystems Engineering - IMTEK, University of Freiburg, Germany
**BioFluidix GmbH, Georges Köhler Allee 106, 79110 Freiburg Germany

Summary
The great vision of 3D printing of human tissue requires [1]:
- 3D printing of an extra cellular matrix (ECM)
- dispensing viable cells into the ECM

In this work an instrument is presented, that is capable of dispensing two different hydrogels (alginate, collagen) and single cells in one run.

The rigid alginate serves as structural framework and soft collagen provides a convenient environment for cells to grow. With the same instrument single cells are printed one by one onto the hydrogel structures with a microfluidic cell dispenser (www.pasca.eu, [2]).

Single Cell Manipulator (SCM)
Main components of the SCM (Fig. 1) are:
- three-axes lab-robot
- machine vision system
- transparent NanoJet™ cell dispenser (Fig. 2 b), c))
- Pipe-Jet™ alginate/collagen dispenser (Fig. 2 a)

Figure 1: Experimental setup with dispensers, optics and substrate.

Figure 2: a) PipeJet™-Technology: A piezo compresses a tube and expels droplets of alginate or collagen. b) NanoJet™-Technology: A piezo deflects a silicon membrane to dispense droplets containing single cells. c) Single cell printing method: unwanted droplets are sucked away, only droplets with single cells are printed. Not to scale.

Figure 3: Two layer alginate square.

Printing of Extra Cellular Matrix (ECM)
Using the PipeJet™-Technology as rapid prototyping method, extra cellular matrices are printed (Fig. 3). Multilayered con-structions of collagen and alginate are printed in defined patterns to support and guide cell growth.

Figure 4: 3-layer alginate square. a) 30 µm b) 100 µm

Figure 5: a) Single dispensed fibroblast cultured within surrounding printed alginate structure (shaded) after 5h of incubation. b) Close up of the cell.

Single Cell Printing Results
After printing of the hydrogel structures, cells are dispensed at controlled positions on the sample. Groups of cells and single cells adhere and grow on a collagen surface within an alginate square. HeLa (cervical cancer) cells were printed in batches of ~50 cells (Fig. 4) or as individual cells in a square alginate confinement. Adherence of cells on in Fig. 4b) indicates viability. A single viable fibroblast (tissue stem cell) printed into an alginate square is shown in Fig. 5.

Conclusion
The presented method has been shown to enable printing of viable single cells of several common cell lines as well as multi-layered collagen/alginate structures. Experiments demonstrate that cells can be grown in these structures.

A combination of the collagen/alginate and cell printing could allow for creating three-dimensional cell tissues in the future.

Acknowledgements
This work has been supported by BMBF (FKZ 16SV50066) and by the European Commission (FP7 GA257073).

References