Topic: Cell and Tissue Engineering or Single Cell Platform **Poster presentation only**

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

J. Schöndube[1], A. Gross[1], S. Rubenwolf[1], S. Niekrawitz[1], P. Koltay[1,2], R. Zengerle[1,3,4] and G. Roth[1,3,4]

[1] Laboratory for MEMS Applications, Department of Microsystems Engineering - IMTEK, University of Freiburg, Georges-Koehler-Allee 103, 79110 Freiburg, Germany,
[2] BioFluidix GmbH, Georges-Koehler-Allee 106, 79110 Freiburg
[3] HSG-IMIT – Institut für Mikro- und Informationstechnik, 78052 Villingen-Schwenningen
[4] BIOSS – Centre for Biological Signalling Studies, Universität Freiburg, Albertstrasse 19, 79104 Freiburg
Jonas.Schoendube@imtek.de

Summary

The great vision of 3D printing of human tissue requires generating an extra cellular matrix with high resolution and filling it with cells of various types. We try to turn this vision into reality and present here our first achievements. We developed an instrument that enables non-contact printing of a **hydrogel** aimed to serve as an **extra cellular matrix** (ECM). The hydrogel is an alginate/collagen composite structure with features down to 300 µm. Rigid alginate serves as structural framework and soft collagen provides a convenient environment for cells to grow. On the same instrument **single cells are printed** one by one onto the hydrogel with a microfluidic dispenser (www.pasca.eu, [1]). Several human cell lines (**HeLa, fibroblasts** and **keratinocytes)** have been deposited and HeLa cells as well as fibroblasts successfully adhered on the substrate.

Motivation

Nowadays artificial tissue is usually created by culturing cells in prefabricated scaffolds [2, 3]. An undefined number of cells is seeded in the scaffold by soaking it into cell solution or by inkjet printing. In contrast, our method aims to print all components in a non-contact manner layer-by-layer. Individual cells are encapsulated and positioned with the help of an optical feedback system. Currently this process is done manually but has already been proven to work in an automated fashion [1]. In summary, our approach differs from others by single cell positioning within a tunable 3D ECM.

Printing of ECM-like Hydrogel

The instrument consists of a three axes robot, optics, an ECM dispenser and a cell dispenser (Fig. 1). ECM dispensing is based on the PipeJet^{TM}-Technology (Biofluidix; Fig. 2a)) with 5 nl – 15 nl droplets of alginate solution (0.5% w/v) printed on collagen coated glass slides. Gelation of the alginate is caused by a liquid thin film of 0.1 M $CaCl_2$ on the glass. Single alginate spots have diameters of 300 - 500 µm and enable printing lines of 500 µm. Up to five layers of alginate have been generated on top of each other (Fig. 3). Collagen is printed with a similar technology and can be applied to cover the alginate (Fig. 4).

Printing of Single Cells onto Hydrogel

Single cells are printed with a transparent NanoJet^{TM} dispenser (Biofluidix; Fig. 2 b)) fabricated from silicon and glass. The optics allows observing the nozzle inside the chip. When a single cell is detected, it can be dispensed on demand (Fig. 5). Cell solution (~10^5 cells/ml) is supplied to a reservoir and droplets of ~100 pl are printed. HeLa cells were printed in batches of ~50 cells (Fig. 5) or as individual cells (Fig. 7) in a square alginate confinement. Adherence of cells on in Fig. 6b) indicates viability. A single viable fibroblast is shown in Fig. 8.

Conclusion

We presented an instrument which enables to print single cells of common cell lines onto printed hydrogel matrices. The multi-layered compound of alginate support and collagen, allows for creating three-dimensional cell positioning, which is a prerequisite for prototyping cellular tissues. Nevertheless ECM-like structures have still to be miniaturized in the future for human 3D tissue prototyping.

Character count: 2649

References

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[3] R. Barry, R. F. Shepherd, J. N. Hanson, R. G. Nuzzo, P. Wiltzius, and J. Lewis, “Direct-Write Assembly of 3D Hydrogel Scaffolds for Guided Cell Growth,” *Advanced Materials*, vol. 21, no. 23, pp. 2407-2410, Jun. 2009.

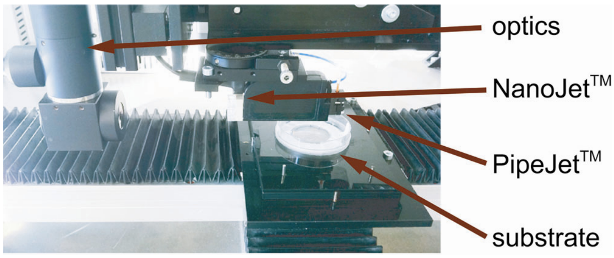

Fig 1. Experimental setup with dispensers, optics and substrate.

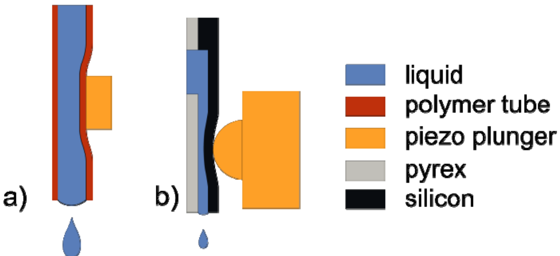

Figure 2. a) PipeJet$^{\text{TM}}$-Technology: A piezo compresses a tube and expels droplets of alginate or collagen. b) NanoJet$^{\text{TM}}$-Technology: A piezo deflects a silicon membrane to dispense droplets containing single cells. Not to scale.

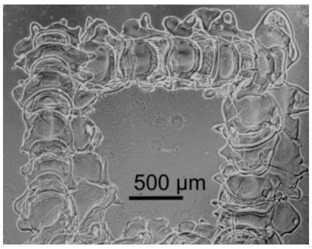

Figure 3. Two layer alginate square.

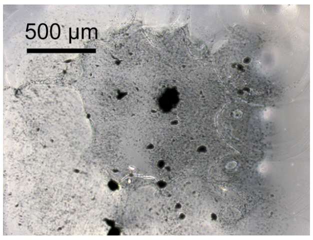

Figure 4. Alginate square covered by collagen, which is stained with carbon black particles.

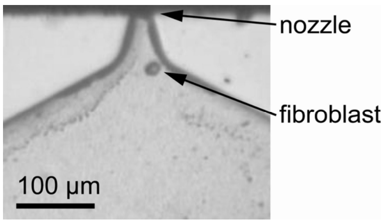

Figure 5. Magnified view on the NanoJet$^{\text{TM}}$ nozzle. The operator controls the dispensing and therewith the number and position of cells to be placed on the substrate.

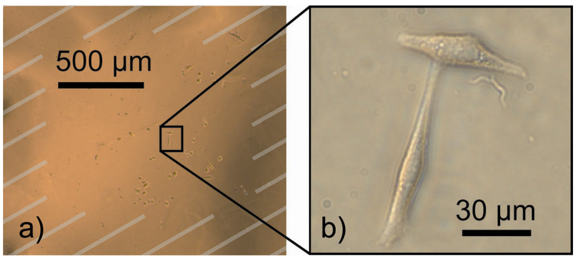

Figure 6. a) Three-layer alginate square (shaded) with ca. 50 HeLa cells after two days of incubation. The transparent alginate is difficult to visualise, due to the submersion of the sample in cell media. b) Close up of two HeLa cells.

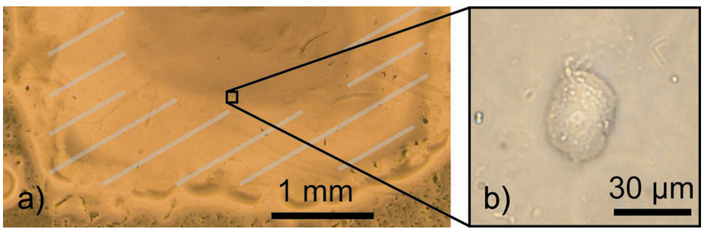

Figure 7. a) Lower half of a three-layer alginate square (shaded) with exactly one HeLa cell after one day of incubation. b) Close up of the single cell.

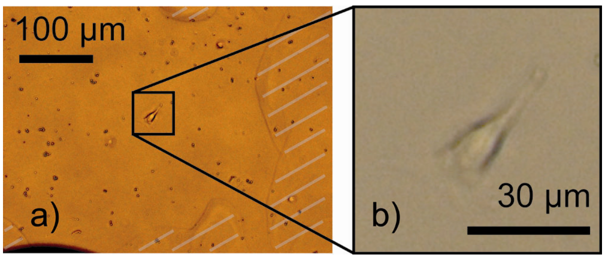

Figure 8. a) Single dispensed fibroblast cultured within surrounding printed alginate structure (shaded) after 5h of incubation. b) Close up of the cell.