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

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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))

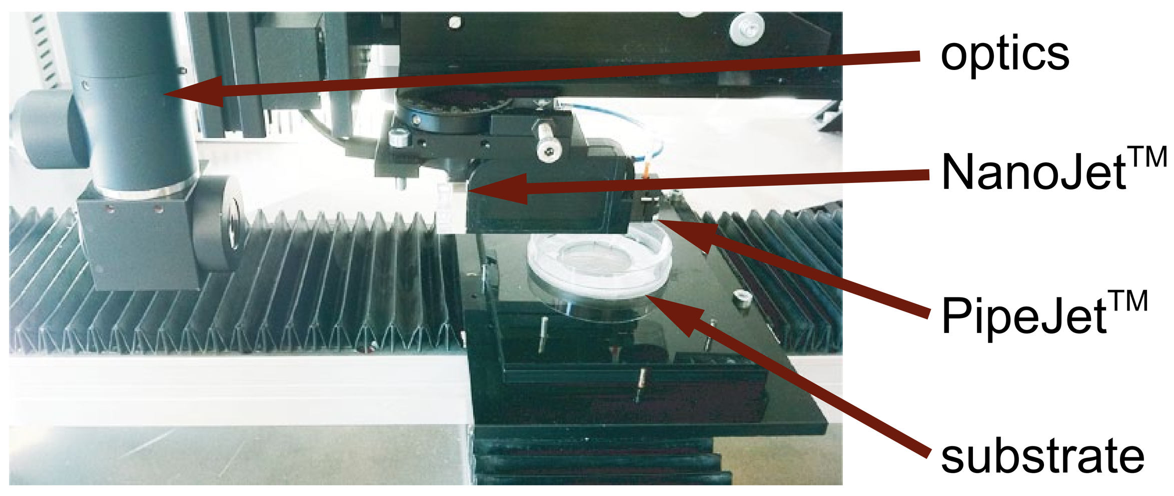

Figure 1: Experimental setup with dispensers, optics and substrate.

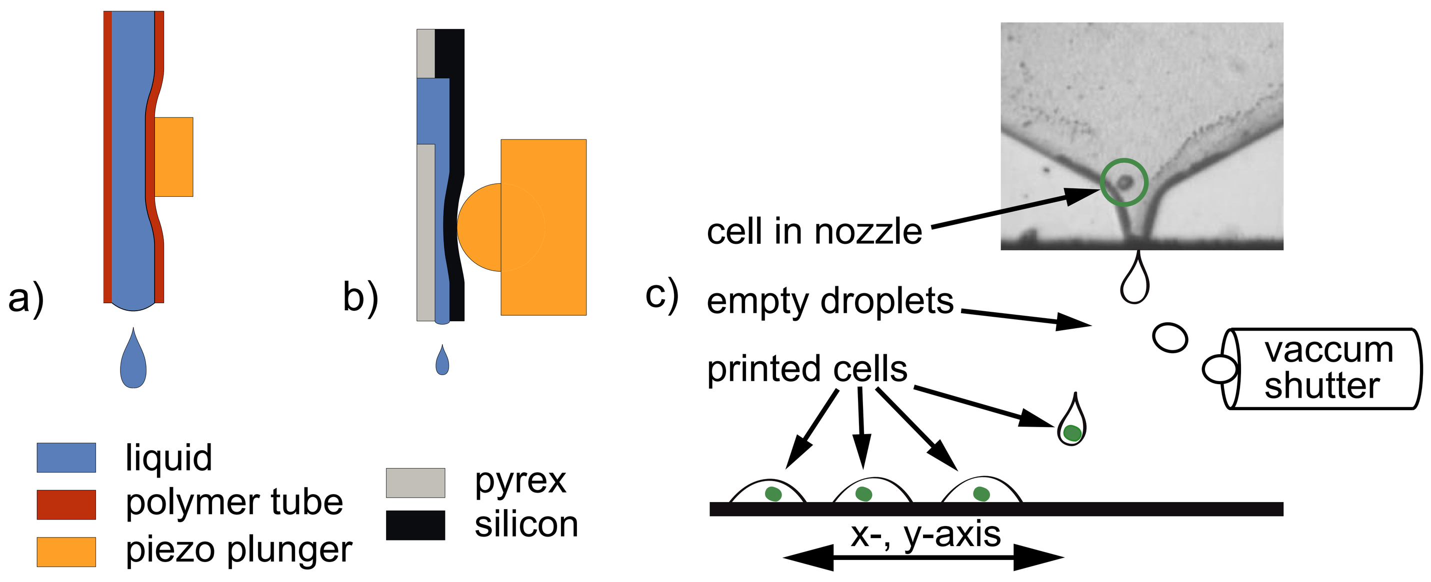

Figure 2: a) PipeJet™-Technology: A piezo compresses a tube and expels droplets of alginate or collagen. b) NanoJet™-Technology: A piezo deflects a silicon membrane to dispense droplets containing single cells. c) Single cell printing method: unwanted droplets are sucked away, only droplets with single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

Printing of Extra Cellular Matrix (ECM)

Using the PipeJet™-Technology as rapid prototyping method, extra cellular matrices are printed (Fig. 3). Multilayered co-structures of collagen and alginate are printed in defined patterns to support and guide cell growth.

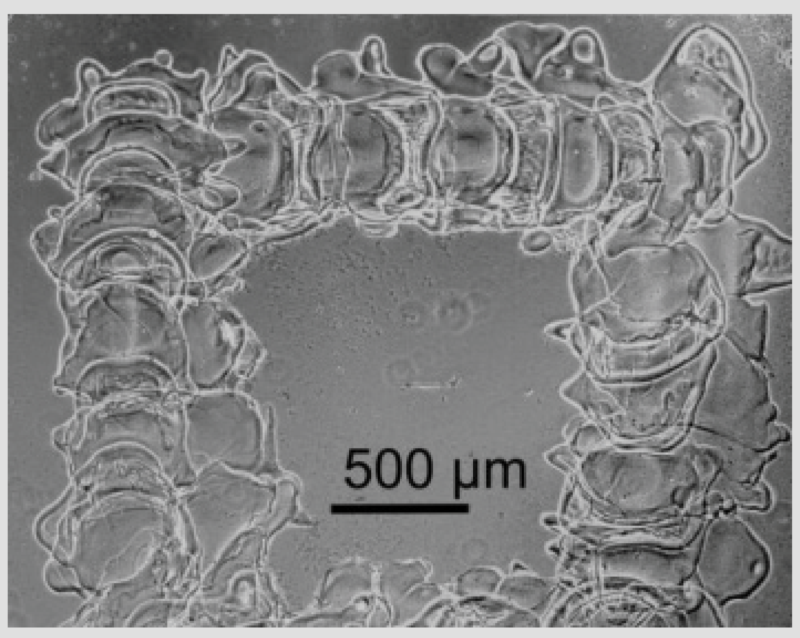

Figure 3: Two layer alginate square.

Single Cell Printing Results

After printing of the hydrogel structures, cells are dispensed at controlled postions on the sample. Groups of cells and single cells adhere and grow on a collagen surface within an alginate square. HeLa (cervial 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.

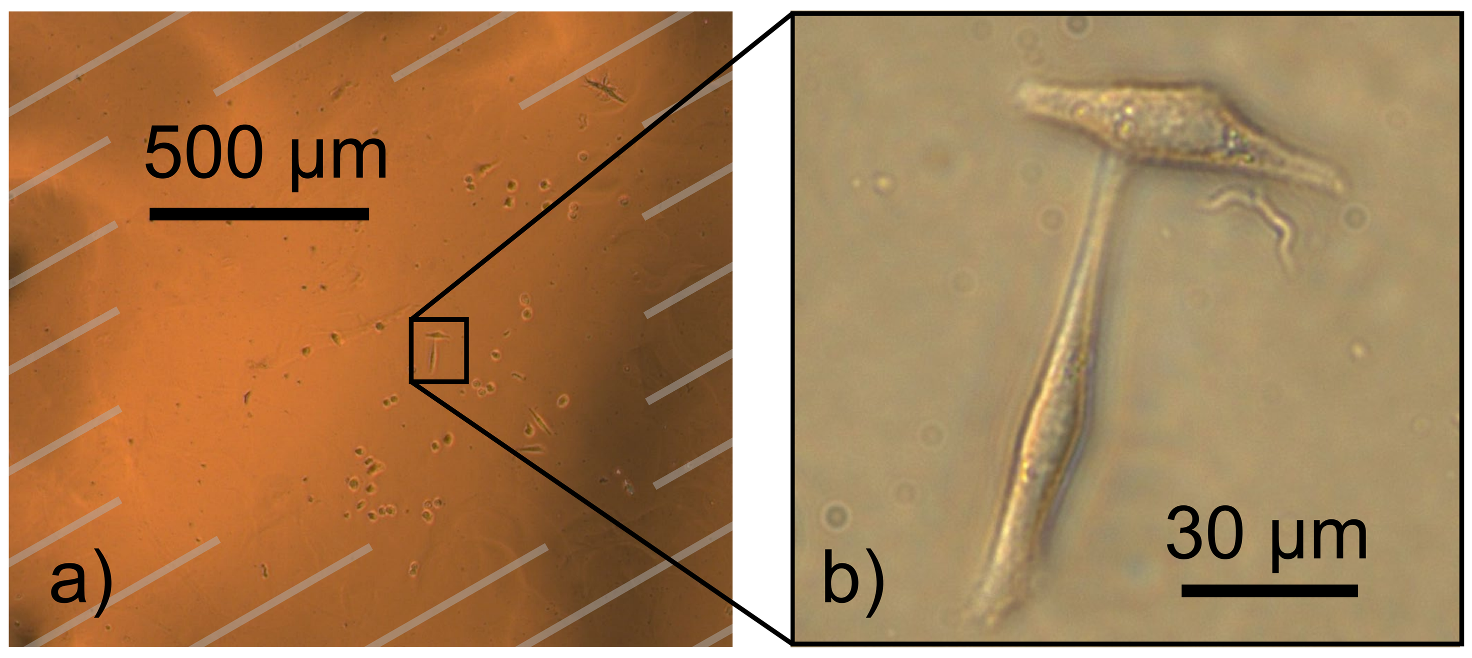

Figure 4: Figure 6. a) Three-layer alginate square (shaded) with ca. 50 HeLa cells after two days of incubation. The transparent alginate is difficult to visualise, due to the submersion of the sample in cell media. b) Close up of two HeLa cells.

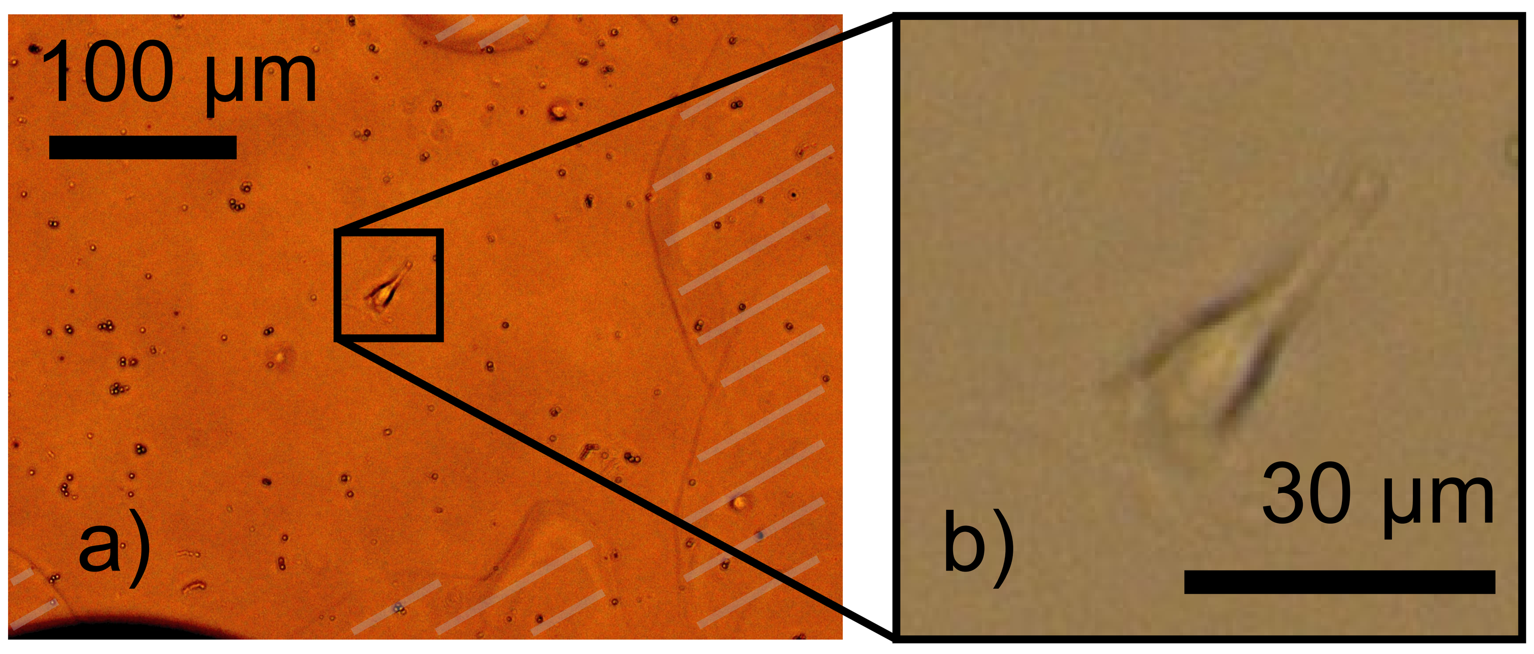

Figure 5: a) Single dispensed fibroblast cultured within surrounding printed alginate structure (shaded) after 5h of incubation. b) Close up of the cell.

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

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References

[1] R. Barry, R. F. Shepherd, J. N. Hanson, R. G. Nuzzo, P. Wiltzius, J. Lewis, *Advanced Materials*, 2009, 21(23), 2407-2410.

[2] Yusof, A., O`Leary, J. J., Zengerle, R., et al., *Lab on a Chip*, 2011, 6-10.

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SEVENTH FRAMEWORK PROGRAMME

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