

Development of microengineered systems to initiate, analyze and control stem cell patterning

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Summary

This thesis is a combination of five main chapters which follow a common theme of analyzing and controlling cell patterning using microengineered platforms, while trying to make these techniques more accessible to the biological community.

In **Chapter 1**, which also serves an introduction, how microengineering and microfluidics can help improve *in vitro* morphogenetic models is highlighted. This introduction summarizes the current state-of-the-art of recently developed *in vitro* morphogenetic models and discusses the current and future roles that microfluidic systems play in these advancements. These models are an attractive alternative to *in vivo* embryonic studies, which lack accessibility and are fraught with ethical issues. However, customized cell culture platforms are needed to unleash their full potential.

In **Chapter 2**, a new, microengineered platform for 3D suspension culture of mouse stem cell aggregates is presented; enabling microscale control of the position and orientation of elongating stem cell aggregates and real-time data acquisition at single cell resolution.

In **Chapter 3**, a microengineered fluorinated ethylene-propylene (FEP)-based cell culture platform is presented which shows excellent optical properties. The microcavities can be utilized in conjunction with brightfield and fluorescence microscopy and support machine learning-based label-free feature extraction. The system's potential for drug and small molecule testing is showcased using 3D mouse embryonic stem cell (mESC) aggregates, which mimic certain aspects of embryonic development.

In **Chapter 4**, a direct photolithography method has been described which enables fabrication of microstructures in bulk poly(methyl methacrylate) (PMMA). The method has been used to create artificial signaling centers on microfluidic chips to control mouse embryonic stem cell patterning. This work opens new avenues for microfabrication of microfluidic and optofluidic systems in the biomedical field.

In **Chapter 5**, a direct deep ultraviolet (DUV) photolithography method, which enables fabrication of microstructures in bulk polystyrene cell culture substrates has been described. Pipelines for generating ink based DUV masks and micropattern virtualization further increase the remarkable straightforwardness of the process. This, combined with the high applicability can significantly improve accessibility of this class of microfabrication techniques to a new and broad biological research community.