Directed assembly and development of engineered tissues using microwell screening platforms

Summary

A long-sought goal in the field of regenerative medicine is to generate complex tissues as use for medical therapies (replacing damaged tissues), disease modelling and drug screening (replacing animal experiments). Classical top-down tissue engineering often involves the use of biomaterial-based scaffolds that act as a matrix template offering guidance and structural support for the cells and the tissues that are formed. Although such approaches bring freedom of geometrical design, the biological complexity of *in vitro*-generated tissues is often limited. More recently, bottom-up tissue engineering has emerged as a methodology to increase the complexity of tissues by exploiting self-assembly and directed development of cellular modules. **Chapter 1** describes the recent technological and biological advancements that push the field of regenerative medicine towards exploiting methods of tissue-authentic and organotypic culture and how to direct and image these cultures in high-throughput. **Chapter 2** then demonstrates an accessible and scalable bottom-up method to build complex three-dimensional tissues up to the centimeter scale using only cells as a building material. Here, the sequential self-assembly of cells and cell-aggregates, that are used as living, self-scaffolding building block materials, allows for the free-form fabrication of complex 3D tissues. To fine-tune the physical and biological properties of the building block, a platform was developed based on non-adherent hydrogel templates that permits high-throughput screening of small molecules and high-content imaging of phenotypical and gene-expression features.

In chapter 3 an microwell screening platform is introduced based on micro-thermoformed round-bottom microwell arrays formed from optically clear cyclic olefin polymer films. The platform is integrated in standard 96-well plates, thus facilitates the manifold formation, screening and automated imaging of organoids. The potential for high-throughput screening is validated by running a small molecule screen to direct differentiation of embryoid bodies into primitive endoderm. Using on-chip high-content imaging, we identify molecules, including modulators of the cAMP pathway, regulating tissue size, morphology and primitive endoderm-gene activity. It is speculated that platforms allowing for the reproducible, controlled and rapid formation of multiple replicates along with high-throughput screening will accelerate the use of organoids as models with greater prediction power for scientific and clinical studies.

In **chapter 4** a succinct overview is given of the processes involved during preimplantation development of the mouse embryo, including the initial cell fate decisions, segregation of those cell populations and morphogenetic events. The “blastoid” is then introduced; a multicellular model formed by a combination of embryonic and trophoblast stem cells to recapitulate preimplantation development more accurately than existing models. Blastoids comprise both the embryonic and extraembryonic compartments and display similar morphology and cell allocation to blastocyst-stage embryos. High-content imaging using the hydrogel microwell screening platform allows for optimizing the formation of the blastoids using titration of cell numbers and soluble factors that modulate developmental pathways such as Wnt. In **chapter 5**, the Prestwick chemical library is employed to screen for compounds that increase the yield of trophoblast stem cells encapsulating embryoid bodies, in order to form blastoids. Another screen using a G-protein coupled receptor

ligand library on embryoid bodies identified several hits, including beta-adrenergic agonists, that promote primitive endoderm formation.

In **chapter 6** themes are discussed about how far we may push engineered multicellular organization in vitro and the potentiality of blastoid cultures including the related ethical concerns. Finally, in the valorization **chapter 7** the potential applications are described for the microwell screening platforms, directed assembly of material-free tissues and the blastoid model.