Dynamic polymer hydrogels as synthetic extracellular matrices for 3D cell culture

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CHAPTER 6

Summary and Perspectives

In vitro cell culture models have been essential tools to support biomedical research prior to *in vivo* studies, especially in the screening new drugs, testing of drug toxicity, exploring the mechanisms of disease and fabrication of microtissue or mini-organ models. A major challenge lies in how to offer cells with a microenvironment that more closely mimics the *in vivo* condition. Synthetic hydrogels that mimic the natural extracellular matrix in the biophysical and biochemical cues it provides to cells are in high demand, however the cell phenotypes as they are observed *in vivo* in numerous cases have yet to be attained. In this thesis, both chemically-defined supramolecular and covalent hydrogels are prepared that are encoded with bioactive peptides/ proteins, dynamics and porous structural features in order to explore how the modification of polymer materials with various biophysical cues affect cell behavior using various readouts. Through the design of a azide-alkyne clickable squaramide-based monomer, we have shown successful introduction of adhesive peptides that can be identified by cells, through an efficient supramolecular co-assembly approach to prepare the materials with the necessary peptide concentrations (**Chapter 2**). We further examine this flexible strategy for coupling of specific peptides to supramolecular monomers to provide matrix interactions for the culture of spheroids based on liver (HepG2) and induced pluripotent stem cells (**Chapter 2** and **Chapter 3**). Also, a dynamic covalent hydrogel system based on thiosulfinate-chemistry to form disulfide bonds was designed and validated to play an important role in supporting the differentiation of induced pluripotent stem cell derived cardiomyocytes. Cell alignment was only observed in dynamic covalent hydrogels within a particular stiffness range

and not on static hydrogels, further emphasizing the need for dynamics of the substrates in biomaterials design (**Chapter 4**). Lastly, gas-forming chemistries that enable control over porosity of the hydrogel materials at the macroscale were examined using the Inverse electron-demand Diels-Alder (IEDDA) reaction with reaction pairs that react with distinct kinetics (**Chapter 5**). Overall, we have engineered dynamic supramolecular and covalent biomaterials using different chemical tools to modulate their biophysical properties that the present to cells, providing guidelines for future biomaterials designs in the field of *in vitro* 3D cell culture.

In **Chapter 2**, a bioactive supramolecular hydrogel based on squaramide monomers is designed and shown to support HepG2 spheroid formation, proliferation and maturation as observed in the gene expression of liver-specific markers. The monomer consists of an azide-functionalized squaramide based scaffold that can be clicked to alkyne-modified RGD peptides through the CuAAC reaction, to provide the RGD-functionalized squaramide monomer. A supramolecular co-assembly approach with native squaramide monomer enabled the formation of bioactive hydrogels with a tunable control of the peptide concentration. Mechanically soft and self-recovering properties of this bioactive hydrogels have been shown in rheological measurements. NIH3T3 cells embedded within this hydrogel are capble of identifing RGD peptides showing cell spreading, branching and migration. In a further step, the bioactive hydrogels support functional maturation of HepG2 cells demonstrating higher gene expression of metabolic enzymes and hepatic markers in comparison to the commonly used Matrigel matrix, together with a compacted, viable and proliferative assembly. Collectively, this work demonstrates that a supramolecular co-assembly approach of squaramide-based monomers is an efficient method to engineer bioactive materials in a simple and controllable way that can be exploited for biomedical applications that require one or multiple bioactive cues.

In **Chapter 3**, a multicomponent supramolecular hydrogel based on squaramide monomers involving two integrin-targeting peptides is designed and applied for human induced pluripotent stem cell expansion in 3D is described. A similar supramolecular co-assembly approach is taken as described in chapter 2, with the exception that three monomers are co-assembled. The introduction of integrin targeting $\alpha v\beta 5$ and RGD into the supramolecular hydrogel materials results in mechanically weaker materials that maintain their self-recovering properties. The combination of peptides AB and RGD boost the growth of hiPSCs when the cells are seeded either as single cells or clumps, with high cell viability, proliferative activity and retention of pluripotency. Moreover, comparable cell expansion was achieved without a source of ROCK-inhibitor through the introduction of these two integrin-binding peptides in the materials. This chapter has demonstrated that successful multicomponent supramolecular co-assembly systems can be designed for different biological requirements in a flexible manner, broadening the application of these biomaterials. In addition, azide moiety on the squaramide monomers leaves the door open for further chemical crosslinking to mechanically manipulate this biomatrix to enable its use for 3D bioreactor culture of hiPSCs.

Chapter 4 describes the design and fabrication of a dynamic, viscoelastic hydrogel for the differentiation of hESC-derived cardiomyocytes. A new crosslinking moiety based on cyclic thiosulfinates used in proteins to form disulfide bonds, is introduced on covalent multi-arm PEG polymers (PEG-4ODT). Because of the lack of stability of the networks using only disulfide bonds, a mixed strategy invoving both dynamic and permanent bonds through the addition of PEG-4VS and PEG-4SH was taken to provide both disulfide and thiol-ene crosslinks within the materials. Rheological experiments verify the rapid gelation process, viscoelasticity and self-recovery properties of these hydrogels. Dynamic hydrogels (25%_dPEG) that consist of 25% dynamic crosslinks and 75% static crosslinks are demonstrated to show long-term stability in presence of PBS

and cell culture medium in swelling ratio measurements. The high stability of these materials under cell culture conditions enables the use of the 25%_dPEG hydrogels to be used as a synthetic matrices. Indeed, encapsulated hESC-CMs within these dynamic hydrogels show elongated morphologies, rapid recovery of cell spontaneous beating and alignment, demonstrating that cardiomyocytes prefer a soft and dynamic matrix for their culture. The significant increase in expression of cardiac genes, cTnT and MYH6, also verify the potential of applying this dynamic hydrogel for cardiomyocytes maturation in 3D. For further study, engineering more complex tissues can be explored within these dynamic materials, for example, co-assembly of cardiomyocytes with cardiac fibroblasts and endothelial cells to more closely mimic heart tissue *in vivo*.

Chapter 5 explores the use of the gas-forming IEDDA bioconjugation reaction on polymer materials to engineer porosity within them. The IEDDA reaction between tetrazine and norbornene on multi-arm PEG polymers was examined to yield elastic hydrogels and with the production of N_2 gas to form macro-sized bubbles in the hydrogel materials. Tetrazine and norbornene were used as reaction pairs because of their favourable reaction kinetics and further tuning of this parameter was achieved by synthesizing tetrazine derivatives containing electron-donating and electron-withdrawing substituents on ring positions 3 and 6. Higher reaction rate and higher PEG polymer concentrations resulted in faster gelation with smaller pore sizes (e.g. TZ3, pore size range = 170-360 μm). The IEDDA-based PEG hydrogels can be prepared with storage moduli ranging from 0.7 kPa to 12 kPa that are stable in the presence of PBS and cell culture medium. Additionally, primary chondrocytes encapsulated in soft IEDDA-based hydrogels displayed high viability and notable cartilage matrix secretion, demonstrating their potential application for cartilage tissue engineering applications. In the future, dynamic crosslinking strategies can be introduced into these modular porosity materials, through the use of interpenetrating or supramolecular polymer materials to prepare iPN or double network hydrogels to prepare a mechanically robust

and dynamic microenvironment for cells that better approximates the biophysical aspect of their microenvironment on several length scales.