Chemically tuning dynamic networks and supramolecular assemblies to enable synthetic extracellular matrices for tissue engineering

Summary

The development of advanced and dynamic hydrogels and bioinks with extracellular matrix (ECM) biomimicry is needed for understanding cellular response to dynamic networks with controlled viscoelasticity and biomimetic fibrous structure. This thesis investigates dynamic covalent chemistry and supramolecular chemistry for creating hydrogels that recapitulate extracellular matrix (ECM) viscoelasticity, stress relaxation, and fibrillar structure. Unlike the ECM, traditional hydrogels are elastic and allowed controlling stiffness. However, the ECM is dynamic and possesses time-dependent properties of viscoelasticity and stress relaxation. Therefore, we investigated dynamic and reversible chemistries for the creation of synthetic analogs of the ECM with controlled dynamicity, viscoelasticity, and structure. We also investigated newly developed dynamic hydrogels 3D bioprinting for the creation of complex life-like structures.

Utilizing imine-type dynamic covalent chemistry (DCvC) in **chapter IV**, we discovered that cross-links with distinct K_{eq} can be used for tuning viscoelasticity. We found that hydrazone has higher viscoelasticity compared to semicarbazone and oxime. Viscoelasticity had an impact on cell morphology, with fibroblasts displaying a round shape in oxime hydrogels as opposed to a spreading shape in hydrazone hydrogels. Similarly, we also found that the reversibility of hydrazone cross-links imparts self-healing and enables 3D bioprinting of complex life-like structures.

Then, we realized that ECM is a supramolecular and self-assembled fibrous structure with controlled viscoelasticity and dynamicity. Utilizing modular mixing/copolymerization of benzene-1,3,5-tricarboxamide (BTA) monomers (**chapter V**) with fast and slow exchange dynamics, we tuned viscoelasticity and stress relaxation in fibrous structure in the range of soft tissues. Fibroblasts and chondrocytes displayed high cell viability and neuronal cells (PC12) and dorsal root ganglion (DRG) could grow within fibrous and dynamic hydrogels. Interestingly, chondrocytes and human mesenchymal stem cells (hMSCs) formed cell aggregates, and more compact spheroids were observed for hMSCs. We attributed the spheroid formation ability of hMSCs within these hydrogels to the dynamicity and viscous properties of the hydrogel. While discovering more about ECM, we found that not only ECM is multicomponent but also ECM is multifunctional. Creating multifunctionality in ECM mimetic structure is desired and supramolecular chemistry offers a biomimetic and modular approach to creating multifunctional ECM for emulating the complexity of ECM. BTA is inspiring due to its one-dimensional (1D) fiber structure; however, large-scale synthesis of multifunctional BTA is the bottleneck. In **chapter VI**, we developed a new desymmetrization strategy that enables the creation of multifunctional and upscale synthesis of BTA architectures and macromonomers. A small library of benzene-1,3,5-activated esters was created and found that benzene-1,3,5-pentafluorophenol tri-ester (BTE-F5Ph) enabled effective desymmetrization and synthesis of BTA with hydrophobic spacers and reactive handles (azide and norbornene). We utilized the

desymmetrization strategy for the creation of telechelic polymeric BTA macromolecules. We discovered that BTA macromolecules form fibrous hydrogels and mechanical properties can be tuned by altering the hydrophobic handles attached on the BTA. Next, after observing some interesting trends in the viscoelastic properties of newly developed BTA fibrous hydrogel, we hypothesize that the length of hydrophobics could dictate the viscoelastic properties of BTA hydrogel. We created a telechelic BTA macromonomer with twelve, sixteen, eighteen, twenty, and twenty-four carbon atoms on the exterior of BTA (**chapter VII**). All macromonomers formed fibrous hydrogels with similar equilibrium storage modulus. We discovered that hydrogels with more than 5 orders of magnitude viscoelasticity can be made by altering the hydrophobic length from twelve to twenty-four. BTA hydrogels were extrudable, and the shape fidelity of 3D printed constructs was greatly improved with the increasing number of carbon atoms. Bioprinted chondrocytes showed high cell viability within BTA hydrogels. This straightforward method of adding or removing a few carbon atoms on BTA overcomes the long-standing challenge of tuning broad-range viscoelasticity in the fibrous hydrogel. Next, we were inspired by the conjunction of self-assembly and covalent fixation of collagen which is believed to be responsible for the toughness and strength of the collagen protein. We proposed a supramolecular/covalent strategy to achieve remarkable mechanical properties. BTA with norbornene (NB) (NB BTA) functionality was made that self-assembles into the fibrillar structure and forms a fibrous hydrogel with controlled viscoelasticity (**chapter VIII**). Intra- and inter-fiber crosslinks were introduced for tuning the hydrogel's stiffness, strength, and toughness. NB-BTA hydrogel was extremely tough, demonstrated recoverable hysteresis, and can withstand 90% compressive strain, and 550% tensile strain. Shear-thinning and self-healing properties of NB-BTA hydrogel enabled 3D printing of a cartilage-like structure. Human mesenchymal stem cell spheroids produced collagen II in bioprinted NB-BTA hydrogel, which strongly shows the development of chondrogenic tissue in bioprinted NB-BTA hydrogel.

In the future, the creation of multifunctional supramolecular monomers with controlled molecular dynamics will be of great interest for achieving next-level control of ECM dynamicity and biomimicry. In addition, molecular control of supramolecular dynamics will enable the development of advanced bioinks with spatiotemporal control of mechanical and biological properties.