Cartilage and bone in concert

Summary

The osteochondral unit comprising of articular cartilage and subchondral bone is located at the extremities of joints and is of utmost importance to facilitate smooth, painless movements. This interface tissue can be affected by diseases such as osteoarthritis, in which both tissues gradually deteriorate. Clinical intervention methods are available, but have only moderate success in the long-term. Due to the lack of long-term clinical solutions, joint replacement surgery is eventually needed. The prothesis have a limited due date which is an issue in especially younger patients (< 60 years).

In the field of tissue engineering, researchers aim to regenerate functional tissue by designing 3D constructs. Here, the tissue engineering triad is based on the combination of cells, biomaterials, and (appropriate) biochemical cues. The biomaterials in osteochondral applications are typically processed with additive manufacturing techniques to create a 3D scaffold, due to the high control over the spatial design. These constructs typically resemble properties found in the tissue interest to control behavior of hosted cell behavior. The choice of biomaterial is important as this directly interacts with the cells. The work described in this thesis focused on improving existing and developing new biomaterials for osteochondral applications. We aimed to enlarge the degree of mimicry by adding biologically relevant functional groups. Moreover, given the important role, the materials were designed having additive manufacturing application in mind.

We summarized in **Chapter 2** the state-of-the-art in terms of additive manufacturing technologies used for osteochondral scaffold fabrication. Ultimately, these scaffolds need to stimulate cartilage and bone formation simultaneously, requiring heterogenous properties across the construct. Given the presence of various gradients, we highlighted the design of polymeric (i.e., thermoplastic, hydrogel, and hybrid) scaffolds comprising gradients prepared with additive manufacturing techniques. In addition, the effect of these systems on OC matrix formation in-vitro and in-vivo was studied. In this literature review, we observed that most used biomaterials had a 'static nature', whereas the extracellular matrix is considered a dynamic environment. Thus, we concluded this chapter by presenting an overview of recently developed dynamic materials, including proposals on how the use of these materials may improve the biomimicry of osteochondral scaffolds.

Due to the heterogeneity in the osteochondral tissue, we wanted to work towards the fabrication of a polymeric construct that stimulates both chondrogenic and osteogenic differentiation in mesenchymal stem cells (**Chapter 3 & 4**). Since synthetic materials lack bioactive properties, we needed to integrate addressable groups that enable the coupling of differentiation-inducing peptides. We decided for this goal to synthesize poly(ε-caprolactone)-azide

(PCLA) and poly(ε-caprolactone)-maleimide (PCLM) as base materials. The azide and maleimide groups enable the specific conjugation of alkyne- and thiol-conjugated molecules, respectively, via orthogonal reactions on the surface.

We first reported in **Chapter 3** the two-step synthesis protocol to convert the terminal hydroxyl (-OH) group of poly(ε-caprolactone) (PCL) into an azide group. We observed during analysis that the solvent affected the formation of the intermediate product. In polar aprotic solvents, such as dimethylformamide or dimethylsulfoxide, a chlorinated intermediate product was obtained. The observed mechanism could be broader applied to azidate thermoplastic polymer comprising (terminal) hydroxyl groups that are only soluble in polar aprotic solvents.

After synthesizing and characterizing PCLM in **Chapter 4**, homogenous PCLA and PCLM scaffolds were prepared via ME-AM. We confirmed that we can modify the surface by coupling alkynated and thiolated dyes, respectively, on the surface of these scaffolds. Subsequently, we assessed the effect of surface-conjugated differentiation-inducing peptides on chondro- and osteogenesis of human mesenchymal stem cells. The biological evaluations showed a small intrinsic effect of the chrondogenic peptide and no apparent effect of the osteogenic peptide during the first 21 days of culture. We ended this chapter by generating a scaffold comprising a polymer gradient with an in-house developed printhead, yet we did not pursue cell experiments due to absence of a clear effect of the peptide in homogenous scaffolds.

The low effect could be explained by a too low density of peptide on the surface. Therefore, a blending strategy was investigated to enhance and tune the density of reactive groups on the surface of scaffolds (**Chapter 5**). We demonstrated by coupling fluorescent dyes via orthogonal chemistry that a broad surface-density could be unlocked. We studied subsequently the effect of peptide density on the osteogenic differentiation of hMSCs. Osteogenic markers were only upregulated at higher density, highlighting the importance to consider the peptide-density when a biofunctionalization strategy is pursued.

In the previous chapters, we designed synthetic scaffolds based on solidified fibers. However, given the hydrated nature of the cartilage, we believe that ultimately the design of a hybrid construct is required for full osteochondral regeneration. Thus, we wanted in the second part of this thesis, focus on the development of hydrogels mimicking features of this tissue (**Chapter 6 & 7**).

We described in **Chapter 6** the two-step synthesis of sulfonated hyaluronic acid. We first introduced a controllable amount of norbornene or maleimide groups on HA. Then, we added a sulfonate group via orthogonal reaction. In addition, we demonstrated the formation of sulfonated hydrogels using photochemical crosslink reactions.

As we concluded in **Chapter 2** that there is a need for dynamic materials in OC applications, we set to synthesize a water-soluble copolymer with sulfonate and aldehyde side groups (**Chapter 7**). We synthesized a small library of copolymers comprising a tunable degree of aldehyde groups (12–64%), according to a simple procedure. Then, we demonstrated that the copolymers could be conjugated with ligands, can rapidly form hydrogels (in seconds) using a poly(ethylene glycol)-di-hydrazide as crosslinker that display rare strain-stiffening behavior, are printable on a microfluidic printing platform, and are biocompatibility. Finally, we demonstrate that more dynamic hydrazone bonds with less dynamic oxime bonds can be replaced, and leverage this ability to selective replace a ligand or de-crosslink the hydrogel.

Taken together, the work described in this thesis delves into the design of biofunctional materials in the context of additive manufacturing and osteochondral regeneration. However, given the vastly different nature of cartilage and subchondral bone, a challenge remains to design one construct that resembles both tissues. We end this thesis by proposing in the general discussion (**Chapter 8**) an outlook on how current issues could be tackled and describe the design of our 'ideal' construct.