Summary

SUMMARY

Articular cartilage injury poses a significant clinical challenge in orthopaedics. Advancements in the recent decades are placing cartilage regeneration in the spotlight, paving the way to overcome limitations of current treatments. In order to improve clinical outcomes and develop new treatment strategies, this thesis aimed to provide models necessary for testing future biomaterial-assisted cell-free repair approaches in the knee joint. The suitability of hydrogels as 3D templates to support infiltration of chondroprogenitor cells, their differentiation, and to deliver biomolecules to enhance these processes were evaluated using spheroids-based migration assay as well as an *ex vivo* mechanically stimulated osteochondral defect explant model.

Cell migration has a critical role in the early process of biomaterial-assisted tissue repair. Multiple biochemical and biophysical factors, reliant on cellular and extracellular matrix (ECM) properties, influence migration efficiency. Cells are dynamically sensitive to biomaterial composition, stiffness and structure as well as to bioactive gradients, which can potentiate cell locomotion and movement. In **Chapter 2** different formulations and cross-linking densities of hyaluronic acid (HA)-based hydrogels were tested *in vitro* and *in vivo*, with the aim to select the most suitable implant template for endogenous regenerative therapies. We found that changes in mechanical properties influenced cell spreading, migration and differentiation. Fibrin conjugation with HA was much more different than the different modulation of the HA-Tyramine cross-linking degree (by using 150, 300 or 600 µM H_2O_2). The H_2O_2 concentration, while keeping HA-Tyramine and horseradish peroxidase concentrations constant, was the major determinant for both cell migration and matrix synthesis during mesenchymal stem cell chondrogenesis. Migration was observed to be inversely correlated with the storage modulus of the gel in the presence of a platelet-derived growth factor BB gradient. This means the softer gel fostered faster migration than the stiffer one. Fibrin-hyaluronic acid (FB/HA) gels, however, always revealed the highest cell migration potential, both in the presence and in the absence of chemoattractant *in vitro*, and also favored chondrogenic differentiation. An osteochondral explant model implanted subcutaneously *in vivo* further confirmed the endogenous cell recruitment, even in the absence of the stimulating factor. This stresses the importance of the microenvironment and the hydrogel substrate in which cells are recruited, as a crucial stepping-stone towards engineering functional musculoskeletal tissues.

As defects in the osteochondral unit often occur in the weight bearing region, it appears intuitive that repair of focal traumatic lesions should take into account mechanical loading as an essential factor influencing osteochondral tissue regeneration. The success of material-based systems depends on the ability of cells and hydrogels to sustain compressive and shear forces during loading. In **Chapter 3** it was demonstrated that low

intensity complex motion applied to chondrocytes-seeded FB/HA gels had a positive effect on maintenance of chondrogenic phenotype and cartilage matrix production. Cellular mechano-transduction was more effective when exogenous fibroblast growth factor-18 variant (FGF-18v) was added to our *in vitro* culture model when subjected to load, which interdependently influences cellular metabolism by increasing the quality of the tissue engineered cartilaginous construct . We showed that chondrocytes could synergistically adapt to a changing biochemical and mechanical microenvironment by modulating the amount of ECM, downregulating matrix degrading enzymes and promoting a functional surface.

Conventional bioreactor studies for cartilage regeneration often do not take the osteochondral tissue into account. With the aim to render biomaterial-assistant osteochondral defect repair technologies clinically feasible, we applied mechanical stimuli to biomaterials in a more confined environment to evaluate their function within the tissue as experienced *in vivo.* In **Chapter 4** we first described the development and validation of an *ex vivo* osteochondral defect model under mechanical compression and shear that provides a representative physiological joint-like environment to allow reproducible prediction of biomaterials performance and of biomolecule treatment efficacy. This model was used in **Chapter 5** to improve our understanding of the mechanisms governing biomaterial-assisted endogenous cell-mediated repair over time. Mechanical loading was identified as inhibitor of cellular infiltration into the wound site, suggesting that the implementation of mechanical stimuli to this system was not necessary at early time point. Interestingly, the addition of chemotactic factors did not counteract this inhibitory effect. Furthermore, the model provided the opportunity to uncover a potential cell migration route at the interface layer of the osteochondral explant; either cells present in the subchondral bone or in the calcified cartilage highly participate in defect restoration, highlighting the importance of the osteochondral unit when evaluating joint tissue repair strategies. The absence of a strong cell response to external mechanical forces at early time point indicated that tuning the extracellular signals over time would be necessary to optimize their use for cellular decision making. These results underline the essence of the use of representative models to provide insight in the optimal time to apply dynamic loading after surgical intervention.

In this thesis we have provided important clues for future improvement of biomaterial-assisted cell-free cartilage repair approaches. In **Chapter 6** it is postulated that a multifactorial approach is pivotal to ameliorate the current strategies. To achieve successful cartilage regeneration, fine tuning of hydrogel design to allow organized endogenous cell infiltration and differentiation, while recapitulating the bulk mechanical properties of the native tissue, needs particular attention, along with a well-coordinated mechanical loading to provide optimal conditions to enhance cartilage remodeling.