SUMMARY

Tissue engineering is a potentially promising treatment strategy to regenerate both functionally and morphologically cartilage tissue that is damaged due to trauma, inherited disease or osteoarthritic degeneration. The ultimate goal of research in this area would be the regeneration of hyaline articular cartilage tissue. This thesis discusses issues regarding the development of a one-step surgical procedure for the treatment of cartilage defects, applying the principles of tissue engineering, i.e. the combination of cells, scaffold materials onto which these cells are seeded, and bioactive cues that should direct these cells into the desired lineage. Based on criteria defined for mesenchymal stem cells by the International Society for Cellular Therapy, we showed in **Chapter 2** that stem cells residing in the dermis and the adipose tissue of human skin have a similar stem cell phenotype and both were able to undergo multilineage differentiation into the osteogenic, chondrogenic and adipogenic lineage. Besides showing a similarity in stem cell characteristics, adipose derived stem cells (ASC) and dermal stem cells (DSC) were also found to express a similar functional chemokine receptor (CKR) profile, which is required in order to allow stem cells to be directly responsive to CK gradients created by wounded tissue. Particularly interesting was the higher CKR expression on the stromal vascular fraction (SVF) isolated from adipose tissue and dermis, when compared to cultured ASC and DSC. This was attributed to the heterogeneity of the stromal vascular cell population (e.g. stem cells, endothelial cells and infiltrating cells such as macrophages). In conclusion, these cells can in principal be considered for implantation in a one-step surgical procedure, based on possessing all the attractive characteristics of MSC. Adipose stem cells are to be preferred, however, due to faster isolation and higher frequencies. Therefore we continued our research with adipose stem cells. In **Chapter 3** we investigated whether the yield and functional characteristics of adipose derived stem cells (ASC) residing in the heterogeneous stromal vascular fraction (SVF) were affected by the adipose tissue harvesting site (THS), which are most frequently used in resection or liposuction procedures, i.e. abdomen and hip/thigh region. We found that the yield of ASC, but not the total amount of SVF per volume, are dependent on the tissue-harvesting site. The SVF isolates derived from abdominal fat contained significant higher frequencies of ASC. When we subsequently cultured cells from both THS, we did not detect any significant difference in growth kinetics and surface marker expression, nor in the osteogenic or chondrogenic differentiation potential of cultured ASC from the two tissue harvesting sites. Regarding this, the abdomen seems to be preferable to the hip/thigh region for harvesting adipose tissue, in particular when considering SVF cells for stem cell based therapies in a one-step surgical procedures for skeletal tissue engineering.

The one-step surgical procedure (OSP) is highly beneficial to the patient, overcoming a second intervention**.** Although the abdomen and hip/thigh regions are the most prevalent anatomical locations for adipose tissue harvesting, others might also contain adipose tissue depots which can be valuable sources of stem cells for application during regenerative therapies. The knee is such a potentially valuable source, containing an infrapatellar (also known as Hoffa's) fat pad. The choice of the infrapatellar fat pad as source for SVF cells would be beneficial for the patient during regenerative knee surgeries, as with a single incision both harvesting of regenerative cells and treatment of the chondral defect could

be realized by application of a cell-loaded scaffold. Hence, in **Chapter 4** we tested whether a one-step surgical procedure for the regeneration of osteochondral tissue is feasible using freshly isolated stromal cells from the infrapatellar fat pad. We confirmed three requirements for the development of a one-step surgical procedure. First we demonstrated that stromal vascular fraction cells can be isolated in clinically relevant quantities from the infrapatellar fat pad. Second, this stromal vascular fraction contained a population of cells resembling ASCs in growth kinetics, phenotypical marker profile and multidifferentiation capacity, using colony-forming unit assays. Moreover, and particularly intended for the one-step procedure, SVF cells seeded in a 3D poly-lactic acid-co-ε-caprolactone scaffold were able to differentiate into the chondrogenic lineage, as was demonstrated by up-regulation of chondro-specific genes, formation of chondrogenic extracellular matrix and production of glycosaminoglycans. We concluded from this study that due to limited and variable availability of these cells, and removal of a healthy structure involved in stabilization and innervation of the knee joint, the SVF from the infrapatellar fat pad might only be applicable for treatment of small focal cartilage defects, whereas for larger osteoarthritic defects subcutaneous adipose tissue depots would be preferable.

Having characterized the ASC as attractive source of mesenchymal stem cells for implantation in this one-step surgical procedure, we then moved on to the second phase of the one-step procedure, that is the triggering of the isolated cells into the chondrogenic lineage. Based on previous research of our group, we had already shown that conventional TGFβ1 addition to the medium can induce chondrogenic differentiation in cultured ASC. In the study presented in **Chapter 5** we investigated the influence of hyperosmolarity and hypoxia, two biochemical cues prevalent under physiological conditions in the joint environment, on the chondrogenic differentiation of adipose derived stem cells, when seeded in a collagen type II gel, and compared this to conventional TGFβ1-induction. The effect of hyperosmolarity and/or hypoxia did not differ significantly on the viability and proliferation of the cultured ASC when encapsulated in a collagen type II gel compared with the conventional TGFβ1-induction. Interestingly, the cultured ASC in the collagen type II gel had a mostly round morphology under all conditions except on TGFβ1-induction, which induced strong aggregation of cells into a big cell nodule. This would imply that hypoxia and hyperosmolarity only mildly induce chondrogenesis contrary to induction by TGFβ1, which results in rapid aggregation and (hypertrophic) differentiation of the cells. Finally, the combination of hyperosmolarity and hypoxia had similar effects on the induction of chondrogenesis compared with TGFβ1-induction, on the genetic level by up-regulation of chondrospecific genes and on protein level by proteoglycan staining and glycosaminoglycan formation. These data might lead to an interesting alternative when considering short-term triggering in a one-step surgical procedure for the treatment of cartilaginous defects.

Knowing these cells are able to be triggered into the chondrogenic lineage, we turned to the third phase of the one-step procedure**.** To implant and keep the triggered cells into the damaged cartilage tissue**,** cells were seeded onto a scaffold material. Next to the multiple characteristics the scaffold material should possess, one of the characteristics of the scaffold material for application in the one-step procedure must be the rapid attachment of the cells to the material, to limit the time-frame of this procedure (ideally

lasting ≤ 2.5 hours). **In Chapter 6**, we tested two radiolucent biodegradable polymeric scaffolds for this purpose: a natural porous Col I/III scaffold and a macroporous poly(L-lactide-co-caprolactone) scaffold. We demonstrated rapid cell attachment (~ 10 min) when the heterogeneous mixture of stromal vascular fraction (SVF) cells was directly seeded onto both scaffold types. We further found a significant difference in attachment of the total number of SVF cells between both scaffold materials. Most of the attached cells from the heterogeneous SVF fraction appeared to be stem cells, since the non-attached cell fraction contained significant lower amounts of stem cells in colony-forming unit assays. Finally we showed that the attached cells were equally capable of differentiating in both the chondrogenic and osteogenic lineage in both scaffold materials by up-regulation of osteo- and chondrospecific genes and by deposition of osteogenic and chondrogenic matrix. We concluded from this study that both scaffold materials were suitable for application in a one-step surgical procedure to treat osteochondral defects. This study concluded testing of the *in vitro* feasibility of all phases of the one-step surgical procedure.

To translate this concept into the clinical setting, we then first tested this concept *in vivo*. In **Chapter 7** we compared the regenerative potential of adipose derived stromal cells (SVF) in a one-step surgical procedure for the treatment of osteochondral defects in a caprine knee defect with the addition of cultured adipose derived stem cells, and the conventional subchondral drilling technique, using a collagen type I/III scaffold. Remarkably after 4 weeks some regeneration could already be observed by immunohistological analysis: the ASC-treated group showed more regenerative potential compared with the SVF-treated group, and the latter gave better results than the acellular defects. After 4 months this regenerative potential clearly augmented, and similar results were obtained comparing both the SVF- and the ASC-treated defects on immunohistological, radiological, biochemical and biomechanical level. Although this was only a pilot study with a limited amount of animals, this *in vivo* study clearly showed the potential use of freshly isolated adipose derived stromal cells in a one-step surgical procedure to regenerate osteochondral defects. Before turning into the preclinical setting, results of this pilot-study should be reproduced with larger defects and long-term follow-up. However results thus far are promising and might pave the way towards preclinical studies investigating the merit of these attractive regenerative cells in the treatment of osteochondral defects.

Chapter 8, the general discussion, describes different topics regarding this one-step surgical procedure and places them in a broader context.