Summary

The research presented in this thesis actually comprises of two parts: in the first part a new intervertebral disc degeneration model was developed and in the second part this model was used to evaluate the regenerative potential of adipose tissue-derived regenerative cells directly injected into the disc in a one-step procedure. For this purpose we needed a model of mild disc degeneration, and therefore a new model of intervertebral disc degeneration was developed. It so happened that the available models all had limitations in terms of size, cell population and the severity of the artificially induced degeneration as compared to human intervertebral disc degeneration. In Chapter 2 we evaluated the cell population present in the goat intervertebral disc at different ages, and showed that notochordal cells are not present in the nuclei pulposi of mature goats, as is true in humans, in who these cells disappear in approximately the second decade of life. Based on previous research from our group we had already shown the comparability of mechanical loads and size of goat spines to human spines, and therefore we decided to evaluate the induction of degeneration using a proteoglycan degrading enzyme, called Chondroitinase ABC (CABC). To evaluate the degeneration the loss of disc height, the signal intensity of the nucleus pulposus on MRI, macroscopic and histologic changes were recorded. The time needed for the degeneration to develop, as well as the determination of the optimal concentration of CABC was evaluated in the studies described in Chapter 3, resulting in a new model of disc degeneration, in which goat intervertebral discs were directly injected with 0.25 U/ml CABC. This concentration results in mild intervertebral disc degeneration after twelve weeks in all discs injected. Biochemical changes and gene expression profiles of the degenerated goat intervertebral discs were also evaluated in a study described in Chapter 4, showing the comparability of the proteoglycan/aggrecan ratio in goat intervertebral discs and also demonstrating the up-regulation of genes involved in the catabolic pathway like MMP-13 and collagen type I, while the expression of Aggrecan was down-regulated. To determine the reproducibility and reliability of the newly developed model the study was repeated and the long term
development of disc degeneration was observed, which is described in Chapter 5. This study indicated that the degeneration could be induced reliably using CABC and that there was no spontaneous recovery after six months. The severity of the degeneration seemed to develop further, as is the case in human disc degeneration, as observed by the development of endplate irregularities and osteophytes.

Based on the studies in the abovementioned chapters we concluded that we had developed a reliable and reproducible large animal model for intervertebral disc degeneration that was suitable for our second purpose: the evaluation of cellular therapy for disc degeneration.

The current developments and technologies were reviewed in Chapter 6, in which the feasibility of a one-step procedure is described by analogy to another study by our group involving spinal fusion. Further, we identified the additional research needed before adipose-derived mesenchymal stem cells can be evaluated in a one-step procedure and address the selection of stem cells from the stromal vascular fraction, the specific triggers needed for cell differentiation and potential suitable scaffolds. Based on in vitro experiments indicating that relevant triggers are present in the micro-environment of the intervertebral disc, we hypothesized that the mere injection of stromal vascular fraction, containing mesenchymal stem cells could lead to regeneration. However, an unexpected advantageous outcome was observed in Chapter 7, describing an experiment evaluating the injection of two different concentrations of SVF in enzymatically degenerated intervertebral discs. A severe inflammatory response was observed, including osteophyte formation, destruction of the endplates and massive cellular infiltration of the intervertebral disc with inflammatory cells leading to gross morphologic destruction of the tissue. In an additional experiment the SVF fraction was injected in both degenerated and healthy discs to exclude effects of the degeneration induction method, which were not found. Also, the SVF was subjected to an additional washing step using a gradient designed to remove red blood cells. Application of this gradient prevented the inflammatory response. Based on this experiment no solid conclusions could be made as to what specific trigger was responsible for the adverse effects observed in our first SVF experiment, however, it did show that the adverse response
could be prevented. In addition, analyzing the discs which did not display an inflammatory response, an up-regulation of aggrecan expression was observed, as well as continued expression of PPAR-γ, a marker for adipose tissue derived cells suggesting, but not proving cell survival in the discs. Cellular therapy probably is most effective in mildly degenerated discs, which in general, have not led to physical complaints by the patient, e.g. pain. One of the future indications might therefore be prevention of degeneration development, for instance in the adjacent discs of a fused segment. There is ongoing debate in the literature whether adjacent segment degeneration exists and whether it is clinically relevant, or that it is a mere symptom of the ongoing process of degeneration that was already present in the degenerated, fused human spine. In Chapter 8 we use a goat fusion model to evaluate the development of adjacent segment degeneration in the further healthy fused goat spines. This allows for evaluation of the development without the presence of ongoing degeneration. The number of goats and the evaluation time was to short to draw solid conclusions however a degenerating effect was observed in the most caudal level. In our opinion, animal model research is the way to go to identify the existence of adjacent segment degeneration. Finally, Chapter 9 discusses the major issues involved in disc degeneration research, including the avascular nature of the disc, identifying it as a unique structure in the body, the relevance of animal models which is essential for studying the degeneration and regeneration process however is not suitable to evaluate the clinical relevance of the problem as the correlation between degenerative signs and symptoms is only limited.