Appendix 1

Summary
Lumbar discectomy is an effective therapy for neurological decompression in patients suffering from sciatica due to a herniated nucleus pulposus (NP). Discectomies however, do not deal with the damaged intervertebral disc (IVD) and high numbers of patients suffer from persisting postoperative low back pain. This has resulted in many strategies targeting the regeneration of the NP. In this thesis we developed a novel tissue engineering strategy to treat patients suffering from an herniated intervertebral disc (IVD). In chapter 2-4 the materials and cells were described and optimized. In chapter 5-6 a model was developed to evaluate the materials in goats in vivo.

In chapter 2 we used rheology to assess the visco elastic properties of the nucleus pulposus (NP) of goats. We used plastic compression to increase the density of collagen type I scaffolds and aimed to imitate the visco elastic properties of the NP. We also assessed the effect of a standard treatment with γ-sterilization on the scaffolds. We found a complex modulus of 22 kPa for the NP which agreed with a collagen density of approximately 23 %. However, the loss tangent, indicative of energy dissipation, was independent of the collagen density and could not be matched to the value of the NP. Treatment by γ-sterilization resulted in an increase of the shear moduli, but also in a more brittle behaviour and a reduced swelling capacity.

In chapter 3 we used alginate as a scaffold material and aimed to mimic the visco-elastic properties of the NP. We also assessed the effects of different alginate stiffnesses on native cells. Alginate scaffolds were prepared by two different techniques (diffusion and in situ gelation) and in concentrations ranging from 1 to 6 %. The 2% alginate scaffolds prepared by diffusion gelation showed the best match to the visco-elastic properties of the NP. However, the visco elastic properties rapidly declined upon incubation in medium. The biosynthetic phenotype of the cells was preserved in alginate, but no differences were found between the various scaffold densities most likely due to the poor adhesiveness of the cells to alginate.

In chapter 4 we assessed the capability of native cells to invade dense collagen scaffolds in vitro. The invasion of cells from the surrounding tissues, the (remnants of the) NP and the annulus fibrosus (AF), is crucial for the use of a-
cellular scaffolds. In this chapter we also assessed if migration could be enhanced by the addition of Histatin-2, a chemokine derived from human saliva that was shown to enhance the migration of fibroblast. We found that migration distance was density dependent and was higher for NP compared to AF cells after 4 weeks of culturing. We also observed a lag phase, before migration occurred. Histatin-2 did not enhance cell migration and this was confirmed in a separate scratch-assay. Although the densities used in this study were lower (1.5 and 3%) compared to the density shown to mimic the NP (23%), we proved that native cells are capable of invading dense collagen scaffolds and the in situ seeding concept might be viable.

Numerous regenerative treatment strategies have being developed targeting the NP. However, accompanying techniques that deal with the damaged AF are increasingly being recognised as mandatory in order to prevent re-herniations and to increase the potential of the NP replacing strategies. In chapter 5 we summarized and discussed all available literature on AF closure techniques of the AF including the attempts performed by commercial parties. We showed that several attempts to repair, support or regenerate the AF have been studied, but a successful clinical application is still far away. In general, tissue engineering strategies lack a vision on the final clinical application and repair therapies lack scientific evidence.

In chapter 6 we designed and tested annulus closure devices intended to be used in our goat nucleus replacement model. First a standardised defect (3 mm) in the lateral region of the AF was created through which a nucleotomy was performed. The AF defect was filled with one of the annulus closure devices (ACD’s) with and without the addition of a collagen nucleus replacement. First studies were performed on goat cadaveric lumbar spines. We showed that ACD’s with four barb rings could withstand the highest axial load. We further showed that the increased range of flexion-extension and lateroflexion due to the discectomy could be restored by implanting an ACD and collagen implant. The positive results were confirmed in a goat pilot study (n=2) after two weeks follow up. However, in a second pilot study (n=8) most ACD’s revealed signs of destruction and/or displacement after 6 weeks follow-up. In addition we found two endplate reactions extending into the subchondral bone, most likely due to continuous
friction of the ACD’s between the vertebrae. The ACD’s showed a tendency to perform better when they were implanted together with a collagen implant.

In the first addendum to chapter 6 we described the development the replacement model in detail. First, a Perspex model of the goat IVD was used to design the instruments and implants with appropriate dimensions. The instruments consisted of metal tubes with ascending diameter to punctuate the AF. Via the largest tube, with an outer diameter of 3 mm, all instruments and implants, except for the ACD can be inserted. In the second addendum to chapter 6, we present the results of the collagen scaffold that was used in adjunct to ACD’s in the goat study. After 6 weeks the disc height index in goats treated with the collagen implants was not significantly decreased compared to untreated control levels. Levels that received a discectomy or ACD alone did show a significant decrease in disc height. Macroscopy revealed that the dense collagen was not yet integrated with the NP tissue and therefore lost during sawing and preparation for histology. Histologic and magnetic resonance imaging score were not useful to evaluate the results after 6 weeks follow up. There was no difference in cell number between the different treatments after 6 weeks.

In conclusion, we showed that the visco elastic properties of the IVD can be imitated with scaffold materials that also allow the invasion of native cells. However, the promising results can not yet be translated to in vivo studies due to the lack of appropriate AF closure techniques.