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General Introduction
Symptomatic lumbar disc herniation occurs in up to 2% of the general population at some point in life [1]. Men are affected more often than woman, with a peak incidence in the fourth and fifth decade of life [1,2]. Since the disease is mainly distributed within the working and employed part of our society, the socio-economic consequences are substantial [3]. In the vast majority of the patients symptoms subside spontaneously within six weeks after presentation and these patients are best off treated conservatively [2]. Another large part will experience a decrease of symptoms in the following months and selection of patients suitable for surgery is therefore still not without dispute [4]. Moreover, the results of surgery are not always favourable in terms of outcome and recurrences [5]. Depending on the exact type and extent of the herniated disc rates of recurrence (of pain), reherniation and reoperation can be as high as 38%, 27% and 21% respectively [5]. It is therefore not surprising that advancements in knowledge, imaging techniques and surgery are all continuously evaluated for their potential in the development of better treatment strategies. In addition, during the past decade a complete new area of research has evolved in medicine: Tissue engineering. The latter yields a great promise for patients suffering from symptomatic lumbar disc herniation and pioneering pre-clinical research is presented in the current thesis.

Disc herniation
The spinal column combines its complex mechanical function with the protection of the most delicate tissue our body harbours: the spinal cord. Failure to fulfil one of its tasks will have dramatic consequences. The spinal column consists of the bony vertebral bodies that articulate with each other by two facet joints posteriorly and the intervertebral disc (IVD) anteriorly. The 33 vertebral bodies are numbered according to their cranio-caudal position: cervical (7), thoracic (12), Lumbar (5), sacral (5) and coccygeal (4). The spinal cord, or below the first lumbar level the cauda equine, is located directly posterior of the IVD. Other borders are the pedicles laterally and the laminae and flavum ligament posteriorly. Exiting nerve roots leave the spinal canal via the intervertebral foramen, which is located between two pedicles behind the posterolateral border of the IVD and anterior of the facet joint.
The IVD is designed to resist the compressive forces yet allowing motion in the otherwise rigid vertebral column. The IVD consists of the gelatinous nucleus pulposus (NP) surrounded by the fibrous annulus fibrosus (AF) and endplates (Figure 1). With aging, a number of changes in the IVD occur, most notably the water content and number of cells decrease, diminishing the capability to cope with its mechanical function [6]. Mechanical demands on the other hand may contribute to the degenerative cascade itself defining a potential vicious circle. The region where the highest stresses are encountered and structural degenerative changes develop most rapidly is the posterolateral part of the AF (Figure 2). Disruption of the layers of the AF at this location results in expulsion of NP material which is often referred to as “disc herniation” or “herniated NP” (HNP). When this happens, the nerve root may become trapped resulting in back pain in combination with radicular symptoms (sciatica). Rarely, but more dramatically, the herniation is located centrally resulting in compression of the cauda equine, the so-called cauda syndrome. Besides the direct neurological consequences, other changes are initiated by disc herniation due to the loss of NP material. Due to the resulting reduction in hydrostatic pressure and subsequent decrease in disc height, facet joints may become overloaded and start to degenerate. Furthermore, the disrupted homeostasis will result in a decreased cell number within the NP and the amount of water-binding proteoglycans they produce declines. This results in further loss of disc height and finally an irreversible cascade of disc degeneration.

Patients suffering from an herniated lumbar IVD typically suffer from (sub)acute low back pain and radicular complaints, a condition called ‘sciatica’. The origin of the low back pain is still not fully understood, but may be generated in either the ruptured AF (which has become more innervated due to degeneration), the degenerated IVD or facet joints.
Figure 1: Image of a formalin embedded healthy human IVD showing the central NP surrounded by the layers of the AF (details: see text)

Figure 2: Image of a human IVD a few weeks after disc herniation shows a large defect of the posterolateral AF at the left side of the picture.
Current treatment modalities

The mainstay of the treatment of disc herniation has always been the removal of the herniated NP material, the so-called discectomy. These procedures are performed since the late 70-ies of the past century and are now the most performed spinal surgical procedures worldwide [7,8]. However, compared to the first described (open) discectomies, many things have been changed. Increased knowledge and the advent of the MRI in the 1990s have resulted in numerous less invasive procedures, abandoning the conventional discectomy. The gold standard nowadays is the microdiscectomy in which every type of disc herniation can be excised through a small incision and limited laminoarthrectomy [7]. An alternative procedure that shows comparable results in experienced hands is the endoscopic transforaminal discectomy [8]. Although the evolution of the conventional disectomies to less invasive procedures has resulted in a decrease of morbidity, still a significant number of patients suffer from recurrences or persisting low back pain. The outcome of patients that undergo a microdiscectomy is not better compared to patients receiving conservative treatment after 1 year follow-up [9]. Considering that the discectomy is directed towards the decompression of the nerve roots and therefore does not deal with the damaged IVD these findings may not be surprising.

Figure 3: schematic drawing of a lumbar discectomy
Chapter 1

Tissue Engineering
In patients suffering from disc herniation, there is an (sub)acute change in mechanics and biology due to the rupture or bulging of AF and the subsequent expulsion of the NP. Although the acute episode is often preceded by some degenerative changes, most (mechanical) changes may still be reversible and the patients might therefore be favorable candidates for early disc repair. This should restore the biomechanical equilibrium within the disc, preserve local homeostasis, and prevent progressive degeneration [10].

Tissue engineering is generally described as “the use of a combination of cells, engineering and materials methods, and suitable biochemical and physio-chemical factors to improve or replace biological functions” [11]. As tissue engineering has quickly emerged as an area of pre-clinical research over the last decade, attractive new strategies that deal with this problem can be developed. The replacement of the lost NP tissue should not only restore local biomechanics but ideally allow disc regeneration in the long-term. To that end cells, being the factories of the extracellular matrix components, are essential. Much research has been performed by seeding scaffolds with either native or stem cells. Native IVD cells, however, are sparse and the use of stem cells requires additional harvesting procedures and time consuming techniques. Therefore the concept of ‘in situ seeding’ has been proposed, meaning the use of a-cellular scaffolds that allow invasion of cells from the surrounding tissue [10]. This concept allows IVD regeneration in a single (one-step) surgical procedure. Ideally, such a scaffold further imitates the biomechanical properties of the NP, allows the invasion of surrounding native cells, and can be used in a single procedure in adjunct to microdiscectomy.

Scope of the thesis
In the current dissertation the concept of ‘in situ seeding’ is further explored, from basic scaffold science till in vivo evaluation. In the first two chapters, scaffold stiffness, which has been shown to strongly influence the biosynthetic response of cells and thus is a crucial factor for successful IVD engineering, is studied. In chapter 2 dense collagen scaffolds are rheologically characterized to find a match in stiffness with native NP tissue. In addition, the effects of sterilization techniques, necessary for final production, are assessed. In chapter 3, another
frequently used scaffold material, alginate, is prepared via several techniques and densities to match to the NP. In this study we also investigate the actual effects of ranging densities on native IVD cells. For the concept of “in situ seeding” the migration of native cells into the scaffold material is a conditio sine qua none. Therefore the capability of IVD cells to migrate into dense collagen scaffolds is assessed in chapter 4. The remainder of the dissertation is directed to the development of an animal model to evaluate the scaffold materials in vivo. It has been discussed that the success of NP replacement therapies might be dependent on an appropriate solution to close the AF defect. In chapter 5 an extensive review is performed to find the literature in which this subject has been addressed. In chapter 6, self-developed AF closure devices are evaluated in a goat model in vitro and in vivo. The results of the collagen scaffolds that are used in addition to the closure devices in the in vivo study are presented in a separate addendum to chapter 6.
References