Intervertebral disc degeneration
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Chapter 7.

General discussion

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Discussion and conclusions

Life expectancy has increased in the past 200 years from 40 years to 80 years, while in the last 2 million years our anatomy and physiology has changed little (1). Our intervertebral discs are subject to ageing and degeneration, and much like any castle, they will crumble given enough time (2). But as one would attempt to extinguish a fire in a castle, we should also attempt to stop painful processes accelerating the deterioration of the IVD, especially when this pain causes grave burdens and loss of quality of life. We must therefore rely on our ingenuity (and therewith science) to compensate and aid our hopelessly ageing bodies to cope with our “unnaturally” increased life expectancy (3).

The combined work in this thesis shows that it is possible to culture a large lumbar IVD. The LDCS is a bioreactor capable of maintaining caprine lumbar IVDs native properties and live cells for up to three weeks in culture. The LDCS is a good alternative for life animal testing, as we are able to answer important basal and translational questions regarding IVD physiology and pathology on cadaveric lumbar IVDs, rather than with in vivo experiments on live goats. The LDCS can be used to get a better understanding of the role of mechanical load in IVD homeostasis. In addition, we were able to implement this model to study the mechanical changes in early DDD and the potentials of quantitative MRI to identify the associated matrix changes.

The LDCS model:

“All models are wrong, but some are useful” (George Box, 1976)” Clearly, the LDCS is a good and very useful model, but not perfect in simulating in vivo conditions. In chapter 2 we show that with the addition of a substantial amount of dynamic axial load in a diurnal rhythm we are able to maintain caprine lumbar IVDs native properties for up to three weeks. However, even with optimal culture conditions and simulated-physiological loading, IVD cells showed signs of

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remodeling or even catabolic response and some creep in the matrix (4). The necessity of some dynamic loading for the vitality of the IVD is a very important finding, and many other IVD culture models have shown beneficial effects of some form of moderate loading and detrimental effects of the absence of loading (5-11). In contrast, a study from Gawri et al. (2011) reports the maintenance of an intact human lumbar IVD in a culture chamber without loading for up to 4 weeks (12). However, only cell viability (and not cell density) and gross matrix content were analyzed.

The LDCS is excellent for answering basal questions regarding IVD (biomechanical) function, studying the processes in the onset of DDD and stringent analyses of the interaction between mechanical load and the cells and matrix of the IVD. Even short-term effects of therapeutics can be studied using the LDCS. However, like any model, the LDCS has multiple limitations. Firstly, it is not suitable for maintaining a large lumbar IVD over a longer culture period than three weeks. Studying processes that would require long-term (>3 weeks) follow-up cannot be performed in the LDCS. Regenerative therapeutics, such as (stem)cell therapy and anti-catabolic agents, which need long follow-up to produce any measurable effect, especially if they require systemic recruitment of excipients, cannot be studied to their fullest potential in the LDCS (13-15). Furthermore, currently the LDCS is only capable of axial loading on the IVDs. The role of torsion and bending, which may influence nutrient exchange, cellular responses and have region-specific response cannot yet be taken into account in our system.

Some important research questions cannot be answered in an ex vivo setting (16). Inherently, since the IVD is outside the body (ex vivo) in a bioreactor, systemic responses, such as immunological processes, recruitment of additional nutrients and cells from the vertebra bone-marrow, migration of vessels and nerves into the endplate and IVD, pain perception etcetera and other collateral effects of various (detrimental) circumstances cannot be simulated or studied in the ex vivo setting (17, 18).

Also, culture of IVDs in the LDCS is laborious, expensive and not without complications such as (load)system malfunction, leakage and infection of specimens. Taken together, the LDCS is a good and useful substitute for some in vivo studies, but it cannot fully replace life animal testing.
Mechanical overloading and degeneration:

In chapter 3 we showed that compared to the SPL condition, high dynamic and static overloading cause degenerative changes in the nucleus and annulus of the disc (19). How this type of accelerated load-induced degeneration compares to the in vivo situation in the human spine is difficult to determine (20-22). All the degenerative responses described in our loading studies have also been reported in the human degenerated IVD (23-33). However, we may have only simulated one single exacerbated subtype of disc degeneration, where there are probably numerous pathways by which the human IVD can deteriorate and become symptomatic DDD.

Also, we can only speculate whether the reported dosage of overloading would result in disc degeneration in the caprine spine in vivo. In the culture set-up of the LDCS, the strictly axial load is delivered solely on the IVD, without the vertebrae attached and without the ligamentous tissues, the motion segments posterior elements, such as the facet-joints and connecting transverse and spinous processes with spinal musculature and even the additional support of the abdomen. This is both a strength and a limitation of the model; it allows for strict study of the IVDs responses to certain (loading) circumstances, and for exact control and monitoring of that force on the IVD: on the other hand, it might impair its validity to the in vivo situation, as the surrounding spinal elements will have their influences on motion segment response. However, the IVD is continuously axially loaded in the LDCS, and therefore in its neutral zone. Effects of surrounding elements would likely only have a greater role if flexion or torsion moments would be introduced.

Loading on the spine in vivo is not strictly axial but multidirectional, magnitude and frequency of loading vary greatly with motion of the spine (34-37). This means that our simulated axial overloading and its effects might not directly correspond with the situation in a living and moving subject. Furthermore, in our ex vivo model the cells in the IVDs cannot recruit additional nutrients or protective excipients (anti-inflammatory, anabolic etc.) in a period of higher stress, simply because the ex vivo condition lacks this systemic feedback regulation (18).
With regard to the loading condition, many factors could be altered which may have varying results. For instance, the cumulative detrimental effects might have been less profound if the period of rest following the overloading had been longer. The overall effect of overloading on the IVD might be relative to the resting period an IVD gets after a period of high loading (38, 39). In addition, varying the frequency and the shape (in our studies always a constant 1 Hz sinusoidal load) of (over)loading might also influence the outcome (40-42). For culture conditions, there is clear evidence that pH, osmotic pressure and glucose concentrations influence outcome of both cell response and IVD mechanics (43-55). Many other culture conditions have been tested in other models, and many additional excipients have been added to culture media (different pH buffers, NSAIDs, (cartilage) substrate and growth factors) in an attempt to improve or alter IVDs’ response to culture (11, 56-59). We have always stuck with one single simple culture medium recipe, because we wanted to focus on the influence of mechanical conditions. The potentially protective or detrimentally effect of changing any of these factors has not been tested, and although in other models results have been reported, we can only speculate on any effect in our bioreactor (57-61). In the light of the presented data in chapter 3 we can conclude that axial overloading compared to SPL loading of a caprine IVD will result in detrimental changes in biomechanics with loss of non-linear poro-elastic recovery behavior, inflammatory and catabolic responses on a cellular level, and loss of matrix structure and content. This essentially confirms that mechanical overloading can indeed induce degeneration of a healthy intervertebral disc.

Static axial overloading and hernia’s:

In chapter 4 we studied the potential relation between static overloading and herniation of the annulus. We found that the posterolateral corners suffers the most damage when the IVD is overloaded with a static axial load (62). Whether the posterolateral part of the annulus is by concept the weakest mechanical site of the outer-annulus or if this weakness is an attainment of a regional degenerative cell response to overloading as accumulated during life, cannot be deduced. What influence the individual changes, e.g. due to ageing, adaption to the mechanical environment and regional specific degenerative changes within the disc, have in the
development of DDD and herniations remains subject to further study (63, 64). We also expect the sagittal posture, the posterior spinal elements and many other factors present in the \textit{in vivo} situation to have some (protective) effect (65-67). We can only conclude that an isolated lumbar caprine IVD loaded with strictly axial static loading will accumulate the most damage in the posterolateral outer-annulus and leave the nucleus relatively spared. Catabolic cell behavior at this site would indeed prime the IVD for herniation (68-71).

\textbf{Biomechanical properties in early DDD:}

In chapter 5 we described that a minor loss of GAG (less than 10\%) from the nucleus due to Cabc-injection, resulted in early stage mild IVD degeneration with significant changes in the poro-elastic properties of the disc. These degenerative changes were most apparent in Cabe treated discs, and less in PBS injected IVDs. We showed that the (changes in) recovery behavior of the IVD could be well characterized by the parameters of a stretched-exponential fit.

We observed that the depressurization from a small GAG loss already affects the mechanical behavior of the discs during physiological range loading (SPL regime). The decreased intradiscal pressure (due to loss of osmotic pressure in the nucleus) accelerates subsidence during load and slows down recovery during unloading (72, 73). Disc degeneration causes a typical change in the stress-strain curve of the IVD, moving from a typical exponential towards a more linear pattern. We propose this change (disappearance of exponential disc height gain and loss) is the hallmark of degeneration, and the distinctive biomechanical characteristic between healthy and degenerated intervertebral discs. It marks the transition of the poro-elastic behavior of the healthy disc (a resultant from both GAG pressurized fluid in the NP region together with the elastic properties of the intact annulus) to the more solid-elastic function of the degenerated disc (in which the porous and fluid-pressurized dampening effects have diminished). This functional difference due to GAG loss is described by the difference in the stretch-constant beta (chapter 5 Fig. 6 and Table 1), which is the major advantage of the stretched exponential fit compared to traditional stiffness and time-constant measures. The changes of the beta parameter with
increasing degeneration found in the current study concur with earlier studies using stretched-exponential fitting for IVD degeneration (74-77).

Other exponential fits have been utilized to describe these highly non-linear IVD biomechanics. In recent studies, the double-void exponential fit has been advocated as well, as it is better able to fit any mechanical “state” of the intervertebral disc (regardless of (de)hydration, osmotic pressurization, degenerative state) (74, 78). This is a clear limitation of the stretched-exponential function as used in chapter 5: if disc behavior becomes too solid-elastic (too linear due to degeneration), the stretched-exponential function will not fit properly. However, the goal of our study was to describe the changes in biomechanical behavior occurring with early degeneration; the initial loss of non-linear poro-elastic behavior. The stretched-exponential fit proved to be able to do so, and due to its simplicity, the parameters in the model can be easily related to actual matrix changes.

Quantitative MRI to detect early DDD:

In chapter 6 we demonstrated that quantitative MRI and MRI techniques such as T1rho and ADC provide objective and more accurate information on the matrix status of intervertebral discs than current routine T1 and T2 imaging (79-82). T1rho is particularly sensitive to small degenerative changes in the IVDs matrix. Enzymatic induction of IVD degeneration resulted in dose-dependent changes in biomechanical parameters (height loss and recovery behavior), loss of GAGs, and mild to moderate degeneration on histological sections.

To our knowledge, this is the first study to directly compare the correlation of quantitative high-resolution T2, T1rho, and ADC maps with actual disc recovery behavior, combined with histology and GAG-content in a lumbar IVD large-animal model. Our region-specific results demonstrate that T1rho nucleus values correlate with GAG content, histological degeneration, as well as disc mechanical properties to a higher degree than T2 and ADC.

Although T1rho is most sensitive in detecting small degenerative changes in the matrix, on the basis of our findings we cannot conclude that it will be superior to T2 on an individual patient level in identifying early DDD. Whether the statistically
significant differences to T2 are clinically relevant has to be further explored. Interestingly, in recent clinical studies T1rho has been found to correlate to the Oswestry and SF-36 pain- and disability scores, significantly better than T2 (83, 84).
**Future direction**

The combined results in chapters 2 through 4 clearly show that diurnal mechanical loading is required for a healthy condition of the intervertebral disc and that overloading of the IVD will detrimentally affect the discs’ cells, matrix and biomechanics. The apoptotic/necrotic, remodeling and inflammatory cell-responses found, could be at the heart of the degenerative cascade of the IVD or could merely be the gene-expressive last sigh of dying or senescent cells. To examine the significance of the found cell-response to overloading, one could condition the culture medium with anti-inflammatory agents to see if this would block the cells’ response and in turn, would protect the IVD matrix from further degradation. However, as we have already discussed, LDCS culture is not without some remodeling effects, especially in long-term culture. Furthermore, the detrimental cell-response to overloading is very broad; many cellular inflammatory, remodeling- and apoptotic pathways seem to be activated simultaneously. This would imply that when attempting to block this adverse cell response, one would have to implement a non-specific or a combination of several specific anti-inflammatory agents to halt these activities or employ stem cells to secrete trophic factors. In addition, the potential protective effects on the matrix would be small during a feasible culture period in the LDCS and therefore very difficult to measure.

Stretched-exponential parameters can distinguish the mechanical behavior of healthy from degenerated IVDs as we have shown in chapter 5. *In vivo* “functional” imaging of the spine with simultaneous (biomechanical) measurements have been conducted; the influence on disc height of sagittal posture, bending and torsion, with and without heavy lifting have been studied using either serial x-rays, CT or MRI (85-91). Combining serial imaging of the spine with measurement of IVD height could yield a functional *in vivo* assessment of the IVDs health. One could propose a standardized MR imaging protocol of the recovering spine (in the sagittal plane) during resting / sleeping after a day of standardized heavy lifting. Subsequent fitting of the recovery curves of individual IVDs with a stretched-exponential function, could potential provide insight in the function of (lumbar) IVDs. A practical challenge would be the relatively long time-constant of the human lumbar IVD and the requirement of the stretched-exponential fit to have at least a multitude of times that time-constant to fit properly. Depending on the degenerative state of their IVDs, this could require subjects to sleep / lie down for 12 or even 16 hours, which might render this option impractical.
In addition to such “functional” assessment of IVDs biomechanics, T1rho with its superior ability to assess ECM content and detect small degenerative changes in the matrix, could be used to measure IVD status accurately and provide a matrix related explanation for the found functional differences. Implementation of T1rho in clinical MR scanners requires only the addition of a new scanning protocol in the software, hardware changes (coils, detectors) are not needed and scan time for structure like the lumbar spine, knee and ankle are comparative to T2.

However, as long as the finding of early DDD in symptomatic patients does not have therapeutic consequences, i.e. same conservative treatment regardless, no radiologist, spine surgeon or rheumatologist will be interested in implementing these superior techniques on a routine basis clinically. Potentially, if randomized controlled trials on the (medicinal) treatment of patients with symptomatic early DDD will be undertaken, these MRI techniques could be of interest for patient-selection and follow-up. If certain disease-modifying, and/or (selective) inflammation-suppressants (biologics) and/or regenerative medicine strategies are found to positively influence the course of symptomatic (early) DDD, the advantages of an exact functional assessment of IVDs and MR sequences like T1rho will be of practical use.

If indeed such a treatment regime is found to be successful, in the light of the findings in this thesis, we can speculate on a better treatment regime for especially young patients with early discogenic low-back pain. With the detrimental processes better identified, there are several ways to improve current “wait-and-see” and “self-management” conservative treatment protocols.

There are many parallels between rheumatoid arthritis (RA) and degenerative disc disease to deny some relation. The exact same cytokines that message joint destruction in RA are found in DDD and osteoarthritis. The same collagenases and (metallo)proteinases (ADAMTS and MMPs) that eat away the cartilage and periarticular bone in the synovial joints in RA, also destroy the IVD and its endplates. And like RA, DDD and multi-joint osteoarthritis are strongly genetically predisposed. I would therefore advocate to consider DDD and multi-joint osteoarthritis as a sort of lingering form of RA. Not necessarily in the strict sense an autoimmune driven process like RA, but at least for DDD also on the basis of genetic “imperfections” combined with detrimental extrinsic factors like overloading, trauma and inadequate nutrition of the IVD. DDD is a far slower form of joint destruction than RA, but like
RA also has symptomatic episodes as an exacerbation of this otherwise lingering inflammatory process, which temporarily accelerates degeneration of the disc or joint.

By perceiving symptomatic DDD more like an RA disease entity, one can easily make the parallel to an according treatment protocol; when a general practitioner is presented with a (young) patient with LBP complaints that could be discogenic, the patient is quickly referred to a rheumatologist for additional diagnostics. When T2 and T1rho MRI show early signs of disc degeneration and other causes of LBP are ruled out, early symptomatic DDD can be diagnosed. Like RA, initial treatment could start (within 6 months of disease onset) with a combination of relatively high dosage disease-modifying-(anti-rheumatic)-drugs (DMARDs) and anti-inflammatory agents to get the disease in remission, i.e. radiologically (PET / T1rho) and clinically (pain and stiffness free). After remission is achieved, dosage and combination of medication can be slowly decreased and at times of exacerbation be increased. Intensive monitoring of disease activity and medication side-effects is imperative for treatment success and safety (92). A physiotherapist should have a role in ensuring that healthy daily physical activities are optimized: patients will need to get educated in the role of extrinsic factors like loading on the spine, posture during daily activity and the necessity of adequate rest (93). Obesity, high cholesterol, diabetes and a sedentary life-style should also be dealt with (94-98).

In conclusion, there are still many fundamental and clinical research questions to be answered, before we will be able to make our intervertebral discs compatible with the burdens of our prolonged life-span. The LDCS is a useful tool in this quest.
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