Intervertebral disc degeneration
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Summary

With clinical evidence emerging on the relationship between intervertebral disc (IVD) degeneration and low-back pain (LBP), and a growing body of knowledge on the fundamental mechanisms involved in IVD degeneration, the need increases for translational platforms to test theories on etiology, diagnostics and possible treatments of degenerative disc disease (DDD).

The primary purpose of the present thesis was to investigate the feasibility of ex vivo culture of a large lumbar intervertebral disc (IVD). We pursued to establish a bioreactor model in which a live intervertebral segment can be sustained under controlled mechanical loading conditions for a prolonged period of time (the Loaded Disc Culture System; LDCS). With the use of this model we aimed to answer fundamental research questions regarding intervertebral disc degeneration on a ‘representative-for-human’ lumbar IVD. With close control of culture conditions and applying a diurnal simulated-physiological loading, we were able to maintain lumbar caprine IVDs in culture for up to three weeks. This model allows us to study biomechanical, cellular and extracellular processes in the IVD simultaneously. Furthermore, it enables us to distinguish between the different regions within the IVD (e.g. nucleus, inner-annulus and anterior/lateral/posterior outer annulus).

The secondary objective with the LDCS was to clarify the role of various mechanical loading conditions on the IVDs native properties. We found that the absence of loading, underloading and overloading of the IVDs have negative effects on mechanical properties, cells and matrix, which are comparable to degenerative changes in human DDD. We found that dynamic and static overloading affects the IVDs regions (nucleus, inner- and outer-annulus) differently. We used the LDCS to investigate if the potential relationship between a sedentary lifestyle and lumbar IVD herniation may be explained based on biomechanics of the IVD. We found static axial overloading to be detrimental especially to the posterior annulus. Furthermore, we studied early degenerative processes in the IVD, for which the LDCS is especially well suited. We observed that for early mild degenerative changes in IVDs biomechanical function and quantitative MR mapping with T1rho provide better information than, for instance IVD height, water-content and T2-weighted imaging.
Specific aims and answers:

Chapter 2.

Is long-term ex-vivo culture of a large lumbar IVD feasible? And is there a need for axial loading to maintain native IVD properties in culture?

In chapter 2 we showed that it is feasible to culture lumbar goat IVDs with cartilaginous endplates in the Loaded Disc Culture System, with conservation of their native properties over a 21-day culture period based on our presented data of cell behavior, matrix status and biomechanical properties. Application of a diurnal axial simulated-physiological loading (SPL) regime proved essential for maintenance of caprine IVDs in culture. Ex vivo culture of large IVDs is challenging and many factors have been identified to be critical for maintenance of IVD properties. Although especially NP cells have been reported to be robust and able to withstand harsh environmental conditions, for preservation of cell phenotype and metabolism a narrow optimum range for glucose, pH, oxygen and osmotic pressure has been reported. These environmental conditions have been studied in other models for their effects on the IVD and optimal range for culture have been described. These conditions could all be adequately controlled to maintain the caprine lumbar IVD in the described custom-designed LDCS.

What is the effect of unloading or low dynamic loading when compared to a simulated-physiological load?

An absence or deficit of axial load on the IVD caused pathological changes in the disc as was evident from a decline in cellular vitality (both viability and density) and changes in gene expression patterns, especially in the NP region.

In the unloaded group, cell viability already drops significantly within the first week of culture, without a significant change in cell density. Although cell viability in the unloaded group seems to stabilize with increased culture duration, the drop in cell density reveals that overall disc vitality is still diminishing.
Continuous low dynamic loading (LDL) could not prevent cell death. Alterations in gene expression in response to different loading conditions were more evident in NP cells than in AF cells. The most pronounced changes could be observed in the unloaded culture group. Absence of mechanical loading led to reduced expression of all anabolic genes, except collagen type 1.

Remodeling genes as well as inflammation-related genes are known to be up-regulated in an adverse response of cells to loading. Both the unloaded and LDL group showed significant up-regulation of the expression of several of these target genes, especially in the nucleus. Histological sections did not reveal changes in matrix staining between day 0 and day 21 of cultured discs. Longer culture periods may be needed to measure significant matrix content loss.

These findings are the resultant of both direct and indirect influences of mechanical loading on the IVD. The observed effects come from a complex system in which cells are within their native matrix environment and interact with loading, cell and matrix deformation and fluid distribution. Cells which in the \textit{in vivo} situation receive abundant mechanical stimuli from the various forces on the IVD, are deprived of these stimuli in the unloaded culture group. A lack of hydrostatic fluctuations in combination with slightly hypo-osmotic medium (when compared to the osmotic pressure in the NP of the disc), causes a different stress equilibrium compared to the physiological situation. Indirect effects may involve a decrease of fluid flow by a deficit of deformation of the IVD in the unloaded and LDL state. This could impair distribution of nutrients towards and waste products from the NP.

\textbf{Chapter 3.}

\textit{Can mechanical overloading cause IVD degeneration?}

The data presented in chapter 3 substantiate the hypothesis that any type of axial overloading will result in degenerative changes in the IVD. With regard to mechanical behavior, we could clearly observe that overall subsidence of the IVDs depended on the amount of loading that the IVD received. IVDs subjected to the static loading regime received on average the highest loading, which was associated with the largest subsidence. Nevertheless, overall deformation was almost as large in the
high dynamic regime. Especially, height recovery was hampered in the overloaded groups. For the high loading regimes, the 8-hour recovery time is too short to regain the water pressed out during the first loading cycle. One may therefore assume that these discs remain in a less hydrated state as compared to the IVDs in the SPL group. This was confirmed by our quantitative matrix measures of water and GAG, which showed loss in NP and AF regions with overloading. On a cellular level, there was significantly more loss in cell viability and cell density, and cell behavior in the overloaded groups shifted towards catabolic/remodeling and inflammatory when compared to the SPL control.

What is the difference in effect of dynamic and static overloading on the nucleus and annulus region?

We observed differences in the onset as well as the pattern of damage throughout the tested discs between overloading regimes. With high dynamic loading, all regions are moderately affected after 21 days, whereas with high static loading especially the outer AF was damaged in some cases already after 7 days of culture. We also observe positive staining for GAGs in the inner- and outer-annulus, likely due to loss and diffusion of GAGs from the NP. Analogous degenerative changes occurred at the cellular level. High dynamic loading caused substantial cell death within 7 days in all disc regions, with cell density dropping significantly after 21 days when comparing to baseline and SPL. The decrease in cell viability and density with high static loading was most pronounced in the AF region.

Chapter 4.

What is the effect of dynamic and static overloading on the nucleus and annulus region? Is there a region (anterior, lateral and posterior) specific response to dynamic and static overloading that could explain the posterolateral predilection of lumbar hernias?

With prolonged axial overloading of caprine lumbar IVD, 1) significant height loss occurs without changes in the exterior pressure distribution over the disc, 2) general cell death and matrix disruption occurs in all disc regions with high dynamic
overloading and 3) static overloading results in a posterior AF region-specific breakdown, with significant cell death and matrix disintegration and relative sparing of the NP region. Therefore, we conclude that the manner of axial overloading (dynamic or static) influences the various regions and structures of the IVD differently.

Most importantly, we found that although the mechanical changes due to dynamic and static overloading of the disc are the same (significant height loss without change in pressure distribution), the type of overloading -dynamic versus static- will affect the biological response within the disc and is region-specific. A region-specific mechanically induced degenerative cycle [39] is triggered specifically in the posterior region of the AF with static overloading. The nucleus is relatively spared, staying hydrated and pressurized, while the posterior region by comparison is weakened by apoptotic and necrotic cell response, which will trigger the vicious cycle of degeneration [39]. Therefore, we conclude that prolonged static axial overloading primes the lumbar caprine IVD for posterolateral herniation. Our findings provide a clear biological rational for the observed predilection of hernia's in the posterolateral corner of the lumbar spine in individuals with a sedentary life-style.

Chapter 5.

**How do the poro-elastic properties of the IVD change in early intervertebral disc degeneration?**

We found that a minor loss of GAG (less than 10%) from the nucleus due to Cabc-injection, caused early stage mild IVD degeneration as scored on histological sections. This mild degeneration was associated with changes in the poro-elastic properties under physiological range loading (SPL regime). These changes could be well characterized by the parameters derived from stretched-exponential fits of the displacement curves during the recovery phase of the diurnal load. Besides the expected increase in the time-constant tau for recovery behavior (axial displacement), the beta also deviates further from 1 (closer to zero) in the Cabc group. This means the fit requires more correction by its stretch constant beta, as the curve is no longer strictly exponential but increasingly linear. The changes of the beta parameter with increasing degeneration found in the current study concur with observations in earlier
studies with stretched-exponential fitting for load-induced IVD degeneration and are likely correlated to GAG loss from the nucleus.

*Can we use exponential fitting to identify dysfunctional disc behavior before height and water are permanently lost?*

Already very early in the degenerative process, we observed an alteration of the mechanical behavior of the disc. Only a minor loss of GAGs causes a change from a more poro-elastic towards a more elastic behavior of the IVD. This change could be quantified by the parameter beta in the stretched exponential model, which was fitted to the recovery curve. This is a major advantage of the stretched exponential fit function compared to traditional height and stiffness measures. Therefore, we concluded that in the current experimental model, the stretched-exponential fit is capable of identifying early degenerative disc changes, before irreversible height and water-content changes occur.

**Chapter 6:**

*How do quantitative T2, T1rho and ADC maps change with mild IVD degeneration?*

All tested quantitative MR mapping, and T1rho in particular, detected the small degenerative changes in the IVDs matrix (loss of GAG) due to Cabc-injection. With the use of a 9.4T MRI we were able to image the lumbar caprine IVD in high anatomical detail. We quantitatively mapped the IVDs 5 distinct regions on T2, T1rho, and ADC images and found significant differences between the NP, inner-AF, and the anterior, lateral and posterior outer-AF. T1rho values pre- and post-loading showed a larger difference than T2, due to T1rho’s larger dynamic range. We only found moderate effects of the degeneration on the ADC values in the (anterior and posterior) outer-AF. Lack of measured changes are most likely due to the effects of ADC’s sensitivity to anisotropy and the IVD culture conditions.
Which MRI technique is superior in quantifying early (regional) IVD degeneration?

T1rho nucleus values correlate strongly with the histological degeneration score ($R^2 = 0.729$) and significantly better than T2 and ADC. T1rho values are more closely linked to actual ECM content and therewith biomechanical function (recovery behavior; the stretch-constant beta) of the disc. Both T2 and T1rho correlated to the Cabc dose-dependent tau increase. T1rho’s stronger correlation to the stretch constant beta, is most likely due to T1rho’s stronger correlation to GAG-content. The stretch-constant beta deviates further from 1 (to zero) when poro-elastic properties are lost and the disc material shifts towards a more linear (solid-elastic) behavior (54). In the case of the IVD, this has been shown to be caused by loss of GAGs (and therewith water) from the NP (73) and structural damage to the disc (55). Taken together, when lower T1rho NP values are found, this is representative for the biomechanical deterioration of the poro-elastic properties of the IVD, which is the first step in the degenerative cascade of DDD.