7.1 Summary

This thesis started with acknowledging the lack of valid and quantitative measures for intervertebral disc degeneration. Current measures rely on subjective scales that are introduced without rigorous validation, and may only describe a non-representative temporary state of the intervertebral disc[1]. This problem severely hampers the progress of research in the field. Therefore, the **aim of this thesis was to identify quantitative functional measures for intervertebral disc degeneration**. As the intervertebral disc has a predominant mechanical function, we investigated the changes in biomechanical response of the intervertebral disc to diurnal loading (**chapter 2**). It was found that a healthy intervertebral disc slowly loses disc height due to the compressive forces during daily activities. However, during the night the disc height was slowly restored. It was shown that this dynamic process changes with degeneration, as the amount of change in disc height and the speed at which this change occurs was related to several traditional measures for disc degeneration. These measures included both qualitative or semi-quantitative measures – MRI, morphology - and quantitative measures of extracellular matrix composition. This resulted in several biomechanical parameters that can be used to quantify degeneration. A significant relation with the mechanical parameters was found for an extracellular matrix measure, namely the proteoglycan/collagen ratio in the nucleus pulposus. The changes in extracellular matrix type are therefore a likely cause of the changes in biomechanical properties.

Proteoglycan loss reduces the osmotic gradient that drives the flow of water into the intervertebral disc. That is why widely accepted models, developed in the 1980s, predict that change in osmotic gradient is the main determinant of the biomechanical changes seen with degeneration. In **chapter 3**, we found that this is only partly the case. By artificially reducing this osmotic gradient, less diurnal loss and gain of disc height was found, similar to the degenerative process, but the time constants increased when the osmotic gradient was reduced. This is in contrast to the degenerated intervertebral disc, as we found in chapter 2 that the time constants are negatively related to degeneration. Therefore, we concluded that the lack of osmotic gradient can only partly explain the changes seen with degeneration, and that with degeneration the permeability of the tissue also increases. These results indicated that the factors that mediate the response to compressive loads are more complex than the classic models predict. Thus, the extracellular matrix content of the nucleus pulposus can be used as an important, but not conclusive measure for disc degeneration.

It was then attempted to determine the additional factors involved in the response to compressive load **(chapter 4)**. To do so, the interaction between water flow, hydrostatic pressure, and viscoelastic deformation was studied under prolonged compressive load and recovery. We confirmed that the osmotic gradient indeed is the main factor that determines water inflow during recovery. However, the recovery of disc height was also in large part determined by viscoelastic extracellular matrix rearrangement that was not related to water inflow, presumably of the annulus fibrosis fibers. The water content of the annulus fibrosis was also strongly related to disc height. Considering that the response to compressive load and recovery is related to degeneration, the intrinsic viscoelastic properties of the

extracellular matrix of both the nucleus pulposus and the annulus fibrosis may potentially be related to intervertebral disc degeneration.

Thus, extracellular matrix properties and content were identified as potential measures for disc degeneration. The traditional methods to measure both are however not satisfactory. The viscoelastic properties of the extracellular matrix are traditionally measured with rheology measurements on small pieces of tissue, dissected from the intervertebral disc. However, as this removes the tissue from its physiological environment, it is unclear if these results are valid for physiological conditions. Therefore, a new device was developed that can measure the viscoelastic extracellular matrix properties of an intervertebral disc *in situ*. This is achieved through indentation in an 18 gauge needle, allowing measurements in the intact intervertebral disc. In **chapter 5**, the first results from these measurements are presented. It was found that the extracellular matrix stiffness of the nucleus pulposus *in situ* is much lower than previously reported based on rheological measurements in isolated tissue. However, with compressive force the extracellular matrix stiffens, and shifts from liquid-like to more solid-like. The developed method used can be implemented in future experiments to determine changes in extracellular matrix properties of both the annulus fibrosis and nucleus pulposus with degeneration.

The methods traditionally used to investigate extracellular matrix composition are histology and biochemical assays. Histology is a qualitative method, which prohibits objective statistical comparison. Biochemical assays are quantitative, but show a high variance and do not provide additional information on distribution of the extracellular matrix over the intervertebral disc. Therefore, in **chapter 6**, a method to quantitatively determine the content and distribution of the extracellular matrix was investigated using a novel imaging method. Fourier Transform Infrared (FTIR) spectroscopy imaging determines the chemical composition of slices of the intervertebral disc by measuring the absorption of infrared light. In intervertebral discs from a caprine *in vivo* study, mildly degenerated intervertebral discs were compared with control discs. In more detail than with the traditional measures we could detect a decrease in proteoglycan, increase in collagen and increasing disorganization of the collagen network, mainly in the nucleus and anterior annulus. This observation was further explored in an investigation on the initiation of disc degeneration, after three weeks of mild overloading in a loaded disc culture system. An overall decrease in the proteoglycan/collagen ratio and an increase in the disorganization (entropy) of the collagen matrix was found in the overloaded discs. This shows that the first steps towards degeneration due to overloading include remodeling of the collagen network. Additionally, we tested the hypothesis that early changes with degeneration are cell-mediated, but this remained inconclusive based on the mixed results.

In conclusion, the biomechanical response to compressive loading of the intervertebral disc is affected by degeneration. Partly, this can be explained by increased permeability and by a reduction in osmotic gradient due to change in extracellular matrix content. However, the viscoelastic properties of the annulus and possibly the nucleus are also strong contributors to the compressive load response. These properties can now be measured *in situ*, which is

physiologically more relevant than previous measurements. The changes in extracellular matrix composition throughout the disc can be measured with greater detail than previously possible using FTIR imaging. Decrease in proteoglycan/collagen ratio and a higher entropy of the collagen matrix are early measurable changes on the way towards disc degeneration.

7.2 Assumptions & limitations

The aim of this thesis was to identify quantitative functional measures of intervertebral disc degeneration. As no golden standard definition for disc degeneration is available, we started with postulating the axiom that the *function* of the intervertebral disc is mechanical. We then analyzed the changes in mechanical properties, and considered these functional changes. This argument includes several subjective assumptions. Most importantly, mechanical function is a perception of the investigator. The cells and matrix in the intervertebral disc will not be aware of any function, and it is deterministic to assume that all tissues in the human body need to have a certain function. But, confronted with the lack of a better option, we assumed that there is an evolutionary advantage in the ability to rotate over multiple axis in the spines, to have a shock damper of a certain stiffness, and to have the forces nicely and evenly distributed over the vertebra endplates.

Besides the assumption of a mechanical function, there are several additional limitations of the research in this thesis that need to be considered. For instance, the discussion in this chapter so far has been limited to the biomechanical properties and the extracellular matrix content of the healthy and degenerating intervertebral disc. However, the vicious cycle of degeneration[2], introduced in chapter 1, contains another important mediator of degeneration: the cell. Only in chapter 6, cells were considered– but only indirectly by reducing the cell activity in a control group. This is partly for practical reasons: the intervertebral disc contains very few cells, and techniques like Polymerase Chain Reaction (PCR) are therefore very difficult to implement[3]. Furthermore, the cellular contribution to intervertebral disc degeneration was covered in great detail in the thesis of fellow PhD student Peeters[4]. Another limitation of this thesis is that the studied methods cannot be implemented *in vivo*. Biomechanical properties cannot be determined directly *in vivo*; FTIR is not feasible without harmful tissue extraction; osmotic properties and water content are only indirectly measurable *in vivo*. However, as discussed in more detail below, the first step towards improvement of current measures that are used *in vivo* is to be able to quantify the functional changes with degeneration. If we can agree on the parameters that change with degeneration, then imaging methods can be developed that are strongly related to these changes. Therefore, fundamental experimental work needs to precede the development of *in vivo* applicable measures.