Jari Rautiainen

NOVEL MAGNETIC RESONANCE IMAGING TECHNIQUES FOR ARTICULAR CARTILAGE AND SUBCHONDRAL BONE

STUDIES ON MRI RELAXOMETRY AND SHORT ECHO TIME IMAGING
JARI RAUTIAINEN

NOVEL MAGNETIC RESONANCE IMAGING TECHNIQUES FOR ARTICULAR CARTILAGE AND SUBCHONDRAL BONE
Studies on MRI relaxometry and short echo time imaging

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University of Oulu Graduate School; University of Oulu, Faculty of Medicine; Medical Research Center Oulu

Abstract

Osteoarthritis is a common joint disease in the adult population characterized by degeneration and limited repair capability of articular cartilage. Cartilage degeneration is also associated with remodeling and sclerosis of the subchondral bone. The relative contributions of these processes are not fully understood partly because of a lack of proper biomarkers for the diagnosis and follow-up of the disease progress. Current diagnostic methods are too insensitive or subjective for reliable and early diagnosis of osteoarthritis.

Novel magnetic resonance imaging (MRI) techniques can be used for indirect assessment of cartilage constituents. In addition, the imaging of the subchondral bone and osteochondral junction has become feasible, which has been very difficult with conventional MRI methods due to the very fast signal ($T_2$) decay in those tissues. Quantitative MRI of cartilage involves measurement of relaxation time parameters ($T_1$, $T_2$, $T_{1p}$, $T_{2p}$, $T_{R AFF}$, $T_{1sat}$) and investigation of how the parameters reflect the properties of the cartilage. SWIFT (Sweep imaging with Fourier transform) is an MRI technique capable of imaging short $T_2$ structures, enabling assessment of the osteochondral junction as well as the subchondral bone. The aim of this work was to investigate osteoarthritic changes occurring in articular cartilage and subchondral bone in experimental animal models of osteoarthritis and degenerated human specimens with quantitative MRI measurements and SWIFT imaging at 9.4 T. Moreover, the feasibility of the different quantitative MRI techniques in the assessment of osteoarthritic changes was studied. For reference, biomechanical, quantitative and qualitative histology, biochemical and micro-computed tomography measurements were conducted.

Novel adiabatic $T_{1p}$ and $T_{2p}$ relaxation time parameters were more sensitive than conventional methods ($T_1$, $T_2$) or continuous wave $T_{1p}$ to both early degenerative changes in a rabbit model of cartilage degeneration and to spontaneous degeneration of human cartilage. SWIFT enabled assessment of the structural properties of the subchondral bone. Moreover, signal changes related to the degree of osteoarthritis were detected at the cartilage–bone interface with SWIFT. These methods may provide new tools for improving the diagnosis of early osteoarthritis. Further studies are needed to investigate the clinical feasibility of these MRI techniques.

Keywords: articular cartilage, calcified cartilage, magnetic resonance imaging, osteoarthritis, relaxation time, subchondral bone, sweep imaging with Fourier transform
Rautiainen, Jari, Nivelruston ja rustonalaisen luun magneettikuvaus. Relaksataatioaikakartoitus ja lyhyen kaikuajan kuvantaminen
Oulun yliopiston tutkijakoulu; Oulun yliopisto, Lääketieteellinen tiedekunta; Medical Research Center Oulu
Oulun yliopisto, PL 8000, 90014 Oulun yliopisto

Tiivistelmä

Magneettikuvausteknologian kehitys mahdollistaa näyttävän nivelsairauden koostumuksesta ja sen muutoksista. Qvantitatiivisen magneettikuvaustekniikan avulla (T₁, T₂, T₁ρ, T₂ρ, TRAFF, T₁sat relaksataatioaikaparametrit) voidaan mittaa epäsuorasti nivelruston koostumusta ja rakennetta. SWIFT (Sweep Imaging with Fourier Transform) on uusi lyhyen kaikuajan magneettikuvausmenetelmä, joka mahdollistaa myös erittäin lyhyen T₂-relaksataatioajan omaavien kudos- ja kudoksien havainnoinnin erilaisissa eläinmalleissa sekä ihmiskudoksissa. Työssä tutkittiin nivelrikossa syntyvien muutosten määrää ja kehitystä nivelruston koostumuksesta sekä raskausaikasta.

Kvantitatiivisista magneettikuvausparametreista adiabaattinen T₁ρ sekä adiabaattinen T₂ρ osoittautuivat tärkeimpiin havaintoihin nivelruston koostumuksesta. SWIFT-magneettikuvaustekniikka puolestaan mahdollisti näkyvän nivelruston koostumuksesta ja kudoksen muutoksista erilaisissa eläinmalleissa sekä ihmiskudoksissa. Työssä tarkasteltiin eri magneettikuvaustekniikoiden herkkyyttä havaita muutokset nivelrustossa sekä raskausaikasta.

Asiasanat: kalkkeutunut rusto, magneettikuvaus, nivelrikko, nivelrusto, relaksataatioaika, rustonalainen luu, sweep imaging with Fourier transform
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Kuopio, March 2015

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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ACLT</td>
<td>Anterior cruciate ligament transection</td>
</tr>
<tr>
<td>AFP</td>
<td>Adiabatic full passage</td>
</tr>
<tr>
<td>AHP</td>
<td>Adiabatic half passage</td>
</tr>
<tr>
<td>AUC</td>
<td>Area under curve</td>
</tr>
<tr>
<td>BV/TV</td>
<td>Bone volume-to-tissue volume ratio</td>
</tr>
<tr>
<td>BW</td>
<td>Bandwidth</td>
</tr>
<tr>
<td>CPMG</td>
<td>Carr-Purcell-Meiboom-Gill</td>
</tr>
<tr>
<td>CT</td>
<td>Computed tomography</td>
</tr>
<tr>
<td>CTRL</td>
<td>Contralateral</td>
</tr>
<tr>
<td>CW</td>
<td>Continuous wave</td>
</tr>
<tr>
<td>DD</td>
<td>Digital densitometry</td>
</tr>
<tr>
<td>DE</td>
<td>Double echo</td>
</tr>
<tr>
<td>DESS</td>
<td>Double echo steady state</td>
</tr>
<tr>
<td>DW</td>
<td>Dry weight</td>
</tr>
<tr>
<td>dGEMRIC</td>
<td>Delayed gadolinium enhanced MRI of cartilage</td>
</tr>
<tr>
<td>ECM</td>
<td>Extracellular matrix</td>
</tr>
<tr>
<td>FCD</td>
<td>Fixed charge density</td>
</tr>
<tr>
<td>FID</td>
<td>Free induction decay</td>
</tr>
<tr>
<td>FLASH</td>
<td>Fast low angle shot</td>
</tr>
<tr>
<td>FOV</td>
<td>Field of view</td>
</tr>
<tr>
<td>FSE</td>
<td>Fast spin-echo</td>
</tr>
<tr>
<td>FT</td>
<td>Fourier transform</td>
</tr>
<tr>
<td>FTIRI</td>
<td>Fourier transform infrared imaging</td>
</tr>
<tr>
<td>GAG</td>
<td>Glycosaminoglycan</td>
</tr>
<tr>
<td>Gd</td>
<td>Gadolinium</td>
</tr>
<tr>
<td>GRE</td>
<td>Gradient echo</td>
</tr>
<tr>
<td>HSn</td>
<td>Hyperbolic secant</td>
</tr>
<tr>
<td>IR</td>
<td>Inversion recovery</td>
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<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
</tr>
<tr>
<td>MT</td>
<td>Magnetization transfer</td>
</tr>
<tr>
<td>MTR</td>
<td>Magnetization transfer ratio</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<td>--------------</td>
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</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
</tr>
<tr>
<td>OA</td>
<td>Osteoarthritis</td>
</tr>
<tr>
<td>OARSI</td>
<td>Osteoarthritis Research Society International</td>
</tr>
<tr>
<td>PG</td>
<td>Proteoglycan</td>
</tr>
<tr>
<td>PI</td>
<td>Parallelism index</td>
</tr>
<tr>
<td>PLM</td>
<td>Polarized light microscopy</td>
</tr>
<tr>
<td>RAFF</td>
<td>Relaxation along a fictitious field</td>
</tr>
<tr>
<td>RF</td>
<td>Radiofrequency</td>
</tr>
<tr>
<td>RFR</td>
<td>Rotating frame of reference</td>
</tr>
<tr>
<td>ROC</td>
<td>Receiver operator characteristic</td>
</tr>
<tr>
<td>ROI</td>
<td>Region of interest</td>
</tr>
<tr>
<td>SAR</td>
<td>Specific absorption ratio</td>
</tr>
<tr>
<td>SNR</td>
<td>Signal-to-noise ratio</td>
</tr>
<tr>
<td>SE</td>
<td>Spin-echo</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SL</td>
<td>Spin-lock</td>
</tr>
<tr>
<td>SR</td>
<td>Saturation recovery</td>
</tr>
<tr>
<td>SWIFT</td>
<td>Sweep imaging with Fourier transform</td>
</tr>
<tr>
<td>$T_1$</td>
<td>Longitudinal relaxation time</td>
</tr>
<tr>
<td>$T_{1Gd}$</td>
<td>$T_1$ in the presence of gadolinium</td>
</tr>
<tr>
<td>$T_2$</td>
<td>Transverse relaxation time</td>
</tr>
<tr>
<td>Tb.N</td>
<td>Trabecular number</td>
</tr>
<tr>
<td>Tb.Sp</td>
<td>Trabecular separation</td>
</tr>
<tr>
<td>Tb.Th</td>
<td>Trabecular thickness</td>
</tr>
<tr>
<td>TE</td>
<td>Time-to-echo</td>
</tr>
<tr>
<td>TI</td>
<td>Time-to-inversion</td>
</tr>
<tr>
<td>TKA</td>
<td>Total knee arthroplasty</td>
</tr>
<tr>
<td>TR</td>
<td>Time-to-repetition</td>
</tr>
<tr>
<td>$T_{1ρ}$</td>
<td>Longitudinal relaxation time in RFR</td>
</tr>
<tr>
<td>$T_{2ρ}$</td>
<td>Transverse relaxation time in RFR</td>
</tr>
<tr>
<td>UTE</td>
<td>Ultrashort echo time</td>
</tr>
<tr>
<td>VOI</td>
<td>Volume of interest</td>
</tr>
<tr>
<td>WW</td>
<td>Wet weight</td>
</tr>
<tr>
<td>ZTE</td>
<td>Zero echo time</td>
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List of original publications

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1 Introduction

Articular cartilage is a highly specialized tissue that enables virtually frictionless movement of the articulating joints and softens peak loads generated during locomotion [1–3]. The tissue has poor regenerative properties due to the absence of blood vessels [1, 3]. However, articular cartilage is very durable as it can remain fully functional for decades or the entire life of an individual. Osteoarthritis (OA) is the most common joint disease, the knee joint being one of the most frequently affected sites [4, 5]. The prevalence of OA is increasing in the elderly population but the pathophysiology of OA is still unclear, partly owing to its complex structure and the interactions of tissue constituents [6]. OA is generally considered as cartilage disease although all joint compartments are affected. It is characterized by progressive degeneration of articular cartilage which starts at the molecular level [4, 7], coupled with sclerosis of subchondral bone and changes at the cartilage–bone interface [8, 9]. In the end stage of the disease cartilage has been lost leaving only the bony surfaces articulating against each other, which causes pain, joint stiffness and disability [4]. The total costs of OA to economies due to medical expenses and early retirements are enormous [10], approximated in Finland to be about EUR 1 billion annually [11].

In the final stage of knee OA the only proper treatment is total knee arthroplasty (TKA) which removes pain and partly restores mobility [12]. Also other more conservative surgical repair techniques and disease modifying drugs have been developed without succeeding to fully restore normal joint function [4]. The reason for this is partly explained by the lack of sensitive biomarkers for the early diagnosis and follow-up of the disease progress or monitoring of the treatment outcome. OA diagnostics is currently based on radiography of the joint-space width or clinical magnetic resonance imaging (MRI), which are too insensitive for reliable and early diagnosis of the disease [13]. Consequently, there is a need for new more sensitive diagnostic imaging methods for assessment of cartilage degeneration.

The development of MRI technology has provided new promising tools for the evaluation of the status of the joint. Noninvasive assessment of cartilage constituents can be implemented by means of quantitative MRI relaxometry which involves measurement of tissue specific relaxation time parameters [14–17]. Direct imaging of bone is instead very difficult with conventional MRI techniques due to very fast signal decay from
calcified structures [18, 19]. Sweep Imaging with Fourier Transformation (SWIFT) is an MRI technique in the ultrashort echo time (UTE) family, capable of capturing a signal from objects having a broad range of relaxation times [20, 21], including tissues with extremely short $T_2$ relaxation time such as subchondral bone.

The aim of this thesis was to study the feasibility of a number of novel MRI techniques for the assessment of degenerative changes in articular cartilage, subchondral bone and the cartilage–bone interface. Quantitative MRI and SWIFT methods were tested in animal models of OA and in human tissue samples suffering varying degrees of degeneration. Furthermore, quantitative MRI techniques were compared to reference methods to study the sensitivity of the methods to the cartilage constituents, histopathological grade and biomechanical properties of cartilage.
2 Articular cartilage and subchondral bone

Articular cartilage is a connective tissue that covers the articulating surfaces in the synovial joints [1–3]. In a knee joint, approximately 2-4 mm thick [22] articular cartilage covers femoral condyles, tibial plateau and patella (Fig 1). It has a highly anisotropic structure with depth-dependent amount and organization of the tissue constituents [3, 23].

![Diagram of knee joint](image)

Fig 1. Anterior (A) and cross-sectional (B) view of a knee joint. Cross-section of articular cartilage (C) reveals depth-dependent orientation of the collagen fibrils.

2.1 Structure and composition of articular cartilage

Articular cartilage is composed of cells and an extracellular matrix (ECM), which includes tissue water and a macromolecular network [2, 3]. Extracellular water comprises up to 80% of the tissue’s wet weight, the rest consisting mostly of large macromolecules such as collagen, proteoglycans (PG) and non-collagenous proteins [2, 3]. Cartilage cells (chondrocytes) contribute to only about 1% of the tissue volume. The interaction of the interstitial water with the ECM components determines the mechanical properties of the tissue [2, 3].

Type II collagen is the most abundant type found in cartilage accounting for about 95% of all collagens [2, 3]. Collagens are organized into a network of fibrils that provides the tissue with its tensile strength properties and attach the large macromolecules and water to the matrix. In total, collagen fibrils contribute approximately 60% of the dry
weight of cartilage. The orientation of the collagen fibrils varies with the depth of the
tissue [24], and is the main determinant of different zones of cartilage.

Proteoglycans are large molecules consisting of protein core and glycosaminoglycan (GAG) side chains [2, 3]. Negatively charged GAGs create the fixed charge density (FCD) of cartilage [23], attracting positive charges and repelling other negatively charged molecules. In articular cartilage, PGs are mostly large aggregating proteoglycan monomers or aggrecans and smaller PGs, which make up approximately 25 to 35% of the dry weight of cartilage [3]. The interfibrillar space of the ECM is primarily filled with aggrecans that are associated with hyaluronic acid and link proteins forming large proteoglycan aggregates [25]. During mechanical loading aggregates prevent the displacement of PGs within the ECM [3].

Chondrocytes, the only type of cells found in the cartilage, are spheroidal in shape and they do not form cell-to-cell contacts within the ECM [3]. Chondrocytes maintain cartilage ECM by synthesizing and degrading cartilage molecules [2, 3]. Due to the avascularity of cartilage, chondrocytes derive their nutrition from the synovial fluid through diffusion. The activity of cells is dependent on the mechanical stresses experienced during loading [26].

The structure of articular cartilage is highly anisotropic. Although the structure varies smoothly, three histological layers with varying composition and organization of tissue constituents are generally distinguishable [2, 3]. The layers are identified according to the organization of the collagen fibril network (Figure 1). The superficial zone is about 5% of the cartilage thickness, the transitional zone 20% and the radial or deep zone 75% in mature cartilage [27]. The composition, organization, mechanical properties of the matrix and cell morphology vary according to the depth from the surface of the articular cartilage [2, 3, 23].

The superficial zone is the thinnest zone of articular cartilage [2, 3]. It has higher concentrations of collagen and water and a lower concentration of PGs relative to other zones. The superficial zone has higher tensile stiffness and strength than deeper zones as the orientation of the collagen fibrils in the superficial zone is parallel to the cartilage surface. Chondrocytes in the superficial zone are more flattened in shape compared to other zones.

Collagen fibrils in the transitional zone are more randomly organized, changing from parallel to surface to perpendicular to the surface in the deeper cartilage [2, 3]. The transitional zone has a higher amount of PGs but lower concentrations of water and collagen than the superficial zone.
The deep zone or radial zone has the highest concentration of proteoglycans and the lowest concentration of water [2, 3]. Chondrocytes are organized in columns perpendicular to the joint surface. Similarly, the primary orientation of the collagen fibrils in the deep cartilage is perpendicular to the cartilage surface. Eventually, the collagen fibrils reach tidemark, the thin 5-µm thick [9], irregular boundary between the calcified and uncalcified cartilage layers.

The zone of calcified cartilage is a mineralized layer at the osteochondral junction, which integrates the uncalcified cartilage to the subchondral bone and transmits forces across the cartilage-bone interface [9]. The thickness of calcified cartilage varies between 20 and 250 µm in healthy human subjects [28]. The calcified cartilage is mainly composed of type II collagen, type X collagen, GAG and chondrocytes [9]. However, chondrocytes in normal calcified cartilage are quiescent and their density is much lower than in the overlying articular cartilage. In addition, while normal articular cartilage is avascular, the zone of calcified cartilage contains vascular channels that originate from the subchondral bone [9]. In healthy subjects, the tidemark limits the vascular invasion and calcification to the calcified cartilage layer. The mechanical strength of the calcified cartilage is lower than that of the subchondral bone, though it is still much stiffer than articular cartilage [29].

2.2 Mechanical properties of articular cartilage

The mechanical properties of articular cartilage are mainly determined by the interaction of collagens and PGs with the interstitial water. PGs are mainly responsible for the compressive stiffness (equilibrium modulus), while the collagen fibril network and water control the dynamic properties [30, 31].

Mechanical parameters can be determined with stress-relaxation, creep or dynamic loading schemes. In the stress-relaxation test, the time-dependent response of the force is tracked after application of an instantaneous load, allowing the tissue to recover to its equilibrium state. In the creep test, the deformation of the tissue is investigated during constant loading. In dynamic loading, the force is cyclically applied for several periods with a certain frequency [32].

Different loading schemes can be implemented in confined, unconfined or indentation geometry [33]. In unconfined compression, a cylindrical cartilage sample detached from the subchondral bone is compressed between two plates allowing free fluid flow from the sides of the sample during mechanical testing. In confined compression, the cartilage
sample is placed in a chamber allowing axial fluid flow through a permeable piston. In indentation, cartilage is loaded with an indenter perpendicular to the cartilage surface. It does not require removal of the cartilage from the subchondral bone. The diameter of the indenter should be over four times larger than width of the sample preventing the edges of the sample from affecting the measurement [34].

Young’s modulus for elastic material is given by

\[ E = \frac{\sigma}{\varepsilon}, \]

where \( \sigma \) and \( \varepsilon \) are the axial stress and strain, respectively. Young’s modulus is typically determined from the slope of a stress–strain plot of a step-wise stress-relaxation test. For indentation geometry, Young’s modulus is obtained by applying a correction factor that takes into account the size and shape of the indenter and the sample size:

\[ E = \frac{\sigma \pi a (1 - \nu^2)}{2 h \kappa}, \]

where \( a \) is the radius of the plane-ended indenter, \( \nu \) is Poisson’s ratio describing the compressibility of the sample, \( h \) is the thickness of the cartilage and \( \kappa \) is a theoretical scaling factor [35]. The dynamic modulus is given by

\[ E_d = \sqrt{E_1^2 + E_2^2} = \frac{\sigma_d}{\varepsilon}, \]

where \( E_1 \) is the storage modulus, proportional to the elastically stored energy in the tissue, \( E_2 \) is the loss modulus that describes the viscous energy dissipated in the loading process and \( \sigma_d \) is the dynamic stress [32]. The dynamic modulus is typically several times higher than the elastic modulus.

### 2.3 Structure and composition of subchondral bone

Bone is a calcified tissue that provides support and protection for organs and enables movement of the limbs. Furthermore, it serves as a storage for minerals such as calcium and phosphorus. Bone undergoes remodeling throughout the lifetime of an individual by reformation and resorption of bone matrix. Morphologically two separate types of bone can be identified in the musculoskeletal system. Hard and dense cortical bone covers the outer layers of bones while the inner parts are more porous trabecular or cancellous bone filled with bone marrow [36, 37]. Subchondral bone is the bone located in the synovial joints under the zone of calcified cartilage, separated by the boundary known as
the cement line [37]. The cortical endplate of an articulating bone is also referred to as the subchondral bone plate.

Bone is a composite material that consists of cells, water, organic and inorganic phase [36]. The mineralized inorganic phase is mainly composed of hydroxyapatite crystals while the organic phase consists mostly of type I collagen, which is produced by osteoblasts, cells that synthesize new unmineralized bone. Osteoblasts differentiate into osteocytes (bone maintaining cells) after they become surrounded by the bone matrix they produce. Hydroxyapatite and the organic phase together form the mineralized bone matrix. Osteoclasts are large multinucleated cells that are capable of resorbing the bone matrix produced by osteoclasts.

The cortical bone consists of cylindrical structures called osteons [36]. Osteons are composed of bone lamellae that surround Haversian canals that include blood vessels and nerves. Vascular channels from the cortical bone can extents into calcified cartilage [37]. Trabecular bone consists of a network of trabeculae, which are connected plate-like structures of bone, aligned principally in the direction of mechanical loading. As it does not contain osteons as the cortical bone, trabecular bone is nourished by bone marrow. Trabecular bone is sensitive to mechanical stresses and is continuously remodeled due to altering loads. The cortical bone is also remodeled, although the rate is significantly higher in the trabecular bone. This adaptive response of bone to external forces is known as Wolff’s law. The medullar cavities between the trabeculae are filled with yellow and red bone marrow. The yellow bone marrow consists mainly of fat while the red marrow is responsible for the production of new blood cells.

2.4 Osteoarthritis

Osteoarthritis, also referred to as degenerative joint disease, may develop idiopathically or due to injury, inflammation or a developmental disorder [4, 5]. OA is most commonly diagnosed in hand, knee, hip and spine joints. Typical clinical symptoms are joint pain and stiffness which are often associated with late-stage OA. The pathophysiology of OA involves progressive degeneration of cartilage accompanied by subchondral bone sclerosis that ultimately results in loss of cartilage tissue [4, 5]. In addition, other joint components, such as menisci, ligaments and synovial fluid are also affected. The disease progress can be roughly divided into three overlapping stages.

The earliest signs of OA occur in the ECM of cartilage at the molecular level [4, 5, 7]. The PG content of cartilage decreases, minor fibrillation may be shown at the cartilage
surface and the water content increases. In addition, the integrity of the collagen network is disrupted causing swelling of the tissue. Together these changes increase the permeability of cartilage, leaving the tissue susceptible to additional damage as the stiffness of the cartilage is reduced. In the early phase, remodeling of the subchondral bone increases the thickness of the subchondral bone plate.

Chondrocytes detect and react to degenerative changes in the tissue by initiating the cellular repair response [4]. The cells degrade damaged matrix components enzymatically and synthesize new ECM macromolecules. The repair response may last for years, even stabilizing the tissue. However, if the degradation process accelerates and breaks the balance, the tissue is vulnerable to further damage.

Failure in the repair response leads to further disorganization of the collagen network, loss of PGs and fibrillation of the cartilage surface [4, 5]. Progressive loss of tissue leads to release of cartilage pieces into joint space, decreasing the thickness of the cartilage. This leads to further thickening of the subchondral bone plate and trabeculae, narrowing the joint space. In addition, osteophytes or cartilaginous tissue may be formed into joint margins. In the cartilage–bone interface, thickening of the zone of calcified cartilage, duplication, and vascular invasion of the tidemark are detected, which further contributes to the thinning of cartilage tissue [9, 38]. Ultimately cartilage tissue is lost, leaving only the dense surface of the subchondral cortical bone.
3 Magnetic Resonance Imaging

The following section covers the essential theoretical aspects of MRI which is based on the physical phenomenon of nuclear magnetic resonance (NMR) [39–41].

3.1 Nuclear magnetic resonance

Spin or nuclear spin angular momentum is an intrinsic quantized physical property of all nuclei [41]. A nucleus with an odd atomic mass number has a nonzero spin and can interact with an external magnetic field, which is the basis of NMR. Nuclei have magnetic dipole moments which align themselves along external magnetic field ($B_0$). A hydrogen atom $^1$H, a proton has a spin number of 1/2 and it has two possible energy states, parallel (α-state) and anti-parallel (β-state) to the $B_0$ field. The energy difference between these states depends on the magnitude of the $B_0$

$$\Delta E = -\gamma \hbar B_0,$$

where $\gamma$ is the gyromagnetic constant (42.58 MHz/T for $^1$H), $\hbar$ is the Planck’s constant divided by $2\pi$. Spins aligned parallel to the $B_0$ have slightly lower energy and it is more favored state at equilibrium compared to the anti-parallel orientation. The number of spins at different energy states is given by the Boltzmann distribution

$$\frac{N_\alpha}{N_\beta} = e^{\frac{\Delta E}{kT}},$$

where $T$ is temperature and $k$ is the Boltzmann constant. The excess of the spins parallel to the static magnetic field creates a net magnetization (polarization) vector $M_0$ which is oriented to the direction of $B_0$ (z direction in the laboratory frame $xyz$ coordinate system) at equilibrium [41]. In the external magnetic field, the magnetic moments are not stationary but precess around the $B_0$. The precession frequency $\omega_0$, also called Larmor frequency is defined as

$$\omega_0 = -\gamma B_0.$$

The Larmor frequency of $^1$H, for example, at 9.4 T is 400.252 MHz. The resonance frequency is also affected by the molecular environment in which the hydrogen atoms are located. In biological tissues, hydrogen atoms in large fat molecules are partly
shielded by the electron clouds from neighboring atoms, reducing the strength of the external magnetic field experienced by the protons. A water molecule has a more simple structure and the shielding effect is much weaker. The difference in the resonance frequencies (chemical shift) between water and fat is 3.5 parts per million, approximately 1.4 kHz at 9.4 T [41].

The equilibrium state can be disturbed by transmitting radiofrequency (RF) energy to the system at the Larmor resonance frequency. The RF pulse creates an additional oscillating magnetic field, noted as $B_1$, perpendicular to the $B_0$ field. For simplicity, the behavior of the $M_0$ is analyzed in the rotating frame of reference (RFR), in which the precessing net magnetization appears stationary as the frame rotates at the Larmor frequency [41]. According to equation (6), the magnetization $M_0$ precesses also about the RF field, which in effect causes the $M_0$ to tilt away from the $z$ axis as long as the RF pulse is applied. If the RF pulse contains off-resonance components, the resulting effective field ($B_{\text{eff}}$) is defined as

$$B_{\text{eff}} = \sqrt{B_1^2 + \Delta B^2},$$

(7)

where $\Delta B$ is the field due to the difference between the resonance and rotating frame frequencies. The magnetization follows the effective field during RF irradiation.

### 3.2 Relaxation

The application of RF pulses at resonance frequency to the spin system at the equilibrium state rotates the net magnetization $M_0$ from the $z$ axis towards the $xy$-plane (transverse plane). The transverse component of the net magnetization can be detected with an RF coil orthogonal to the $xy$-plane. This NMR signal induced in the coil is called the free induction decay (FID), emitted by the system after the application of the RF pulse, as the system returns to the equilibrium state through different relaxation processes [42, 43].

#### 3.2.1 $T_1$ relaxation

The spin-lattice or longitudinal relaxation time constant, $T_1$, characterizes the recovery of the longitudinal magnetization back to the equilibrium state. The molecular motion of the spins creates local magnetic field fluctuations, thus spins in different molecular environments experience slightly different magnetic fields. These dipole-dipole interactions, which are the main relaxation mechanisms in tissues, causes spins to exchange energy
with their surroundings (lattice), until the thermal equilibrium is reached. Spectral density is a quantity that describes the frequency distribution of the molecular motions

$$J(\omega) = \frac{\tau_c}{1 + \omega^2 \tau_c^2},$$  \hspace{0.5cm} (8)

where $\tau_c$ is the correlation time that characterizes the time scale of the molecular tumbling. The dependency of $T_1$ on the correlation time is described as:

$$\frac{1}{T_1} \propto B_{xy}^2 J(\omega),$$  \hspace{0.5cm} (9)

where $B_{xy}$ is the field present in the xy-plane. Thus, $T_1$ relaxation is the most effective, when magnetic field fluctuations occur near the Larmor frequency.

\[ Fig\ 2.\ Inversion\ and\ saturation\ recovery\ sequences.\]

One of the most common ways to measure $T_1$ is to use the inversion-recovery (IR) sequence (Fig. 2). The magnetization is inverted with a 180° inversion pulse followed by recovery of the net magnetization with $T_1$ relaxation rate. After a certain time period (time-to-inversion, TI) a 90° pulse is applied, which flips the longitudinal magnetization to the transverse plane for measurement. The magnetization follows the equation

$$M = M_0(1 - 2e^{-TI/T_1}).$$  \hspace{0.5cm} (10)

$T_1$ can be estimated by repeating the experiment with several TI values, however the repetition time (TR) between the inversion pulses must be long enough for full recovery of the longitudinal magnetization. $T_1$ can also be determined with a saturation recovery (SR) sequence that utilizes repeated 90° pulses (Fig. 2). The behavior of the magnetization resembles that of the IR sequence (10):
Thus, signal is dependent on TR. In saturation recovery, the magnetization reaches a steady state and enough repetitions must be allowed to stabilize the steady state before measuring the signal.

### 3.2.2 $T_2$ relaxation

Spin-spin or transverse relaxation time, $T_2$ characterizes the decay of the transverse magnetization that results from the energy exchange between the spins. Magnetic field fluctuations caused by molecular tumblings and static magnetic field inhomogeneities result in a loss of phase coherence between the spins due to the slight variations in the precession frequencies. No energy is lost to the surroundings. In addition, $T_2$ relaxation is enhanced by diffusion as spins may change place and experience different local magnetic fields. $T_2$ is always equal or faster than $T_1$ relaxation. $T_2^*$ is a time constant that takes into account all the effects contributing to the decay of transverse magnetization and is defined as

$$\frac{1}{T_2} = \frac{1}{T_2} + \frac{1}{T_2'}, \quad (12)$$

where $T_2'$ captures the relaxation due to magnetic field inhomogeneities. $T_2$ is also dependent on the correlation time:

$$\frac{1}{T_2} \propto B_z^2 \tau_c, \quad (13)$$

where $B_z$ is the field present in the z-axis. Transverse relaxation is more effective at slow tumbling rates.

In the ideal case, the transverse magnetization (FID signal) follows $T_2$ decay but due to field inhomogeneities the decay is characterized by the $T_2^*$ time constant. The phase coherence of the spins can be refocused in a simple spin-echo (SE) experiment which starts with a 90° RF pulse that flips the magnetization to the transverse plane (Fig. 3). The dephasing of the spins is reversed with a refocusing 180° inversion pulse and after a certain time period (time-to-echo, TE) a signal called the spin echo or the Hahn echo [44] is produced, as the spins refocus. However, the refocusing is not complete due to diffusion. The magnetization follows exponential decay
\[ M = M_0 e^{-TE/T_2}. \] (14)

The measured signal intensity depends on the echo time, and thus the \( T_2 \) relaxation time can be determined by repeated measurements at different TE values. Insensitivity to diffusion can be obtained with the Carr-Purcell-Meiboom-Gill (CPMG) sequence which utilizes repeated refocusing pulses separated by an inter-pulse delay that form a train of echoes (Fig. 3).

**Fig 3.** Spin-echo, double echo, CPMG and gradient echo sequences. In the spin-echo experiment, the echo is formed after refocusing inversion pulse. Gradient echo is formed through dephasing of the spins with negative gradient followed by rephasing with positive gradient.

The gradient echo (GRE) experiment utilizes gradient magnetic fields instead of RF pulses for the refocusing of the spins. A negative gradient is applied after the initial excitation pulse (typically less than 90°), which causes rapid dephasing of the transverse magnetization (Fig. 3). Reversal of the first gradient causes rephasing of the spins which produces a gradient echo after TE. The signal follows a \( T_2^* \) decay

\[ M = M_0 e^{-TE/T_2^*}. \] (15)
Usually shorter echo times can be achieved with GRE compared with SE based techniques.

### 3.2.3 Rotating frame relaxation

Relaxation processes may occur also in the so-called rotating frame of reference which can be studied with spin-lock (SL) methods [45]. Effectively spin-locking experiments probe relaxation at very low magnetic fields, utilizing the higher signal-to-noise ratio (SNR) of high magnetic fields. In a classical spin-locking experiment, spins are locked to the transverse plane with a continuous wave (CW) RF pulse $B_{1SL}$ after the initial 90° tip. The magnetic moments start to precess around the spin-lock field at $\omega = -\gamma B_{1SL}$. During the spin-lock pulse, magnetization relaxes exponentially with a longitudinal relaxation time constant in the rotating frame, $T_{1\rho}$ ("T1 rho"), along the applied $B_{1SL}$ field [46]. Thus, $T_{1\rho}$ approximates to $T_1$ at very low magnetic fields. The magnetization during SL follows the equation

$$M = M_0 e^{-T_{SL}/T_{1\rho}},$$

where $T_{SL}$ is the duration of the spin-lock pulse. For the quantification of $T_{1\rho}$, $T_{SL}$ is varied in repeated measurements. $T_{1\rho}$ is dependent on the molecular motion:

$$\frac{1}{T_{1\rho}} = K \left( \frac{3}{2} \frac{\tau_c}{1 + 4\omega_{eff}^2 \tau_c^2} + \frac{5}{2} \frac{\tau_c}{1 + \omega_0^2 \tau_c^2} + \frac{\tau_c}{1 + 4\omega_0^2 \tau_c^2} \right),$$

where $K$ is a constant including information on the local field fluctuation and $\omega_{eff}$ is the effective SL field, which is equal to $\gamma B_{1SL}$ in the case of on-resonance excitation [47]. The equation (17) shows that $T_{1\rho}$ is sensitive to slow molecular motion. $T_{1\rho}$ dispersion, the SL measurement with varying $B_{1SL}$ provides information about the frequency distribution of the molecular motions. At low spin-lock power, the $T_{1\rho}$ relaxation time approaches $T_2$ relaxation, although $T_{1\rho}$ is always greater than $T_2$. Spin-locking experiments can also be performed with off-resonance pulses with lower RF pulse power as the magnetization follows and relaxes along the effective RF field, defined by the equation (7).

Another way to induce and quantify $T_{1\rho}$ relaxation is to use adiabatic RF pulse trains (Fig. 4) by varying the number of pulses [48]. Adiabatic RF pulses cover a broad range of resonance frequencies by utilizing time dependent frequency and amplitude modulation [49]. The spin-locking can also be achieved with adiabatic pulses as the
magnetization follows the effective field during the adiabatic sweep, assuming that the so-called adiabatic condition is fulfilled which requires that the effective field is much larger than the rate of change of its orientation. Hyperbolic secant (HS$n$) pulses are common types of adiabatic pulses for performing an adiabatic full passage pulse (AFP) (180° flip) or adiabatic half-passage (AHP) (90°) frequency sweeps. By changing the stretching factor $n$ (= 1, 4, 8 . . .), relaxation rates can also be altered for the dispersion measurement. The advantage of adiabatic pulses is that the RF power can be distributed sequentially in time while still covering a broad frequency range with a clean profile compared with hard pulses. Furthermore, they are relatively insensitive to $B_0$ or $B_1$ inhomogeneities [49].

**Fig 4.** Pulse sequences for adiabatic $T_{1\rho}$, adiabatic $T_{2\rho}$, RAFF and inversion-prepared MT methods.

The transverse relaxation component in RFR is called $T_{2\rho}$, which characterizes the relaxation of magnetization perpendicular to $B_{1SL}$ [50]. If the RF pulse is applied perpendicular to the magnetization, relaxation is dominated by the $T_{2\rho}$ time constant. In an adiabatic $T_{2\rho}$ measurement, the magnetization is flipped to the transverse plane with an AHP pulse which is followed by an AFP pulse train, similar to that in the adiabatic $T_{1\rho}$ experiment (Fig. 4). After the pulse train, an inverse AHP pulse brings the magnetization back to the $z$ axis.

Relaxation along a fictitious field (RAFF) is another RFR relaxation component that works in the doubly rotating frame or higher rank rotating frame [51–53]. The relaxation time $T_{RAFF}$ is different from the $T_{1\rho}$ or $T_{2\rho}$ constants. In the RAFF experiment, the
frequency swept sine and cosine amplitude and frequency modulated pulses violate the adiabatic condition generating a fictitious magnetic field. $T_{RAFF}$ relaxation occurs along the fictitious field that can be utilized for spin-locking. The magnetization follows the equation

$$M_{\pm} = M_{\pm 0}e^{-t/T_{RAFF}} - M_{SS}e^{-t/T_{RAFF}},$$

where $M_{\pm 0}$ are the initial magnetizations before the application of sine/cosine pulses (with and without inversion), and $M_{SS}$ is the steady-state magnetization [51].

### 3.2.4 Magnetization transfer

Magnetization transfer (MT) loosely means exchange of the magnetization between the free and bound water pools [41, 54]. With MT experiments, it is possible to study the interactions between the macromolecular and free water pools. Bound protons are associated with macromolecules and have very short transverse relaxation time. Free or more mobile water molecules have a much narrower resonance peak and a longer $T_2$ relaxation time. The bound water pool has a broader resonance peak and therefore it can be irradiated with an off-resonance RF pulse (off-resonance to the narrow free water peak). As a result, the saturated magnetization of the macromolecular spins will move into the free pool through exchange, reducing the free water signal. The strength, duration and frequency offset of the saturation RF pulse affect the amount of signal reduction. During an MT experiment, the free water pool also experiences some saturation, which has to be taken into account in the analysis of MT effects. A common way to quantify the MT effect is to calculate the magnetization transfer ratio ($MTR$)

$$MTR = 1 - \frac{M_{sat}}{M_0},$$

where $M_{sat}$ and $M_0$ are the magnetizations of the mobile protons with and without saturation, respectively. Another possible way to quantify the MT effect is to measure $T_1$ during off-resonance saturation ($T_{1sat}$) (Fig. 4) [55]. The magnetization follows the behavior described in equation (18) [55]. In conventional MT experiments, the steady state is difficult to achieve as the duration of the saturation pulse is typically limited due to RF energy or pulse duration limits, causing instability to signal fitting. With an inversion preparation, the dynamic range of the MT measurement and signal fitting can be improved.
3.3 MRI pulse sequences

For imaging purposes, the NMR signal must be localized using linearly changing magnetic field gradients [41]. Frequency and phase encoding, as well as slice selection gradients are used for mapping of spatial frequencies of the image and the data is gathered into the k-space, which is the raw MRI data matrix. Fourier transform of the k-space produces the final magnetic resonance image. Before spatial localization, the magnetization can be prepared and set to carry e.g. $T_1$, $T_2$, $T_1\rho$, or other weighting (Fig. 4). The final image can be encoded (readout) with a basic sequence such as SE or GRE. However, they are not generally feasible in their simplest form because of too long acquisition times. Fast spin echo (FSE) or turbo FLASH (fast low angle shot, (GRE)) sequences allow collection of multiple echoes within a single TR period by applying evenly spaced $180^\circ$ or gradient pulses that form an echo train and drastically speed up the acquisition. With two refocusing pulses, the acquisition is named dual or double echo (DE) sequence (Fig. 3).

3.3.1 Ultrashort echo time sequences

UTE MRI techniques are able to capture signal from very short $T_2/T_2^*$ components [18, 56, 57]. In conventional MRI, typical echo times are a few milliseconds whereas in UTE, the echo times are in the range of 10 $\mu$s. For imaging of short $T_2$ species with bound water protons, such as deep regions of cartilage and subchondral bone, an echo time of a few milliseconds is already enough for almost all of the signal to disappear due to fast $T_2$ relaxation, making signal detection impossible. Although the sequence family is termed UTE here, UTE also refers to particular ‘UTE’ sequences.

In the UTE sequence, the signal acquisition starts after the excitation RF pulse, during ramping up of the gradients (Fig. 5). TE is defined as the time between the midpoint of the RF pulse and the start of the acquisition and is limited only by the ability of the MRI device to switch between transmit and receive modes. In addition, the length of the RF pulse is a trade-off between bandwidth (BW) and RF power, as shorter pulses require a higher power to maintain the same bandwidth. Before UTE data acquisition, preparation pulses can be embedded into the sequence. Long inversion pulses may be used for nulling of the long $T_2$ components, for example fat or free water signals. UTE generally utilizes radial acquisition although spiral trajectories for k-space sampling have been introduced [58]. For reconstruction of non-Cartesian data, the
k-space data is typically converted into a Cartesian grid and reconstructed by 2D or 3D Fourier transformation (FT).

Zero echo time (ZTE) is another technique in the UTE family in which readout gradients are already ramped up before the RF excitation [59, 60]. A short, high BW rectangular pulse is immediately followed by data acquisition (Fig. 5) resulting in almost zero TE. In ZTE experiments, gradients are stepped gradually instead of ramping them up and down, which produces silent scans with short TR. The short delay, a dead time between excitation and acquisition is mostly caused by the ability of the hardware to switch between transmit and receive modes. This delay, causing an undersampling of the center of the k-space has to be taken into account in the image reconstruction by acquisition oversampling [59].

SWIFT is a 3D sequence that allows a nearly zero TE, of a few μs, due to interleaved excitation and signal acquisition [20, 21, 61, 62]. The pulse sequence of the SWIFT contains repeated broadband frequency sweep HSₙ pulses, which are divided into short segments when the RF power is on (Fig. 5). The FID signal is collected between the excitation pulse segments. Similar to ZTE, the signal acquisition is done in the presence of a frequency encoding gradient, which is stepped in small increments after each HSₙ pulse. Each gradient step collects a frequency encoded radial projection (also called view or spoke), from different angle in a spherical 3D volume. The acquisition points are typically distributed on a spiral trajectory in the k-space. SWIFT images can be reconstructed using a 3D back-projection algorithm or Cartesian gridding of the
k-space data and subsequent 3D Fourier transform [63]. Due to the stepped nature of the gradients, SWIFT is also a silent sequence [20].

Before the final image reconstruction, the collected FID signals have to be processed with the so-called cross-correlation method due to their contamination with the frequency components of the transmit RF pulse [20, 21, 61, 62]. The measured response \( r(t) \) of the spin system to the RF pulse \( x(t) \) is considered as linear and they are related through convolution of the unit impulse response \( h(t) \) (i.e. the true FID) and \( x(t) \). Convolution in the time domain corresponds to a complex multiplication in the frequency domain of the Fourier transformed signals:

\[
R(\omega) = H(\omega)X(\omega),
\]

The frequency spectrum of the system \( H(\omega) \) is obtained using correlation, which can be performed in the frequency domain:

\[
H(\omega) = \frac{R(\omega)X^*(\omega)}{|X(\omega)|^2},
\]

where \( |X(\omega)| \) is the modulus of \( X(\omega) \). As a result, all contaminant signals are in the imaginary part of \( H(\omega) \) and the real part of \( H(\omega) \) corresponds to the spin density profile \( M_0 \) of the system. The signal intensity of SWIFT depends only on \( T_1 \) and the proton density as

\[
M = M_0 \frac{1 - e^{-TR/T_1}}{1 - e^{-TR/T_1} \cos(\theta)} \sin(\theta),
\]

where \( \theta \) is the nominal flip angle [20]. Thus, \( T_1 \) can be measured with SWIFT by varying the flip angle [64, 65]. The maximum signal intensity with the chosen TR is obtained with an optimum flip angle (so-called Ernst angle) of \( \theta_{\text{opt}} \):

\[
\cos(\theta_{\text{opt}}) = e^{-TR/T_1},
\]

If the flip angle is much lower than the optimum flip angle, the \( T_1 \)-weighting becomes negligible and the image contrast depends mainly on the proton density \( M_0 \) [20].

Fat and water signals can also be selectively suppressed with SWIFT by embedding saturation pulses between excitation pulses at a selected resonance at appropriate intervals [66]. SWIFT acquisition can be also modified to carry \( T_{1P} \) or RAFF weighting [67, 68].
3.4 Quantitative MRI of articular cartilage

MRI provides means for non-invasive assessment of articular cartilage. Several different quantitative MRI methods have been proposed and studied in cartilage for assessment of cartilage constituents and degeneration [13–17]. In addition, semi-quantitative scoring systems have also been developed for the evaluation of the status of the joints [13]. The scoring systems are based on the evaluation of morphologic changes of cartilage, such as depth of lesions, thickness and volume of cartilage. Morphological evaluation is usually based on gradient echo or double echo steady state (DESS) imaging [13]. However, the volume and the thickness of cartilage varies between individuals and morphologic changes are not always evident in the early stages of OA. Thus, quantitative MRI of cartilage is potentially more sensitive to early OA changes than semi-quantitative MRI evaluation.

\[ T_2 \] relaxation time mapping is one of the first quantitative MRI methods applied for the assessment of cartilage. The structure and integrity of the collagen fibril network and tissue water content are the main components affecting \( T_2 \) relaxation time in the cartilage [69–83]. In the normal articular cartilage, \( T_2 \) maps or \( T_2 \)-weighted images demonstrate a laminar appearance, corresponding to different histological cartilage zones [69–74, 77, 79]. The laminar appearance is related to the dipolar coupling of the spins associated with the collagen fibrils, which reduces the signal intensity and has the following orientation dependence, also known as the magic angle effect:

\[
\frac{1}{T_2} \propto 3\cos^2(\theta) - 1, \tag{24}
\]

where \( \theta \) is the angle between \( B_0 \) and the vector joining the two nuclei [84]. The signal intensity is greatest at the minima of this term, namely at 54.7°, the ‘magic angle’. The effect is also seen in other highly organized joint structures like menisci and tendons. \( T_2 \) elevation due to cartilage degeneration, associated with increased tissue water content and disorganization of the collagen network has been reported in numerous studies \textit{in vitro} and \textit{in vivo} [75, 78, 81–83, 85–87].

Delayed gadolinium-enhanced MRI of cartilage (dGEMRIC) is a technique that uses gadolinium-based anionic contrast agent (Gd-DTPA\(^2^-\)) for the assessment of negatively charged GAGs or the FCD of cartilage. Several studies have validated and shown strong associations of the dGEMRIC method with the PG content of cartilage, or with cartilage degeneration [80, 86, 88–97]. The accumulation of the contrast agent is increased in the
regions where GAG content has decreased, reducing also the $T_1$ relaxation time. Thus, there is an inverse relationship between the concentration of the contrast agent and the GAG content of the cartilage, which can be assessed by $T_1$ relaxation time mapping. Estimation of the concentration of the contrast agent requires measurement of $T_1$ in the absence of gadolinium, or $T_1$ can also be assumed to be constant [98]. In vivo dGEMRIC measurement requires intravenous or intra-articular injection of the gadolinium followed by delay to allow the contrast agent to diffuse into the cartilage [99].

Pre-contrast $T_1$ relaxation time has not shown orientational dependence in cartilage [70, 100]. $T_1$ relaxation time profiles have shown decreasing trends from the surface to the deep cartilage, similar to the distribution of water in the cartilage [86, 101]. Indeed, a strong correlation has been found between $T_1$ and the water content of cartilage [101]. $T_1$ has also shown significant elevation with cartilage degeneration [86].

The MT effect in cartilage is dominated by the collagen macromolecules [102–105], although the PGs may also provide some contributions [102]. It has been reported that the MTR parameter is not sensitive enough for monitoring changes in damaged cartilage after repair procedures [106]. However, $T_{1sat}$ relaxation time measured with the inversion prepared MT protocol has demonstrated sensitivity to trypsin-induced PG depletion in bovine cartilage [107]. Chemical exchange saturation transfer (CEST), a specific type of MT technique, exploits the dependence between the frequency of the off-resonance saturation and the extent of the MT effect [108]. Certain frequencies are associated with specific molecules such as GAG, which contain exchangeable protons. The GAG content of articular cartilage has been successfully investigated with the CEST (gagCEST) method [109–111].

$T_{1p}$ has been proposed as a more sensitive biomarker for early cartilage degeneration than conventional $T_1$ or $T_2$ mapping due to the high sensitivity of RFR relaxation to slow macromolecular motion. Studies with CW-$T_{1p}$ have associated the elevation of $T_{1p}$ with cartilage degeneration, specifically to GAG loss and changes in FCD of cartilage [112–117, 117–128]. However, CW-$T_{1p}$ has also been shown to be dependent on the magic angle effect (less than $T_2$), suggesting some contribution of the collagen network to the relaxation [129–131]. Furthermore, $T_{1p}$ has been found to be more sensitive than $T_2$ in revealing cartilage degeneration [124, 132–134]. CW-$T_{1p}$ measurements in vivo are typically performed at spin-lock frequencies around 500 Hz due to RF power (specific absorption ratio, SAR) limitations. $T_{1p}$ dispersion studies in cartilage have demonstrated the laminar appearance in $T_{1p}$ weighted images or relaxation time maps at low spin-lock frequencies when $T_{1p}$ approximates $T_2$, but with reduced effect with
increasing frequency [79, 115]. There are potentially several relaxation mechanisms that contribute simultaneously to $T_{1\rho}$ relaxation in cartilage, including dipolar interaction and chemical exchange between protons on the GAG side chains and extracellular water. However, the relative contributions of these relaxation mechanisms are not yet fully understood [45, 79].

Adiabatic $T_{1\rho}$, adiabatic $T_{2\rho}$ and RAFF methods have not yet been widely applied in cartilage research. The advantage of the methods relates to the relaxed RF power requirements compared to CW type SL methods [49, 51]. Furthermore, adiabatic pulses are more insensitive to $B_0$ or $B_1$ field variations [49]. In addition, adiabatic $T_{1\rho}$ has been shown to be more insensitive to sample orientation than CW-$T_{1\rho}$ [130]. In the initial study, adiabatic $T_{1\rho}$ and RAFF were sensitive to trypsin-induced degeneration in bovine articular cartilage [107]. Although studies on adiabatic $T_{2\rho}$ in cartilage are lacking, its potential as a biomarker has been demonstrated in other tissues, for example in the assessment of brain pathologies [135]. Theoretical descriptions of adiabatic $T_{2\rho}$ and RAFF relaxation have shown evidence of dependence on both dipolar and exchange-related relaxation channels, although the relative contributions depend on the tissue investigated [48, 50, 51].

In addition to the quantitative methods listed above, diffusion weighted imaging (DWI) techniques have also been applied for the assessment of articular cartilage [103, 136–140]. DWI utilizes diffusion gradients in the pulse sequence, which causes a non-refocusing phase accumulation in diffusing spins. By varying the gradient strength, an apparent diffusion coefficient (ADC) can be determined. ADC has been suggested as indicator for early cartilage degeneration [103]. Apart from $^1$H imaging, sodium ($^{23}$Na) MRI is a potential method for quantitative assessment of cartilage as sodium ions are associated with the GAG molecules of PGs, nearly directly related to the FCD of cartilage. The method has been validated to show differences in the GAG content, however, $^{23}$Na MRI has limitations due to a poor signal-to-noise ratio (SNR) and special hardware requirements [45, 141–143].

3.5 MRI of subchondral bone

Bone has much lower proton density in comparison with cartilage and due to the calcified nature of the tissue, it has a very short $T_2$ relaxation time, at a few hundred $\mu$s [19, 144]. Consequently, direct imaging of bone with conventional methods is extremely difficult as the majority of the signal decays before data acquisition is possible. Free or
relatively mobile water protons are located in the small pores such as the Haversian
canals of the cortical bone whereas bound protons are associated with collagen [19, 144].
Thus, bone contains different proton pools that affect its $T_2$ relaxation time.

MRI techniques capable of imaging extremely short $T_2$s such as UTE, ZTE and
SWIFT enable the direct assessment of bone tissue properties [56, 145–148]. The
NMR signal of the cortical bone has been shown to correlate with the mechanical
properties of the bone [149]. In addition, the $T_2^*$ relaxation time of the cortical bone
has been quantified using UTE which allows echo times down to approximately 8 $\mu$s
[145, 150]. Furthermore, UTE has been used for quantification of the cortical bone
water concentration and the ratio of long and short $T_2$ image signal intensities, which
have been correlated with bone porosity and bone mineral density, respectively [151].

Traditionally the trabecular bone microstructure has been assessed indirectly by
imaging the fatty bone marrow, which has much higher proton density than bone. In
previous studies, strong linear correlations between high-resolution MRI and micro-
computed tomography (micro-CT) have been reported [81, 152–156]. High resolution
ZTE imaging has demonstrated imaging of bone microstructure with high correspon-
dence to histology [147]. SWIFT imaging has been initially applied for bone and dental
imaging [20, 66, 148, 157]. SWIFT allowed depiction of tumor invasion into cortical
and trabecular bone while using water and fat suppression schemes [152].

UTE studies of the osteochondral junction have shown a high intensity signal
band in the cartilage-bone interface which likely originates from the zone of calcified
cartilage and the deepest regions of uncalcified cartilage [57, 158–162]. The dual
inversion recovery UTE sequence allows acquisition of images with and without long $T_2$
suppression, which difference (subtraction) enhances short $T_2$ components in the image
[159, 160].
4 Aims of the thesis

The specific aims of the study were:

1. To investigate the sensitivity of novel quantitative MRI relaxation time parameters for assessment of cartilage degeneration in a rabbit model of early cartilage degeneration
2. To evaluate cartilage degeneration in osteoarthritic human osteochondral specimens with novel quantitative MRI techniques
3. To investigate the feasibility of the SWIFT MRI technique for the assessment of spontaneous repair of osteochondral lesions in an equine model
4. To evaluate the cartilage–bone interface in osteoarthritic human specimens with the SWIFT MRI technique
5 Materials and methods

This thesis consists of three independent studies (I-III) and unpublished data. All materials and methods used in the studies are summarized in Table 1.

Table 1. The summary of materials and methods in studies I-III and in unpublished data.

<table>
<thead>
<tr>
<th>Study</th>
<th>Material</th>
<th>MRI parameters</th>
<th>Reference methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Rabbit femoral condyles, ACLT ( n = 8 ), contralateral joints ( n = 8 )</td>
<td>( T_{1p, ad} ), ( T_{2p, ad} ), ( T_{RAFF} ), ( T_2 ) (DE &amp; CPMG), ( T_{sat} )</td>
<td>Histology (Safranin-O staining, DD, PLM, Mankin grading)</td>
</tr>
<tr>
<td>II</td>
<td>Human osteochondral samples (tibia), early OA ( n = 5 ), advanced OA ( n = 9 )</td>
<td>( T_{1p, ad} ), ( T_{2p, ad} ), ( T_{RAFF} ), ( T_2 ) (DE and SE), ( T_{sat} ), ( T_1 ), ( T_{1Gd} )</td>
<td>Histology (Safranin-O staining, DD, PLM, OARSI grading), FTIRI</td>
</tr>
<tr>
<td>III</td>
<td>Equine osteochondral ( n = 5 ), chondral ( n = 5 ) lesions</td>
<td>SWIFT (with fat and water suppression schemes), FSE, gradient echo sequence</td>
<td>Micro-CT, histology (Safranin-O staining)</td>
</tr>
<tr>
<td></td>
<td>Unpublished human osteochondral samples (tibia), ( n = 16 )</td>
<td>SWIFT (with fat and water suppression and 3 kHz off-resonance saturation schemes)</td>
<td>Micro-CT, histology (Mason’s trichrome and Safranin-O stainings, OARSI grading)</td>
</tr>
</tbody>
</table>

5.1 Materials

In studies I-III and in unpublished data, three different sample sets and species (rabbit, human and equine) were used. The following sections include detailed description of the materials and preparation procedures.
5.1.1 **Rabbit samples**

In study I, unilateral anterior cruciate ligament transection (ACLT) was performed to the left knee of skeletally mature New Zealand White rabbits (*Oryctolagus cuniculus*, age 14 months, *n* = 8). The right knees were used as a contralateral (CTRL) control group. Four weeks after the ACLT, the knee joint were harvested and frozen for storage at -20°C. In the sample preparation, the joints were first thawed to room temperature in phosphate buffered saline (PBS) which was followed by removal of the distal ends of the femurs including the femoral condyles of the joints. The animal experiments were carried out according to the guidelines of the Canadian Council on Animal Care and were approved by the committee on Animal Ethics at the University of Calgary.

5.1.2 **Human samples**

In study II, human osteochondral plugs of 6 mm in diameter (*n* = 14) were collected from the tibial plateaus of TKA patients. The samples were stored at -20°C. The experiments were approved by the Ethical Committee of the Northern Ostrobothnia Hospital District, Oulu, Finland (191/2000). The same samples were used in the unpublished dataset which included two additional specimens (*n* = 16).

5.1.3 **Equine samples**

In study III, chondral (*n* = 5) and osteochondral lesions (*n* = 5) of 6 mm in diameter were surgically created on the fourth carpal bone of the intercarpal joints of 2-year-old horses (*n* = 7). Osteochondral lesions penetrated to a depth of 3–4 mm in the subchondral bone while chondral lesions were restricted to the articular cartilage. The animals were sacrificed after spontaneous healing of the lesions for 12 months. Osteochondral plugs of 14 mm in diameter containing the lesion and adjacent tissue were collected, marked with a landmark cut followed by storage at -20°C. The procedures were approved by the Utrecht University Animal Ethics Committee.

5.2 **MRI measurements**

All MR studies were conducted in a 9.4 T vertical bore magnet (Oxford 400 NMR, Oxford Instruments, Witney, England) and by using Varian DirectDrive (Varian, Palo
Alto, California, USA) console (VnmrJ 3.1A). In all imaging experiments, a 19-mm quadrature RF volume transceiver (RAPID Biomedical, Rimpar, Germany) was used. The samples were placed inside a Teflon test tube filled with perfluoropolyether to minimize susceptibility effects from the tissue–air interface and for a $^1$H signal free background. In studies I and II, a magnetization preparation block was coupled to an FSE readout ($TR = 5$ s, $TE_{eff} = 5$ ms, echo train length = 4, $256 \times 128$ matrix size, 62.5 $\mu$m depth-wise resolution, FOV $16 \times 16$ mm, 1-mm slice thickness). In study I, slices (two slices in total) were positioned to the lateral and medial femoral condyles and the readout was modified accordingly to multi-slice FSE imaging acquiring both of the slices immediately after the preparation pulses. Slices were positioned at the sites of biomechanical testing, in study I to the load-bearing cartilage region at the apexes of the femoral condyles. The resolution over the depth of the cartilage in each slice was 62.5 $\mu$m for all relaxation parameters. The orientation of the samples was controlled by placing the cartilage surface of the samples parallel to $B_0$ in study I and, perpendicular to $B_0$ in studies II and III.

5.2.1 $T_1$ measurements

In study II, the dGEMRIC technique was applied for the assessment of cartilage degeneration. Pre-contrast $T_1$-mapping was performed before immersion of the specimens into 1 mM Gd-DTPA$^{2-}$ (Magnevist$^\text{TM}$, Bayer Healthcare, Montville, NJ) with a saturation recovery FSE sequence ($TE = 10$ ms, $TR = 80, 160, 320, 640, 1280, 2560, 5120$ ms). Post-contrast $T_1$-maps ($T_1$Gd) were acquired at 24 hours after equilibration.

5.2.2 $T_2$ measurements

In studies I and II, $T_2$-mapping was performed with an adiabatic double echo technique (DE-$T_2$). Two HS1-AFP pulses were placed between AHP pulses ($TE = 4, 8, 16, 32, 64, 128$ ms, $TR = 5$ s). For comparison of different $T_2$ mapping methods, $T_2$ relaxation time was also measured with CPMG sequence (CPMG-$T_2$, train of hard 180° pulses, pulse duration = 115 $\mu$s, inter-pulse delay = 1 ms) in study I, and with spin echo preparation (SE-$T_2$) in study II ($TE = 4, 8, 16, 32, 64, 128$ ms, $TR = 5$ s in both studies).

In study III, the FSE sequence ($TE = 3.5$ ms; $TR = 2.5$ s, $19 \times 19$ mm$^2$ FOV, $256 \times 128$ matrix size, 75 $\mu$m depth-wise resolution) was used for acquiring a single 1-mm thick slice covering the lesion site of the equine specimens.
5.2.3 Rotating frame relaxation measurements

In studies I and II, adiabatic $T_1_ρ$ was measured with a train of 0, 4, 8, 12 and 24 HS1 AFP pulses (pulse duration = 4.5 ms, $γB_{1,max} = 2.5$ kHz) [163]. Measurement of adiabatic $T_2_ρ$ was performed by embedding the same AFP pulse train as in adiabatic $T_1_ρ$ experiment between AHP pulses (pulse duration = 4.5 ms, $γB_{1,max} = 2.5$ kHz) [50]. $T_{RAFF}$ was acquired with a train of 0, 2, 4 and 6 sine/cosine modulated RAFF pulses (pulse duration = 9 ms, $γB_{1,max} = 625$ Hz) [51].

In study I, on-resonance CW-$T_1_ρ$ was measured using hard CW spin-lock pulses with durations of 0, 10, 20, 40, 80 and 160 ms ($γB_1 = 1$ kHz) embedded between HS1 AHP pulses [164]. $T_1_ρ$ dispersion was investigated in study II with similar CW pulse setup at spin-lock frequencies of 125, 250, 500 and 1000 Hz.

5.2.4 Magnetization transfer measurements

As an MT experiment, $T_{1, sat}$-mapping was performed in studies I and II. $T_{1, sat}$ relaxation time was measured using 10 kHz off-resonance CW hard pulse irradiation ($γB_1 = 250$ Hz) with durations of 0.1, 0.3, 0.8, 2.0 and 4.0 s (study I), both with and without an inversion preparation to increase the dynamic range [55]. In study II, the CW irradiation pulse durations were 0.1, 0.2, 0.4, 0.8, 1.6, 3.5 and 7.0 s.

5.2.5 Gradient echo imaging

In study III, a 2D gradient echo sequence ($TE = 1.33$ ms, $TR = 15$ ms, 20° flip angle, 30×30 mm² FOV, 256×256 matrix size, 117 μm in-plane resolution, imaging time 30 seconds) was used for acquiring a single 1-mm thick slice covering the lesion site of the equine specimens.

5.2.6 SWIFT

In study III, SWIFT MR imaging was performed with 144 000 views, 35³ mm³ FOV, $BW = 62.5$ kHz, 256 complex points, 137 μm isotropic resolution and a nominal flip angle of approximately 5°, which was optimized for each sample. Fat saturation was implemented by placing an HS4 inversion pulse ($BW = 1$ kHz) centered on the fat resonance frequency after every 16 views [66]. Suppression of the water spins was done
similarly at the water resonance frequency. In addition, images without saturation were acquired with identical timing parameters. The imaging time was approximately 40 minutes per SWIFT dataset.

The samples of study II including 2 additional samples were also imaged with SWIFT MRI (unpublished data). SWIFT imaging was performed with 96000 views, FOV $40^3$ mm$^3$, $BW = 62.5$ kHz, 384 complex points, 104 $\mu$m isotropic resolution and a flip angle of approximately 5-7°. SWIFT images without suppression schemes and, with fat and water saturation were obtained similarly as described for study III. Furthermore, to suppress short $T_2$ spins, saturation pulses were centered 3 kHz off-resonance to the water resonance frequency. The scan time was approximately 30 minutes per single SWIFT dataset.

The SWIFT images were reconstructed using custom-made LabVIEW software [63]. For each suppression scheme, image acquisition was performed at the water resonance frequency. During image reconstruction, water suppression images (fat images) were adjusted for correct frequency shifts to focus the signal of fat.

5.3 Reference methods

5.3.1 Micro-computed tomography

In study III, a high-resolution micro-computed tomography (micro-CT) desktop scanner (Skyscan 1172, Bruker microCT, Kontich, Belgium) was used for the assessment of the microstructure of the subchondral bone in equine specimens. Datasets with 20 $\mu$m isotropic resolution were acquired using 100 kV tube voltage. Similarly, the human specimens ($n = 16$) were imaged with the micro-CT at 28 $\mu$m resolution (unpublished data). The micro-CT images were reconstructed using NRecon software (Bruker microCT).

5.3.2 Biomechanical testing

In studies I-II, the biomechanical properties of cartilage were determined using indentation testing with a plane-ended indenter of 1 mm in diameter. Before the mechanical testing, the thickness of the cartilage was measured with a high-resolution ultra-sound system [165]. Equilibrium elastic modulus ($E_{eq}$) was obtained from stress-relaxation tests (3 x 5% step in study I, 4 x 5% step in study II, 15 min relaxation after each step).
Dynamic moduli \( (E_{\text{dyn}}) \) were determined from sinusoidal loading tests (1 Hz frequency, amplitudes of 4% and 1% of cartilage thickness in studies I and II, respectively).

### 5.3.3 Microscopic techniques

In studies I and II, anisotropy of the collagen fibrils and PG content of articular cartilage were determined with polarized light microscopy (PLM) [166] and digital densitometry (DD) [167, 168], respectively. Microscopic sections were obtained at the sites of MR imaging and biomechanical testing (four sections/condyle in study I and three sections/sample in study II). Unstained 5 µm thick sections were used in PLM measurement to obtain the parallelism index (PI), an indicator for collagen fibril anisotropy. PI was determined by measuring the signal intensity of the light at seven different polarizer settings at 15° steps (0-90°). The signal intensity change follows a sinusoidal function and using least square fitting, maximum and minimum signal intensities can be determined from the equation. PI is determined as

\[
PI = \frac{I_{\text{max}} - I_{\text{min}}}{I_{\text{max}} + I_{\text{min}}},
\]

where \( I_{\text{max}} \) and \( I_{\text{min}} \) are maximum and minimum signal intensities, respectively. A PI value of 0 indicates random arrangement while PI of 1 indicates fibrils running parallel to each other.

For DD, 3 µm thick microscopic sections were stained with Safranin-O, which is a cationic dye that binds stoichiometrically to negatively charged GAG side chains of the PGs [169]. According to the Beer-Lambert law, optical density (OD) or absorbance (A) is linearly proportional to the concentration of the absorbing molecule

\[
A = -\ln \frac{I}{I_0} = \varepsilon cl,
\]

where \( I \) and \( I_0 \) are the intensities of the light transmitted through and entering the sample, respectively, \( \varepsilon \) is the molecular absorption coefficient, \( c \) is the concentration of the absorbing molecule and \( l \) is the thickness of the sample. Thus, the amount of Safranin-O staining is linearly proportional to the PG content of articular cartilage.

In study II, Fourier transform infrared imaging (FTIRI) was used for quantification of collagen and PG contents of the cartilage. In infrared (IR) spectroscopy, a spectrum of IR light is directed through a sample which is absorbed by chemical bonds in molecules at
specific wavelengths. Therefore, the IR absorption is related to the chemical composition of a sample.

The human samples were imaged using Hyperion 3000 FTIR Microscope (Bruker Inc, Germany). The spectral resolution and pixel size were 4 cm$^{-1}$ and 25 $\mu$m, respectively. The spectral region of 950–1720 cm$^{-1}$ was analyzed and an offset baseline correction was performed for all spectra prior to analysis. Collagen and PG contents were estimated by integrating over the area of the amide I absorbance peak (1585–1720 cm$^{-1}$) and the carbohydrate region (985–1140 cm$^{-1}$), respectively [170, 171].

5.3.4 Histological evaluations

In studies I and II, Safranin-O stained microscopic sections were histopathologically graded with Mankin [172] and Osteoarthritis Research Society International (OARSI) grading [173] systems, respectively. In Manking grading, the following criteria are evaluated: cartilage structure (0–6), cell population (0–3), distribution of Safranin-O stain (0–4) and tidemark integrity (0–1), with zero indicating normal appearance. The scale in OARSI grading is 0–6, which indicates the depth of progression of osteoarthritic changes in the cartilage. Grade 0 represents intact normal cartilage and grade 6 complete loss of articular cartilage with bone deformation. In both studies, grading was performed by three independent observers and grades were averaged.

5.3.5 Morphologic assessment of cartilage repair

In study III, the specimens underwent overall qualitative morphologic assessment using specific criteria for articular cartilage, subchondral bone and cartilage-bone interface. The assessment of the MRI images included evaluation of the integrity of articular cartilaginous surfaces as well as the signal intensity within the cartilage and the repair tissue. Furthermore, the areas of chondral and osteochondral lesions were investigated to evaluate the degree of repair outcome, ranging from completely filled defect with cartilage repair tissue to no repair tissue with exposed subchondral bone. In addition, the continuity and/or discontinuity between the native and repair tissue was assessed at the cartilage-bone interface. In the subchondral bone, sclerosis, integrity and bone marrow edema were evaluated at the lesion sites and the adjacent native tissue.
5.3.6 **Biochemical analyses**

In study II, the total water content of cartilage was determined biochemically. First, the articular cartilage was detached from the subchondral bone, followed by measurement of the wet weight (WW) of the cartilage. The measurement was repeated three times and averaged. Finally, specimens were freeze-dried, re-weighted (DW) and the water content was determined as [165]

\[
\left(1 - \frac{DW}{WW}\right) \times 100\%.
\]  

(27)

5.4 **Data-analysis**

For further analysis of the results, the specimens in different studies were divided into groups using the following criteria. In study I, samples were divided into ACLT \( n = 8 \) and CTRL \( n = 8 \) groups. In addition, medial and lateral condyles were also treated as separate groups. In study II, the samples were divided into two groups according to OARSI grading with grade = 1.5 as cut-off point [174]. The samples with an OARSI grade less than 1.5 were considered as the early OA group \( n = 5 \) and samples with an OARSI grade higher than 1.5 as the advanced OA group \( n = 9 \). In study III, specimens with chondral \( n = 5 \) and osteochondral lesion \( n = 5 \) were considered as separate groups.

5.4.1 **Relaxation time and microscopic ROI analyses**

MRI relaxation time maps were calculated using mono-exponential two-parameter fitting on a pixel-by-pixel basis for \( T_1, T_2, CW-T_{1p}, T_{1p, adiab}, \) and \( T_{2p, adiab} \) measurements. For \( T_{RAFF} \) and \( T_{1 sat} \) (inversion-prepared MT) maps, a three-parameter mono-exponential fitting with steady-state was applied [51, 55] (equation (18)). In studies I and II, 3 mm wide full-thickness regions of interest (ROI) were selected from cartilage at the site of biomechanical testing for determination of mean ± standard deviation (SD) values for each relaxation time parameter. Furthermore, in study I the full-thickness cartilage ROI was split into superficial and deep ROIs for zonal analysis. In study II, full-thickness ROIs were used for the determination of mean depth-wise profiles of relaxation times in cartilage, which were interpolated to a normalized length of 10 points due to the variable thickness of the cartilage. The thicknesses of the full-thickness ROIs were
compared against the thicknesses derived from microscopy images to avoid inclusion of non-cartilage material in the ROI. Similar depth-wise profiles were calculated for the PLM, DD and FTIRI measurements. All data and ROI analysis was performed using Aedes software (http://aedes.uef.fi) and custom routines written in MATLAB (MATLAB R2010a, Math-Works, Natick, MA).

5.4.2 Assessment of short $T_2$ spins

To evaluate the cartilage–bone interface, images of short $T_2$ components were obtained by subtracting SWIFT images with and without 3 kHz off-resonance suppression pulses (Fig 6). By subtracting SWIFT images with and without water suppression (fat blurred due to chemical shift), both long and short $T_2$ components are visualized, while the signal of fat is subtracted in the process. Combination of fat and water suppression (fat in focus) SWIFT images results in a lack of short $T_2$ components but both fat and water signals are in focus in the image.
Fig 6. The summary of signal components in different SWIFT images with or without signal suppression schemes. The images can be further subtracted or combined to obtain images of the short $T_2$ component or to improve contrast between subchondral bone and marrow.

5.4.3 Estimation of structural bone parameters

In addition to qualitative assessment of the equine specimens in study III, structural bone parameters were determined from SWIFT and micro-CT data using CTAn software (Skyscan). Bone volume-to-tissue volume ratio (BV/TV), trabecular thickness (Tb.Th), trabecular separation (Tb.Sp) and trabecular number (Tb.N) parameters were calculated from cylindrical volumes of interest (VOI) ($2 \times 3 \text{ mm}^3$) that were placed in the trabecular bone of the lesion site and adjacent bone, in the same locations for SWIFT and micro-CT data. Prior to the analysis, the resolution of SWIFT and micro-CT images was matched by downsampling the micro-CT data to 137 $\mu\text{m}$ resolution. Furthermore, the orientations of the datasets were co-registered using axial rotation with the help of surgical landmarks.
To improve contrast between marrow and bone, combination images of fat and water saturated SWIFT data were calculated (Fig 6). In addition, the gray scale values in the SWIFT data were inverted to obtain similar contrast compared to micro-CT images. Datasets were binarized with the global thresholding method [175], with both modalities having their own separate values (Fig 7).

5.4.4 Assessment of signal-to-noise ratio

In study III, signal-to-noise ratio was determined for FSE, GRE and SWIFT images (without saturation, fat and water saturation data). Orientation parameters of the FSE sequence were used to calculate matching slices from the SWIFT data co-registered with the micro-CT data. Also the field of view of SWIFT and GRE sections was matched to FSE data. SNR was defined as the ratio of mean signal of tissue ROI to the SD of the background ROI. Signal ROIs were determined for articular cartilage, subchondral bone plate and trabecular bone. For a comparison between the MRI sequences, SNRs were
corrected with the voxel size and square root of the imaging time. Furthermore, the values were normalized to SNR of cartilage in the FSE image.

### 5.4.5 Measurement of cartilage thickness

The thickness of articular cartilage in the equine specimens was determined from matched SWIFT (without saturation), FSE, GRE and Safranin-O stained sections (average of 9 slices). The thickness in the lesion site and adjacent intact cartilage was obtained as an average value from 2 mm wide ROIs that were manually segmented using a custom made Aedes plugin. Finally, group mean thickness values were determined for chondral and osteochondral lesion samples.

### 5.5 Statistical analysis

In study I, the non-parametric Kruskal-Wallis post-hoc test was used for investigating differences in MRI relaxation time and biomechanical parameters between femoral condyles in the ACLT and CTRL joints. Pearson’s linear correlation analysis was used for studying relationships between the MRI findings and biomechanical properties, PG content (optical density) and collagen fibril anisotropy (PI), by pooling the ACLT and CTRL groups. Full-thickness ROI values were used in the correlation analysis between MRI and biomechanical parameters and superficial ROIs between MRI and histology.

In study II, differences in the MR imaging parameters and reference methods between early and advanced OA groups were evaluated with the Mann-Whitney U-test. As in study I, Pearson’s linear correlation analysis was performed for pooled data between MRI relaxation time parameters (full-thickness ROI) and biomechanical parameters, water content of cartilage, optical density, PI, OARSI grade and FTIR-derived PG and collagen contents.

For studies I and II, relative changes (%) in MRI relaxation times between CTRL and ACLT, and between early OA and advanced OA groups were calculated as

\[
\frac{\text{mean}(\text{degenerated}) - \text{mean}(\text{reference})}{\text{mean}(\text{reference})} \times 100\%. \quad (28)
\]

Moreover, the ability of the MRI parameters to differentiate between the two groups was investigated using receiver operating characteristics (ROC) analysis. The area under
the curve (AUC) values (0–1) are an indicator of the sensitivity and specificity of a method, 1 indicating 100% sensitivity and specificity and 0.5 a random guess.

In study III, Pearson linear correlation coefficients between SWIFT and micro-CT derived structural bone parameters were calculated. Furthermore, Wilcoxon signed rank tests were performed to study differences between modalities in the thicknesses of articular cartilage.

All statistical analyses were performed with SPSS Statistics 19.0 (IBM, Armonk, NY) and MATLAB R2010a. Values of P < 0.05 were considered indicative of a statistically significant difference.
6 Results

6.1 Quantitative MRI of cartilage degeneration

6.1.1 Reference methods

In study I, ACLT resulted in significantly reduced PG content in the superficial part of the cartilage in the medial and lateral femoral condyles of the rabbit joints in comparison to the contralateral joints (Fig. 8). Full-thickness cartilage defects were not found in the ACLT joints. Collagen fibril anisotropy (PI) and $E_{eq}$ were also significantly reduced in the lateral compartment of the ACLT joint (Table 2). In addition, $E_{eq}$ and $E_{dyn}$ were also significantly reduced in the medial compartment of the ACLT joint. There was also a significant difference in Mankin grades in both condyles between ACLT and CTRL groups.

![Graphs showing changes in PG content and collagen fibril anisotropy in ACLT and CTRL joints.]

Fig 8. Normalized depth-wise profiles (0 indicates cartilage surface, 1 cartilage-bone interface) of PG content (optical density) and collagen fibril anisotropy (PI) of rabbit articular cartilage in lateral and medial femoral condyles in ACLT and CTRL group as determined by DD and PLM, respectively. Grey background indicates a statistically significant difference (Kruskal-Wallis test, $p < 0.05$)

In study II, OARSI grades of the human cartilage specimens ranged from 0.9 to 1.5 and 1.6 to 4.1 in the early and advanced OA groups, respectively (Table 2). The relative
Table 2. Mean values ± SD of biomechanical parameters histopathological grades for rabbit and human tissue specimens in different groups. Mankin and OARSI grading systems were used for rabbit and human specimens, respectively.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Rabbit medial condyle (n = 8)</th>
<th>Rabbit medial condyle (n = 8)</th>
<th>Rabbit lateral condyle (n = 8)</th>
<th>Rabbit lateral condyle (n = 8)</th>
<th>Human early OA (n = 5)</th>
<th>Human advanced OA (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E_{eq}$ (MPa)</td>
<td>1.3 ± 0.5</td>
<td>0.6 ± 0.4</td>
<td>1.1 ± 0.5</td>
<td>0.6 ± 0.4</td>
<td>1.2 ± 0.3</td>
<td>0.2 ± 0.3</td>
</tr>
<tr>
<td>$E_{dyn}$ (MPa)</td>
<td>3.5 ± 1.2</td>
<td>2.0 ± 0.6</td>
<td>3.4 ± 1.4</td>
<td>2.9 ± 1.3</td>
<td>6.8 ± 1.7</td>
<td>1.9 ± 2.3</td>
</tr>
<tr>
<td>Mankin grade</td>
<td>1.4 ± 1.1</td>
<td>4.8 ± 2.6</td>
<td>0.6 ± 1.0</td>
<td>4.5 ± 4.1</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>OARSI grade</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1.3 ± 0.3</td>
<td>3.2 ± 1.0</td>
</tr>
</tbody>
</table>

Kruskal-Wallis post-hoc test (rabbits), Mann-Whitney U-test (humans) $^1 p < 0.05$, $^2 p < 0.01$

Water content of cartilage showed an increasing trend (early OA 74.3 ± 2.5%, advanced OA 77.2 ± 4.6%), while $E_{eq}$ and $E_{dyn}$ were significantly reduced in the advanced OA group. There were no statistically significant differences in FTIRI-derived collagen or PG contents (amide I and carbohydrate absorptions, respectively) while DD-derived PG content (optical density) and collagen anisotropy were significantly lower in the deep cartilage of the advanced OA group in comparison with early OA group (Fig. 9).
Fig 9. Mean depth-wise normalized MRI relaxation time parameter ($T_{1\rho, \text{adiab}}$, $T_{2\rho, \text{adiab}}$, CW-$T_{1\rho}$ ($\gamma B_1 = 125 – 1000$ Hz), $T_{\text{RAFF}}$, DE-$T_2$, $T_1$, $T_{1\text{Gd}}$, $T_{1\text{sat}}$) and reference method (FTIR amide I and carbohydrate absorptions, DD (PG content), collagen anisotropy (PI)) profiles in human specimens in early and advanced OA groups. Depth value 0 indicates cartilage surface and grey background a statistically significant difference (Mann-Whitney U-test, $p < 0.05$)

6.1.2 Relaxation time measurements

In study I, there was a statistically significant increase in adiabatic $T_{1\rho}$ and adiabatic $T_{2\rho}$ relaxation time parameters in the superficial part of cartilage of both femoral condyles in the ACLT joints (Table 3). In the lateral compartment, CW-$T_{1\rho}$ and DE-$T_2$ were also significantly longer in the ACLT group.
Table 3. Mean MRI relaxation time ($T_1\rho$, $T_2\rho$, CW-$T_1\rho$ ($\gamma B_1 = 1$ kHz), $T_{RAFF}$, DE-$T_2$, CPMG-$T_2$, $T_{1sat}$) values $\pm$SD in the superficial cartilage ROI of rabbit femoral condyles in ACLT and CTRL groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Medial CTRL ($n = 8$)</th>
<th>Medial ACLT ($n = 8$)</th>
<th>Lateral CTRL ($n = 8$)</th>
<th>Lateral ACLT ($n = 8$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_{1p, adiab}$</td>
<td>79.9 ± 6.0</td>
<td>102.3 ± 32.5$^1$</td>
<td>75.3 ± 4.4</td>
<td>109.8 ± 61.5$^1$</td>
</tr>
<tr>
<td>$T_{2p, adiab}$</td>
<td>26.3 ± 2.9</td>
<td>37.4 ± 20.4$^1$</td>
<td>26.8 ± 2.5</td>
<td>44.9 ± 31.1$^1$</td>
</tr>
<tr>
<td>CW-$T_1\rho$, $\gamma B_1 = 1$ kHz</td>
<td>21.6 ± 8.2</td>
<td>33.2 ± 14.9</td>
<td>19.9 ± 5.2</td>
<td>31.7 ± 10.6$^1$</td>
</tr>
<tr>
<td>$T_{RAFF}$</td>
<td>18.5 ± 7.6</td>
<td>19.3 ± 4.5</td>
<td>15.7 ± 3.5</td>
<td>16.4 ± 3.6</td>
</tr>
<tr>
<td>DE-$T_2$</td>
<td>20.6 ± 3.2</td>
<td>26.2 ± 4.1</td>
<td>22.0 ± 3.8</td>
<td>36.4 ± 22.1$^1$</td>
</tr>
<tr>
<td>CPMG-$T_2$</td>
<td>29.9 ± 2.8</td>
<td>39.3 ± 21.0</td>
<td>30.6 ± 5.5</td>
<td>57.6 ± 50.5</td>
</tr>
<tr>
<td>$T_{1sat}$</td>
<td>338.6 ± 46.7</td>
<td>409.4 ± 103.1</td>
<td>345.6 ± 46.8</td>
<td>420.3 ± 175.4</td>
</tr>
</tbody>
</table>

Kruskal-Wallis post-hoc test $^1p < 0.05$

Fig 10. Representative MRI relaxation time maps of $T_1\rho$, $T_2\rho$, CW-$T_1\rho$ ($\gamma B_1 = 1$ kHz), $T_{RAFF}$, DE-$T_2$, CPMG-$T_2$, $T_{1sat}$ parameters of from rabbit lateral femoral condyle of ACLT and CTRL groups.

In full-thickness analysis of rabbit cartilage, adiabatic $T_1\rho$ and adiabatic $T_2\rho$ showed a statistically significant prolongation in both medial and lateral condyles in the ACLT group compared to the CTRL joints (Table 4). Moreover, DE-$T_2$ was significantly longer in the lateral compartment of the ACLT group. The prolongation in relaxation times is also evident in the representative relaxation time maps from the lateral femoral condyle (Fig. 10). There were no statistically significant differences in the deep cartilage. In CW-$T_1\rho$ and $T_{RAFF}$ relaxation time maps, banding artifacts were shown in cartilage that originate most likely due to $B_0$ field inhomogeneities.
Table 4. Summary of the average MRI relaxation times in the full-thickness ROI ($T_1^{ρ, adiab}$, $T_2^{ρ, adiab}$, CW-$T_1^{ρ}$ ($γB_1 = 125 − 1000$ Hz), $T_{KAFF}$, DE-$T_2$, CPMG-$T_2$, SE-$T_2$, $T_1$, $T_{1Gd}$, $T_{1sat}$) values ± SD (in ms) in rabbit and human cartilage at different sites and groups.

<table>
<thead>
<tr>
<th>Parameter (ms)</th>
<th>Rabbit</th>
<th>Rabbit</th>
<th>Rabbit</th>
<th>Rabbit</th>
<th>Human</th>
<th>Human</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>medial</td>
<td>lateral</td>
<td>medial</td>
<td>lateral</td>
<td>early OA</td>
<td>advanced OA</td>
</tr>
<tr>
<td>CTRL</td>
<td>(n = 8)</td>
<td>(n = 8)</td>
<td>(n = 8)</td>
<td>(n = 8)</td>
<td>(n = 5)</td>
<td>(n = 9)</td>
</tr>
<tr>
<td>$T_1^{ρ, adiab}$</td>
<td>75.6 ± 5.6</td>
<td>88.6 ± 15.6</td>
<td>71.9 ± 5.1</td>
<td>94.5 ± 35.5</td>
<td>116.1 ± 10.5</td>
<td>153.0 ± 23.8</td>
</tr>
<tr>
<td>$T_2^{ρ, adiab}$</td>
<td>21.8 ± 1.6</td>
<td>27.8 ± 10.5</td>
<td>23.0 ± 2.5</td>
<td>35.8 ± 22.1</td>
<td>32.7 ± 4.2</td>
<td>53.2 ± 11.6</td>
</tr>
<tr>
<td>CW-$T_1^{ρ}$, $γB_1 = 1$ kHz</td>
<td>19.7 ± 7.3</td>
<td>28.7 ± 12.8</td>
<td>18.9 ± 4.6</td>
<td>27.2 ± 10.5</td>
<td>61.2 ± 5.5</td>
<td>80.2 ± 21.3</td>
</tr>
<tr>
<td>CW-$T_1^{ρ}$, $γB_1 = 0.5$ kHz</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>45.8 ± 4.1</td>
<td>69.3 ± 15.0</td>
</tr>
<tr>
<td>CW-$T_1^{ρ}$, $γB_1 = 0.25$ kHz</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>35.0 ± 6.1</td>
<td>54.7 ± 12.1</td>
</tr>
<tr>
<td>CW-$T_1^{ρ}$, $γB_1 = 0.125$ kHz</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>25.0 ± 7.0</td>
<td>40.2 ± 13.2</td>
</tr>
<tr>
<td>$T_{KAFF}$</td>
<td>15.9 ± 5.5</td>
<td>16.5 ± 3.2</td>
<td>13.8 ± 2.9</td>
<td>14.1 ± 2.4</td>
<td>31.1 ± 8.3</td>
<td>50.6 ± 11.8</td>
</tr>
<tr>
<td>DE-$T_2$</td>
<td>18.1 ± 2.7</td>
<td>23.0 ± 10.1</td>
<td>19.3 ± 3.1</td>
<td>29.6 ± 14.4</td>
<td>20.9 ± 3.9</td>
<td>34.8 ± 8.3</td>
</tr>
<tr>
<td>CPMG-$T_2$</td>
<td>25.8 ± 2.5</td>
<td>29.9 ± 11.5</td>
<td>25.5 ± 4.1</td>
<td>41.6 ± 26.3</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>SE-$T_2$</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>20.0 ± 4.0</td>
<td>32.0 ± 8.8</td>
</tr>
<tr>
<td>$T_1$</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1390.4 ± 86.9</td>
<td>1566.7 ± 21.0</td>
</tr>
<tr>
<td>$T_{1Gd}$</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>482.1 ± 53.2</td>
<td>434.6 ± 98.5</td>
</tr>
<tr>
<td>$T_{1sat}$</td>
<td>325.3 ± 34.1</td>
<td>374.5 ± 66.1</td>
<td>330.1 ± 34.0</td>
<td>381.5 ± 116.7</td>
<td>444.7 ± 37.9</td>
<td>582.8 ± 96.4</td>
</tr>
</tbody>
</table>

Kruskal-Wallis post-hoc test (rabbits), Mann-Whitney U-test (humans) $^1p < 0.05$, $^2p < 0.01$
In study II on spontaneously degenerated human cartilage obtained from knee replacement surgeries, all quantitative MRI parameters except $T_{1,Gd}$ and CW-$T_1\rho$ ($\gamma B_1 = 1$ kHz) showed a statistically significant difference between early and advanced OA groups in full-thickness cartilage ROI analysis (Table 4), which is also evident in the representative relaxation time maps (Fig. 11). Zonal analysis revealed significant prolongation of the relaxation times, especially in the superficial part of the cartilage (Fig. 11).

In the early OA group, $T_2$ relaxation time maps demonstrated the typical tri-laminar appearance which was also evident in adiabatic $T_{2\rho}$, $T_{RAFF}$ and CW-$T_1\rho$ maps with spin-lock frequencies below 1 kHz (Fig. 11). $T_{1\rho}$ dispersion was demonstrated at CW-$T_1\rho$ maps with different spin-locking fields, the $T_2$-like appearance shown most evidently at $\gamma B_1 = 125$ Hz. In the adiabatic $T_{1\rho}$, CW-$T_1\rho$ ($\gamma B_1 = 1$ kHz) and pre-contrast $T_1$ maps relaxation times showed a decreasing trend towards deep cartilage.
Fig 11. Representative MRI relaxation time maps of $T_1Gd$, $T_1sat$, $T_1\rho$, adiab., $CW-T_1\rho$, $T_2\rho$, adiab., $DE-T_2$, $SE-T_2$, $T_1$, $T_2$, $T_1Gd$, $T_2sat$ parameters for human cartilage specimens in early and advanced OA groups. Reference methods (FTIR amide I (collagen) and carbohydrate (PG) absorptions, DD (PG content), collagen anisotropy (PI)) reveal superficial PG loss in the early OA specimen. The sample with more advanced degeneration shows surface fibrillation, loss of both PGs and collagen as well as disorganization of the collagen fibril network.

6.1.3 Sensitivity analysis

The highest relative changes in the rabbit ACLT model of study I were obtained with adiabatic $T_2p$ and $T_2$ measured with DE and CPMG sequences in the lateral femoral
condyle, approximately 50–60% (Table 5). For adiabatic $T_1\rho$ and CW-$T_1\rho$ the changes were in the range of 30–40%, respectively. $T_{RAFF}$ and $T_{1sat}$ were prolonged only by 2% and 16% in the lateral femoral condyle, respectively. In the human specimens of study II, the prolongation of adiabatic $T_2\rho$, $T_{RAFF}$, DE-$T_2$ and SE-$T_2$ and CW-$T_1\rho$ ($\gamma B_1 = 125 – 500$ Hz) was about 60% while the change in adiabatic $T_1\rho$, CW-$T_1\rho$ ($\gamma B_1 = 1$ kHz) and $T_{1sat}$ was about 30%. All parameters, except $T_{1Gd}$, which was shortened, experienced prolongation as expected.

Considering both studies I and II, ROC-analysis showed the highest AUC values for adiabatic $T_1\rho$ and adiabatic $T_2\rho$ parameters (Table 5). In study II, high, over 0.8 AUC-values were obtained also for CW-$T_1\rho$, $T_{RAFF}$, DE-$T_2$, SE-$T_2$, $T_1$ and $T_{1sat}$.

Table 5. Summary of the relative changes (%) in the relaxation time parameters ($T_{1\rho, adiab}$, $T_{2\rho, adiab}$, CW-$T_1\rho$ ($\gamma B_1 = 125 – 1000$ Hz), $T_{RAFF}$, DE-$T_2$, CPMG-$T_2$, SE-$T_2$, $T_1$, $T_{1Gd}$, $T_{1sat}$) and the AUC values for rabbit and human specimens.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Rabbit lateral condyle</th>
<th>Rabbit medial condyle</th>
<th>Rabbit lateral condyle</th>
<th>Human lateral condyle</th>
<th>Human medial condyle</th>
<th>Human</th>
<th>AUC value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_{1\rho, adiab}$</td>
<td>31%</td>
<td>17%</td>
<td>32%</td>
<td>0.88</td>
<td>0.78</td>
<td>0.98</td>
<td></td>
</tr>
<tr>
<td>$T_{2\rho, adiab}$</td>
<td>55%</td>
<td>28%</td>
<td>63%</td>
<td>0.80</td>
<td>0.77</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>CW-$T_1\rho$, $\gamma B_1 = 1$ kHz</td>
<td>44%</td>
<td>45%</td>
<td>31%</td>
<td>0.84</td>
<td>0.72</td>
<td>0.82</td>
<td></td>
</tr>
<tr>
<td>CW-$T_1\rho$, $\gamma B_1 = 0.5$ kHz</td>
<td>–</td>
<td>–</td>
<td>52%</td>
<td>–</td>
<td>–</td>
<td>0.96</td>
<td></td>
</tr>
<tr>
<td>CW-$T_1\rho$, $\gamma B_1 = 0.25$ kHz</td>
<td>–</td>
<td>–</td>
<td>56%</td>
<td>–</td>
<td>–</td>
<td>0.98</td>
<td></td>
</tr>
<tr>
<td>CW-$T_1\rho$, $\gamma B_1 = 0.125$ kHz</td>
<td>–</td>
<td>–</td>
<td>61%</td>
<td>–</td>
<td>–</td>
<td>0.91</td>
<td></td>
</tr>
<tr>
<td>$T_{RAFF}$</td>
<td>2%</td>
<td>4%</td>
<td>63%</td>
<td>0.58</td>
<td>0.61</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>DE-$T_2$</td>
<td>53%</td>
<td>27%</td>
<td>67%</td>
<td>0.77</td>
<td>0.58</td>
<td>0.98</td>
<td></td>
</tr>
<tr>
<td>CPMG-$T_2$</td>
<td>62%</td>
<td>16%</td>
<td>–</td>
<td>0.83</td>
<td>0.66</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>SE-$T_2$</td>
<td>–</td>
<td>–</td>
<td>60%</td>
<td>–</td>
<td>–</td>
<td>0.87</td>
<td></td>
</tr>
<tr>
<td>$T_1$</td>
<td>–</td>
<td>–</td>
<td>13%</td>
<td>–</td>
<td>–</td>
<td>0.87</td>
<td></td>
</tr>
<tr>
<td>$T_{1Gd}$</td>
<td>–</td>
<td>–</td>
<td>-10%</td>
<td>–</td>
<td>–</td>
<td>0.64</td>
<td></td>
</tr>
<tr>
<td>$T_{1sat}$</td>
<td>16%</td>
<td>15%</td>
<td>31%</td>
<td>0.61</td>
<td>0.77</td>
<td>0.87</td>
<td></td>
</tr>
</tbody>
</table>

### 6.1.4 Association with structure and function

In study I, there was moderate negative correlation between quantitative MRI parameters and equilibrium modulus (Table 6). While $T_{RAFF}$ was not significantly correlated with $E_{eq}$, there was a significant but weak correlation to dynamic modulus. Also CW-$T_1\rho$ and $T_{1sat}$ were significantly correlated to $E_{dyn}$. There were no significant correlations
between MRI parameters and PG content or collagen anisotropy as determined from DD and PLM measurements, respectively.

In study II, all quantitative MRI parameters, except $T_{1\text{Gd}}$ were significantly correlated with histopathological OARSI grades and biomechanical parameters ($E_{eq}$ and $E_{dyn}$), particularly adiabatic $T_{1p}$, adiabatic $T_{2p}$, CW-$T_{1p}$ ($\gamma B_1 = 250 – 500$ Hz), $T_{RAFF}$ and $T_{1\text{sat}}$ (Table 6). There were significant correlations between the water content of cartilage and adiabatic $T_{1p}$, CW-$T_{1p}$ ($\gamma B_1 = 250 – 500$ Hz) and $T_{1\text{sat}}$. The same parameters correlated also with optical density while collagen anisotropy correlated with DE-$T_2$ and CW-$T_{1p}$ ($\gamma B_1 = 125$ Hz). Collagen content as represented by FTIR-derived amide I absorption correlated significantly with $T_1$ relaxation time while carbohydrate region, representing PG content, correlated with CW-$T_{1p}$ ($\gamma B_1 = 250$ Hz), DE-$T_2$, $T_2$, and $T_{RAFF}$. Furthermore, $E_{eq}$ and $E_{dyn}$ correlated significantly with OARSI grade, while $E_{eq}$ also correlated with optical density and $E_{dyn}$ with water content of cartilage (Table 7). There was also a significant correlation between $E_{eq}$ and FTIR-derived PG content, and between optical density and OARSI grade.
### Table 6. Summary of the linear correlation coefficients between MRI relaxation time parameters ($T_1\rho$, $T_2\rho$, $T_1Gd$, $T_1sat$) and reference methods ($E_{eq}$, $E_{dyn}$, OD, PI, OARSI, water content (%), amide I (collagen) and carbohydrate (PG) absorptions).

<table>
<thead>
<tr>
<th>Parameter Species</th>
<th>$E_{eq}$</th>
<th>$E_{dyn}$</th>
<th>OD</th>
<th>PI</th>
<th>OARSI</th>
<th>Water FTIR</th>
<th>FTIR Amide I</th>
<th>FTIR Carbohydrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_1\rho$, $T_2\rho$, $T_1Gd$, $T_1sat$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human</td>
<td>0.24</td>
<td>0.85</td>
<td>0.56</td>
<td>0.39</td>
<td>0.13</td>
<td>0.81</td>
<td>0.62</td>
<td>0.22</td>
</tr>
<tr>
<td>Rabbit</td>
<td>-0.30</td>
<td>0.13</td>
<td>-0.32</td>
<td>0.29</td>
<td>0.13</td>
<td>-0.31</td>
<td>0.14</td>
<td>-0.20</td>
</tr>
<tr>
<td>Human</td>
<td>-0.38</td>
<td>0.13</td>
<td>-0.44</td>
<td>0.25</td>
<td>0.22</td>
<td>-0.44</td>
<td>0.22</td>
<td></td>
</tr>
<tr>
<td>Rabbit</td>
<td>-0.38</td>
<td>0.13</td>
<td>-0.44</td>
<td>0.25</td>
<td>0.22</td>
<td>-0.44</td>
<td>0.22</td>
<td></td>
</tr>
<tr>
<td>Human</td>
<td>-0.64</td>
<td>0.13</td>
<td>-0.67</td>
<td>0.13</td>
<td>0.63</td>
<td>0.60</td>
<td>0.30</td>
<td></td>
</tr>
<tr>
<td>Rabbit</td>
<td>-0.42</td>
<td>0.13</td>
<td>-0.41</td>
<td>0.13</td>
<td>0.13</td>
<td>-0.20</td>
<td>0.13</td>
<td></td>
</tr>
<tr>
<td>Human</td>
<td>-0.80</td>
<td>0.13</td>
<td>-0.72</td>
<td>0.13</td>
<td>0.80</td>
<td>0.59</td>
<td>0.34</td>
<td></td>
</tr>
<tr>
<td>Rabbit</td>
<td>-0.42</td>
<td>0.13</td>
<td>-0.41</td>
<td>0.13</td>
<td>0.13</td>
<td>-0.20</td>
<td>0.13</td>
<td></td>
</tr>
<tr>
<td>Human</td>
<td>-0.74</td>
<td>0.13</td>
<td>-0.59</td>
<td>0.13</td>
<td>0.74</td>
<td>0.40</td>
<td>0.37</td>
<td></td>
</tr>
<tr>
<td>Rabbit</td>
<td>-0.04</td>
<td>0.35</td>
<td>-0.31</td>
<td>0.14</td>
<td>-0.31</td>
<td>0.14</td>
<td>-0.31</td>
<td></td>
</tr>
<tr>
<td>Human</td>
<td>-0.73</td>
<td>0.13</td>
<td>-0.57</td>
<td>0.13</td>
<td>0.69</td>
<td>0.47</td>
<td>0.38</td>
<td></td>
</tr>
<tr>
<td>Rabbit</td>
<td>-0.47</td>
<td>0.13</td>
<td>-0.22</td>
<td>0.10</td>
<td>-0.22</td>
<td>0.10</td>
<td>-0.22</td>
<td></td>
</tr>
<tr>
<td>Rabbit</td>
<td>-0.43</td>
<td>0.13</td>
<td>-0.23</td>
<td>0.12</td>
<td>-0.23</td>
<td>0.12</td>
<td>-0.23</td>
<td></td>
</tr>
<tr>
<td>Human</td>
<td>-0.65</td>
<td>0.13</td>
<td>-0.49</td>
<td>0.24</td>
<td>-0.52</td>
<td>0.59</td>
<td>0.31</td>
<td></td>
</tr>
<tr>
<td>Human</td>
<td>0.48</td>
<td>0.52</td>
<td>0.45</td>
<td>-0.09</td>
<td>-0.40</td>
<td>-0.46</td>
<td>0.16</td>
<td>0.28</td>
</tr>
<tr>
<td>Human</td>
<td>-0.76</td>
<td>0.13</td>
<td>-0.81</td>
<td>0.13</td>
<td>0.53</td>
<td>0.57</td>
<td>0.42</td>
<td></td>
</tr>
<tr>
<td>Rabbit</td>
<td>-0.48</td>
<td>0.13</td>
<td>-0.41</td>
<td>0.13</td>
<td>-0.20</td>
<td>0.13</td>
<td>-0.20</td>
<td></td>
</tr>
<tr>
<td>Human</td>
<td>-0.71</td>
<td>0.13</td>
<td>-0.68</td>
<td>0.13</td>
<td>0.64</td>
<td>0.45</td>
<td>0.60</td>
<td></td>
</tr>
<tr>
<td>Human</td>
<td>-0.81</td>
<td>0.13</td>
<td>-0.81</td>
<td>0.13</td>
<td>0.53</td>
<td>0.57</td>
<td>0.42</td>
<td></td>
</tr>
<tr>
<td>Rabbit</td>
<td>-0.48</td>
<td>0.13</td>
<td>-0.41</td>
<td>0.13</td>
<td>-0.20</td>
<td>0.13</td>
<td>-0.20</td>
<td></td>
</tr>
</tbody>
</table>

1 *p < 0.05, 2 *p < 0.01.
Table 7. Summary of the linear correlation coefficients between biomechanical parameters ($E_{eq}$ and $E_{dyn}$) and other reference methods (OD, PI, OARSI, water content (%), amide I (collagen) and carbohydrate (PG) absorptions). In addition, other reference methods are correlated with all other parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Species</th>
<th>$E_{eq}$</th>
<th>$E_{dyn}$</th>
<th>OD</th>
<th>PI</th>
<th>OARSI grade</th>
<th>Water content (%)</th>
<th>FTIR Amide I</th>
<th>FTIR Carbohydrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E_{eq}$</td>
<td>Human</td>
<td>1.00</td>
<td>0.92(^2)</td>
<td>0.61(^1)</td>
<td>0.36</td>
<td>-0.90(^1)</td>
<td>-0.44</td>
<td>0.38</td>
<td>0.55(^1)</td>
</tr>
<tr>
<td></td>
<td>Rabbit</td>
<td>1.00</td>
<td>0.63(^2)</td>
<td>0.25</td>
<td>-0.30</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>$E_{dyn}$</td>
<td>Human</td>
<td>0.92(^2)</td>
<td>1.00</td>
<td>0.46</td>
<td>0.43</td>
<td>-0.90(^2)</td>
<td>-0.59(^1)</td>
<td>0.31</td>
<td>0.43</td>
</tr>
<tr>
<td></td>
<td>Rabbit</td>
<td>0.63(^2)</td>
<td>1.00</td>
<td>0.39(^1)</td>
<td>-0.16</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>OD</td>
<td>Human</td>
<td>0.61(^1)</td>
<td>0.46</td>
<td>1.00</td>
<td>-0.20</td>
<td>-0.55(^1)</td>
<td>0.1</td>
<td>0.12</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td>Rabbit</td>
<td>0.25</td>
<td>0.39(^1)</td>
<td>1.00</td>
<td>-0.07</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PI</td>
<td>Human</td>
<td>0.36</td>
<td>0.43</td>
<td>-0.20</td>
<td>1.00</td>
<td>-0.43</td>
<td>-0.26</td>
<td>-0.18</td>
<td>-0.06</td>
</tr>
<tr>
<td></td>
<td>Rabbit</td>
<td>-0.30</td>
<td>-0.16</td>
<td>-0.07</td>
<td>1.00</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>OARSI grade</td>
<td>Human</td>
<td>-0.90(^1)</td>
<td>-0.90(^1)</td>
<td>-0.55(^1)</td>
<td>-0.43</td>
<td>1.00</td>
<td>0.57(^1)</td>
<td>-0.20</td>
<td>-0.38</td>
</tr>
<tr>
<td>Water content %</td>
<td>Human</td>
<td>-0.44</td>
<td>-0.59(^1)</td>
<td>0.10</td>
<td>-0.26</td>
<td>0.57(^1)</td>
<td>1.00</td>
<td>-0.30</td>
<td>-0.34</td>
</tr>
<tr>
<td>FTIR Amide I</td>
<td>Human</td>
<td>0.38</td>
<td>0.31</td>
<td>0.12</td>
<td>-0.18</td>
<td>-0.20</td>
<td>-0.30</td>
<td>1.00</td>
<td>0.84(^2)</td>
</tr>
<tr>
<td>FTIR Carbohydrate</td>
<td>Human</td>
<td>0.55(^1)</td>
<td>0.43</td>
<td>0.26</td>
<td>-0.06</td>
<td>-0.38</td>
<td>-0.34</td>
<td>0.84(^2)</td>
<td>1.00</td>
</tr>
</tbody>
</table>

\(^1p < 0.05, \(^2p < 0.01\)
6.2 Evaluation of osteochondral structures with short $T_2$ imaging

6.2.1 SWIFT imaging of osteochondral repair in equine model

In study III, spontaneously healed osteochondral and chondral lesions in equine joints resulted in different healing outcomes according to histological evaluation. All chondral lesions (n = 5) demonstrated poor cartilage repair (Fig. 12) while four osteochondral lesions showed healing. One sample with osteochondral lesion healed poorly by filling the lesion only with fibrous tissue that reached deep into the subchondral bone (Fig. 13).

**Fig 12.** Chondral lesion in equine joint with poor cartilage repair after spontaneous healing of 12 months demonstrated by SWIFT (A. non-saturated, B. fat-saturation, C. combination image of fat and water saturation images, D. water saturation) and micro-CT, Safranin-O stained microscopy section, FSE and GRE images. With SWIFT, the cartilage lesion and thinning of the subchondral bone plate is clearly depicted (white arrow). In addition, the signal intensity in the subchondral bone is higher in SWIFT images in comparison with conventional FSE and GRE techniques.

SWIFT MRI enabled a differentiation of spontaneous healing outcomes of osteochondral and chondral lesions by simultaneous assessment of articular cartilage and subchondral bone. SWIFT produced high signal intensity throughout cartilage and subchondral bone, both with or without fat saturation (Fig. 12 & 13). In cartilage, the signal intensity remained constant at the lesion sites and adjacent intact tissue. Bone marrow was clearly visualized in water saturated SWIFT data and by combining it with the fat saturated image, the contrast in the subchondral bone was improved allowing a calculation of the bone parameters. Combined SWIFT images demonstrated striking visual similarities between SWIFT and micro-CT (Fig. 12 & 13). The correlation of BV/TV and Tb.Th parameters between SWIFT and micro-CT derived values was high
while Tb.Sp and Tb.N parameters correlated only moderately with SWIFT derived values (Table 8). However, an overestimation was noted in SWIFT-derived BV/TV and Tb.Th values and an underestimation of Tb.Sp values.

![Image](image_url)

Fig 13. Osteochondral lesion in equine joint with poor repair after spontaneous healing of 12 months. SWIFT (A. non-saturated, B. fat-saturation, C. combination image of fat and water saturation images, D. water saturation) and micro-CT, Safranin-O stained microscopy section, FSE and GRE images reveal fibrous tissue penetrating deep into the subchondral bone (white arrows). With SWIFT, the fibrous tissue is separated from bone marrow with fat and water suppression schemes (black arrows). In addition, the signal intensity in the subchondral bone is higher in SWIFT images in comparison with conventional FSE and GRE techniques.

Table 8. Linear correlation coefficients of microstructural bone parameters between SWIFT and micro-CT derived values.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Linear correlation coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone volume/tissue volume ratio</td>
<td>$R = 0.83^2$</td>
</tr>
<tr>
<td>Trabecular separation</td>
<td>$R = 0.79^2$</td>
</tr>
<tr>
<td>Trabecular thickness</td>
<td>$R = 0.68^2$</td>
</tr>
<tr>
<td>Trabecular number</td>
<td>$R = 0.49^1$</td>
</tr>
</tbody>
</table>

$^1p < 0.05, ^2p < 0.01$

In the osteochondral sample with poor healing and fibrous infiltration, SWIFT depicted a high intensity signal from fibrous tissue that corresponded with the void in the micro-CT image at the lesion site (Fig. 13). The fibrous tissue was further differentiated from marrow by applying fat saturation to the SWIFT image. Thinning and sclerosis of the subchondral bone plate as well as cartilage defects were also clearly detected at the lesion site (Fig. 12 & 13). At the cartilage–bone interface, a high intensity signal band, typical of the osteochondral interface with UTE sequences, was not present. In SWIFT
without any signal suppression schemes, the microstructure of the trabecular bone was blurred due to the chemical shift of fat. By applying fat or water saturation pulses, the signal contributed by marrow and water protons was efficiently removed, respectively (Fig. 12 & 13).

The normalized signal-to-noise ratio in SWIFT was high in articular cartilage, subchondral bone plate and trabecular bone in comparison to matched FSE images, except with water saturation, as expected (Table 9). With respect to GRE, the SNR of cartilage was comparable with that of SWIFT, but was reduced to one fourth in the subchondral bone plate. The SNR of trabecular bone was high in SWIFT datasets compared to FSE or GRE with a clear reduction when fat saturation was used with SWIFT.

Table 9. The normalized signal-to-noise ratios in SWIFT (without any saturation pulses, with fat and water saturation), FSE and GRE images in articular cartilage, subchondral bone plate and trabecular bone.

<table>
<thead>
<tr>
<th>Sequence</th>
<th>Articular cartilage</th>
<th>Subchondral bone plate</th>
<th>Trabecular bone</th>
</tr>
</thead>
<tbody>
<tr>
<td>SWIFT</td>
<td>1.8</td>
<td>0.9</td>
<td>1.2</td>
</tr>
<tr>
<td>SWIFT fat sat.</td>
<td>1.7</td>
<td>0.7</td>
<td>0.8</td>
</tr>
<tr>
<td>SWIFT water sat.</td>
<td>0.4</td>
<td>0.5</td>
<td>1.3</td>
</tr>
<tr>
<td>GRE</td>
<td>1.8</td>
<td>0.2</td>
<td>0.6</td>
</tr>
<tr>
<td>FSE</td>
<td>1.0</td>
<td>0.1</td>
<td>0.9</td>
</tr>
</tbody>
</table>

The thicknesses of articular cartilage at the lesion sites and adjacent tissue measured from SWIFT images were comparable to those obtained from GRE or microscopy images (Table 10). In FSE images, the signal from the deep regions of cartilage was lacking, resulting in a trend towards thinner cartilage at the adjacent intact cartilage, however, this was not statistically significantly as compared to other modalities.

Table 10. The thickness of articular cartilage ± SD (mm) at lesion sites and adjacent intact cartilage of equine specimens as determined from SWIFT, GRE, FSE and microscopy images.

<table>
<thead>
<tr>
<th>Modality</th>
<th>Chondral lesion (n = 5)</th>
<th>Osteochondral lesion (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intact (mm)</td>
<td>Lesion site (mm)</td>
</tr>
<tr>
<td>SWIFT</td>
<td>0.71 ± 0.18</td>
<td>0.55 ± 0.30</td>
</tr>
<tr>
<td>GRE</td>
<td>0.65 ± 0.17</td>
<td>0.62 ± 0.24</td>
</tr>
<tr>
<td>FSE</td>
<td>0.47 ± 0.10</td>
<td>0.55 ± 0.28</td>
</tr>
<tr>
<td>Microscopy</td>
<td>0.69 ± 0.04</td>
<td>0.48 ± 0.25</td>
</tr>
</tbody>
</table>

The differences between the modalities were studied using Wilcoxon signed rank test. Statistically significant differences were not found.
6.2.2  **SWIFT signal at the cartilage-bone interface**

In SWIFT subtraction images of human specimens, a high-intensity signal was observed at the osteochondral junction in relatively intact samples with mild OA and OARSI grades (Fig. 14 A & B, white arrows). The bright line appeared in the region of the calcified cartilage, which was shown in microscopy sections stained with Masson’s trichrome and Safranin-O. The samples with low OARSI grade demonstrated normal subchondral bone in the micro-CT images, while the Safranin-O stained sections revealed superficial PG loss in the cartilage.

With the advancement of OA, the high intensity signal band was lacking in the SWIFT images which included both long and short $T_2$ components (Fig. 14 C-E). The SWIFT images with only short $T_2$ components showed thinning or diffuse thickening of the high intensity signal band. The samples with OARSI grades higher than 3.0 demonstrated sclerosis of subchondral bone, increased roughness of the zone of calcified cartilage, duplication of tidemarks and PG loss, which were quite visible in the microscopy and micro-CT images. Furthermore, fibrillation of the cartilage surface was also seen in SWIFT images. However, similarly to study III, the signal intensity inside articular cartilage did not show significant alterations with the progression of cartilage degeneration.
<table>
<thead>
<tr>
<th>SWIFT</th>
<th>SWIFT</th>
<th>Masson’s trichrome</th>
<th>Safranin-O</th>
<th>Micro-CT</th>
</tr>
</thead>
<tbody>
<tr>
<td>(long + short $T_2$)</td>
<td>(short $T_2$)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>OARSI = 1.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>OARSI = 2.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>OARSI = 3.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>OARSI = 3.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>OARSI = 4.1</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig 14. SWIFT subtraction images of the human cartilage–bone interface reveal signal changes during the progression of osteoarthritis. Masson’s trichrome, Safranin-O staining and micro-CT images reveal PG loss, sclerosis of the subchondral bone, duplication of tidemarks and thickening of the zone of calcified cartilage. In SWIFT, a high-intensity signal (white arrows) is observed at the osteochondral junction in relatively intact cartilage samples. During advancement of OA, loss, thinning or diffuse thickening of the high-intensity signal was observed in SWIFT difference images (black arrows).
7 Discussion

In this thesis, novel MRI techniques were tested on animal models of OA and human articular cartilage and subchondral bone. With quantitative MRI relaxation time mapping, the biochemical status of cartilage may be assessed indirectly and noninvasively. Moreover, SWIFT MRI enables imaging of tissues with very short $T_2$ relaxation time, which are found in deep cartilage and bone. Assessment of those structures is very difficult with conventional MRI. Thus, quantitative MRI techniques may advance knowledge about OA pathology and provide research tools for development and follow-up of new treatments for OA. In addition, application of the novel MRI methods in clinical decision-making may help to identify patients with early stage OA. The main findings of the study are discussed in more detail in the following sections.

7.1 Quantitative MRI of degenerated articular cartilage

The comparison of the different relaxation parameters has previously been difficult as only a few studies have systematically compared several quantitative MRI parameters within the same OA model [105, 107, 176, 177]. In the present study, the sensitivity of multiple quantitative MRI relaxation time parameters for the assessment of cartilage degeneration was investigated in two OA models. Conventional $T_1$ and $T_2$ relaxation time mapping as well as novel rotating frame relaxation parameters ($CW-T_1$, adiabatic $T_1$, adiabatic $T_2$, RAFF) and an MT experiment ($T_{1\text{sat}}$) were tested in a rabbit ACLT model of early cartilage degeneration 4 weeks post-transection. Degenerative changes were also investigated with quantitative MRI in osteoarthritic human specimens with varying degrees of degeneration. The ultimate aim was to sensitively detect degenerative changes with quantitative MRI in the biochemical composition of cartilage at early stage of the disease.

To evaluate the MRI findings, the degeneration of articular cartilage in studies I and II was investigated with quantitative and qualitative histology and biomechanical and biochemical measurements. In both studies, degenerative changes were demonstrated by diminished mechanical properties of cartilage in the degenerated sample groups as compared to controls, and confirmed with quantitative and qualitative histology. In study I, DD and PLM measurements revealed PG depletion and disruption of the collagen network limited to the superficial part of the cartilage, suggesting early cartilage...
degeneration in the femoral condyles of ACL transected rabbits. Similar degenerative changes in cartilage have also been reported in previous studies with the ACLT rabbit model four weeks post-ACLT [126, 178, 179]. These spatially limited degenerative changes further emphasize the importance of the zonal evaluation of articular cartilage with reference methods or quantitative MRI. No full-thickness cartilage erosion was observed in the present or previous works [126, 178, 179] which is relevant when studying early OA changes. To compare with humans, the anatomy of the rabbit knee joint and post-ACLT disease progression are similar, although the rate of OA progression is much faster in rabbits [7].

In study II, human osteochondral specimens with a wide range of degeneration were collected from the tibiae of TKA patients. Although healthy (OARSI grade 0) cartilage was not found, the samples with an OARSI score less than 1.5 represent very mild degeneration [173]. Early OA samples demonstrated PG loss in the superficial cartilage, however without statistical significance in comparison with the advanced OA group. The advanced OA specimens (OARSI grade > 1.5) showed more severe PG loss, surface fibrillation, and disruption of the collagen fibril network, which is consistent with previous work on human specimens [2, 4, 174]. As there was a considerable variation among the human samples, it may have resulted in a higher spread of the MRI relaxation and reference parameter values and thus resulted in improved correlations between them compared to study I. However in study I, the changes in the PG content and collagen fibril anisotropy were very small and limited only to the most superficial part of cartilage, leading to some averaging in the ROI analysis, which may potentially explain the difference between the two studies.

In studies I and II, $T_2$ relaxation time was mapped using the adiabatic DE technique. For comparison, $T_2$-mapping was also performed with CPMG and SE preparations in studies I and II, respectively. DE-$T_2$ was sensitive to cartilage degeneration by showing significant prolongation only in the lateral femoral condyle of the rabbits, which however showed more severe degeneration. In study I, CPMG-$T_2$ also showed a trend towards elevated values but without statistical significance. $T_2$-relaxation time maps of intact articular cartilage typically show a laminar appearance associated with the histological cartilage zones, which has been reported in many animal and human studies [69–74, 77, 79, 180]. In study I, however, rabbit cartilage was relatively thin, making it difficult to detect the laminar structure in $T_2$-maps while in human cartilage specimens both $T_2$-methods demonstrated the typical tri-laminar structure in the early OA group. Consistent with previous studies, a significant elevation due to cartilage
degeneration was noted with both DE-\(T_2\) and SE-\(T_2\) methods in the advanced OA group of human specimens [75, 78, 81–83, 85–87, 181].

\(T_2\) relaxation time has been reported to be sensitive to both collagen content and orientation, as well as to water content of the cartilage [69–83]. In study I, collagen fibril anisotropy (PI) was increased in the lateral condyle and was also detected with DE-\(T_2\) by an increase in the relaxation time. However, the correlation of DE-\(T_2\) or CPMG-\(T_2\) with PI or \(E_{\text{dyn}}\) was not statistically significant in study I. In the human sample study, only DE-\(T_2\) correlated significantly with both PI and \(E_{\text{dyn}}\) parameters. There were also significant correlations between \(T_2\) methods and \(E_{\text{eq}}\), which have also been reported previously [73]. Similarly, \(T_2\) methods correlated moderately with FTIR-derived PG-content. DE-\(T_2\) and SE-\(T_2\) also correlated significantly with the OARSI grade, the correlation being stronger with DE-\(T_2\). The reason for the better sensitivity of DE-\(T_2\) might originate from improved robustness of the adiabatic inversion pulses in comparison to the hard pulse CPMG or SE-based \(T_2\), improving estimation of the relaxation time.

The dGEMRIC technique is a well-established method for evaluating the GAG content of cartilage, which has been demonstrated \textit{in vitro} and \textit{in vivo} [80, 86, 88–97]. In the present study, the \(T_1\) relaxation time was mapped after 24-hour equilibration of the human specimens in gadopentetate contrast agent. The advanced OA samples showed significant PG depletion in the deep cartilage and there was a decreasing trend in \(T_{1\text{Gd}}\) relaxation time in all cartilage zones in the advanced OA group in comparison to the early OA group. However, the changes in \(T_{1\text{Gd}}\) between the groups were not statistically significant. Moreover, there were no significant correlations between \(T_{1\text{Gd}}\) and any of the reference methods including that between \(T_{1\text{Gd}}\) and PG content, although the depth-wise profiles of \(T_{1\text{Gd}}\) and PG content appeared similar in both groups. In addition, the \(T_{1\text{Gd}}\) relaxation time maps also resembled FTIRI and Safranin-O maps. Similarly, in a previous study the dGEMRIC technique could not distinguish between early hip OA and reference cartilage [182]. \(T_1\) relaxation time was also mapped prior to immersion into contrast agent and was significantly elevated in the advanced OA group. Previously, pre-contrast \(T_1\) relaxation time has been linked to the water content of cartilage [101] and in this study it demonstrated significant correlations with \(T_1\) and \(E_{\text{eq}}, E_{\text{dyn}}\) and OARSI grade.

MRI relaxation parameters in the rotating frame of reference such as \(T_1\rho, T_2\rho\) and \(T_{\text{RAFF}}\) utilize spin-locking RF pulses and the relaxation processes are sensitive to magnetic field fluctuations in the kHz range [48, 51]. Thus, the parameters are
sensitive to a broad range of slow macromolecular motion, which are relevant in many pathologies such as OA.\( T_{1\rho} \) relaxation time has been the most intensively studied RFR parameter for cartilage and it has been suggested as biomarker for early cartilage degeneration. Furthermore, it has been associated with the PG content of cartilage [45, 104, 112–114, 116, 117, 120, 123, 124, 127, 132]. Traditionally \( T_{1\rho} \) has been measured with CW RF pulses, although RFR relaxation measurement can also be implemented with adiabatic type RF pulses.

In the present study, CW-\( T_{1\rho} \) detected early cartilage degeneration in the lateral femoral condyle in the rabbit ACLT model and was also significantly elevated in advanced OA human samples. Similar to the present findings, CW-\( T_{1\rho} \) was significantly elevated at three weeks in the lateral femoral condyles in a previous study with the rabbit ACLT model [126]. At 6 weeks post-ACLT, both compartments had significantly higher \( T_{1\rho} \) values compared to the control joint, although there was no clear quantitative histological evidence of the progression of OA [126]. In previous clinical studies at 3 T, CW-\( T_{1\rho} \) with spin-lock power \( \gamma B_1 = 500 \) Hz was significantly increased in knee OA patients compared with healthy controls as well as in patients with post-traumatic cartilage degeneration 1 and 2 years after ACL reconstruction [123, 124, 127]. Similarly at 1.5 T, CW-\( T_{1\rho} \) (\( \gamma B_1 = 500 \) Hz) was elevated due to arthroscopically confirmed cartilage degeneration in the knee joint [118].

The CW-\( T_{1\rho} \) dispersion phenomenon was evident in study II, showing features of a tri-laminar appearance at spin-locking powers less than 1 kHz. In the CW-\( T_{1\rho} \) (\( \gamma B_1 = 1 \) kHz) map, relaxation times were decreasing from surface towards deep cartilage. In addition, PG content as measured with DD was correlated significantly with CW-\( T_{1\rho} \) at \( \gamma B_1 = 250-500 \) Hz while at \( \gamma B_1 = 125 \) Hz CW-\( T_{1\rho} \) correlated significantly with collagen fibril anisotropy. These findings are consistent with reports associating \( T_{1\rho} \) to the PG content of cartilage as well as with the relaxation theory, as the \( T_{1\rho} \) relaxation time theoretically approaches \( T_2 \) with decreasing spin-locking frequency [45]. However, at 1 kHz spin-locking field, one would also expect significant correlation with the PG content, which was not found in the present study. Possible tissue erosion of PG depleted superficial cartilage in the advanced OA samples (study II) may also affect the correlation and explain this unexpected finding. With respect to other reference parameters, CW-\( T_{1\rho} \) correlated significantly with \( E_{eq}, E_{dyn}, \) water content and OARSI grade, the correlations being highest at spin-lock frequencies of 250 and 500 Hz. In contrast, a previous study reported no significant correlation of histological Mankin grade with \( T_{1\rho} \) or \( T_2 \) on osteoarthritic human specimens. However, the OARSI grading
system has a larger dynamic range for early OA grades in comparison with the Mankin grading [173], which may partly explain the discrepancy.

Of the rotating frame MRI contrasts, adiabatic $T_{1\rho}$ and adiabatic $T_{2\rho}$ have not yet been widely tested in cartilage. Previously adiabatic $T_{1\rho}$ has been found to be sensitive to trypsin induced PG depletion in bovine articular cartilage [107]. In studies I and II, both adiabatic $T_{1\rho}$ and adiabatic $T_{2\rho}$ were sensitive to degeneration in both medial and lateral condyles of rabbits as well as degeneration in human specimens, as demonstrated by significant elevation of the relaxation times. Adiabatic $T_{1\rho}$ relaxation times were decreasing towards deep cartilage while a laminar appearance was shown in adiabatic $T_{2\rho}$ maps in the early OA specimens. In study II, both relaxation parameters were highly correlated with $E_{eq}$, $E_{dyn}$, and OARSI grade. Moreover, significant correlations were found with PG content and there was also a significant relationship between adiabatic $T_{1\rho}$ and water content of cartilage. On the other hand, there were no association between PG content or collagen anisotropy and adiabatic relaxation parameters in study I. The decrease in the PG content and changes in collagen fibril anisotropy were small and limited only to the superficial part of cartilage, which may have led to averaging in the ROI analysis.

RAFF is an attracting RFR parameter with low SAR properties [51–53], which has been shown to be a potential biomarker for cartilage degeneration [107]. In the present study, RAFF was not sensitive to early cartilage degeneration in rabbit specimens while in study II there were significantly prolonged relaxation times in the advanced OA group of human specimens. In addition, RAFF correlated significantly with $E_{eq}$, $E_{dyn}$, OARSI grade and the FTIR-derived PG content in study II. The sample orientation with respect to $B_0$ was different between the studies which might at least partially explain the difference in the findings. Furthermore, the cartilage degeneration induced by trypsin in the previous study was much more severe than the post-ACLT degeneration in the rabbit specimens.

In the present study, the MT effect in cartilage was investigated with an inversion prepared MT experiment and measurement of the $T_{1\text{sat}}$, the relaxation time during off-resonance saturation. With inversion preparation, the dynamic range of the measurement is increased, improving the parameter mapping and thereby reducing MT pulse length compared to conventional ways of measuring MT [55]. $T_{1\text{sat}}$ showed an elevated trend in study I, but without statistical significance. In study II, $T_{1\text{sat}}$ was significantly elevated in the advanced OA group, similar to a previous study on trypsin digested bovine cartilage in comparison to control groups [107]. Furthermore, $T_{1\text{sat}}$ was highly correlated with the
$E_{eq}$, $E_{dyn}$ and OARSI grade and there were also significant correlations with water and PG contents. In previous studies, MT in cartilage has been linked to changes in the collagen content and to interaction of mobile water with collagen or collagen-bound water [102]. As in the present study, some studies have reported minor contributions of PGs to MT in cartilage [183, 184].

The presented multiparametric measurement of quantitative MRI parameters is not feasible in a clinical setting, for example, due to imaging time limitations and thus the most sensitive MRI parameters should be identified. In studies I and II, the ROC analysis was performed to investigate the accuracy of the quantitative MRI parameters to separate between the two sample groups. In previous clinical studies, CW-$T_{1p}$ has been reported as a more sensitive biomarker for cartilage degeneration than $T_2$ [123, 132, 133, 185]. This has also been demonstrated with the ROC analysis [133, 134], which is in agreement with the present results. In the present study, the AUC values of adiabatic $T_{1p}$, adiabatic $T_{2p}$ and CW-$T_{1p}$ relaxation parameters were high (0.8-1) in both the rabbit ACLT model and osteoarthritic human specimens in the full-thickness ROI analysis.

In studies I and II, the highest relative changes ranging from 50 to 60% were obtained for CW-$T_{1p}$, adiabatic $T_{2p}$ and $T_2$ relaxation parameters in the full-thickness analysis of cartilage. In study II, RAFF was also elevated over 60% in the advanced OA group. For adiabatic $T_{1p}$ the change was approximately 30% in both studies. The relative changes of adiabatic $T_{1p}$, RAFF and $T_{1sat}$ are similar to those found in a previous study [107]. The present results together with the ROC analysis indicate a high sensitivity and specificity of adiabatic RFR methods for cartilage degeneration.

In the present work, the relatively small number of samples in studies may have limited the statistical power of comparisons between the groups. Furthermore, completely intact cartilage specimens were not studied in study I or II. In the ACLT rabbit model, the contralateral knees likely do not represent fully intact tissue [186] and obtaining intact human cartilage specimens is typically difficult. However, the MRI techniques could separate between the sample groups and with healthy control tissues the changes could have been further emphasized. The MRI experiments were conducted in a non-physiological environment at very high 9.4 T $B_0$ field strength, and consequently the MRI techniques investigated require clinical validation studies. In study I, another limitation was the relatively thin cartilage on rabbit femoral condyles, approximately 10 imaging pixels, which is comparable, however, to the resolution achieved in human studies on clinical scanners. The advanced OA samples in study II may have experienced
tissue erosion which may have an effect on the zonal analysis of cartilage specimens. In study I, the cartilage degeneration was much milder and the tissue erosion was not so significant. Moreover, relaxation times in the superficial cartilage may have been influenced by small amounts of residual PBS solution since specimens in studies I and II were thawed in PBS prior to experiments, which may have led to longer relaxation times than expected. Nonetheless, perfluoropolyether was used to minimize this effect. Furthermore, the thicknesses of the full-thickness ROIs in study II were compared against the thicknesses derived from microscopy images. Although the segmentation of cartilage ROIs was done manually, the intra- and inter-operator variation was not investigated in the present study. However, the segmentation of the ex vivo specimens is quite straightforward and it was done consistently for each sample using the criteria specified in each study.

The comparison between CW-$T_{1\rho}$ and adiabatic $T_{1\rho}$ was limited since the SAR-values were not matched. With adiabatic pulses the peak spin-lock power was 2.5 kHz which equals approximately to 1090 Hz RMS power, which in turn is close to the CW-$T_{1\rho}$ experiment ($\gamma B_1 = 1$ kHz) in studies I and II. In clinical human studies, the use of CW-$T_{1\rho}$ has been limited to relatively low spin lock powers ($\gamma B_1 \leq 500$ Hz) due to SAR limitations, especially at high magnetic field strengths. Adiabatic $T_{1\rho}$ and $T_{2\rho}$ measured using HS$n$ pulse trains also require relatively high peak RF pulse power to maintain adiabatic condition, which may limit their usefulness at high $B_0$. However, both techniques have been successfully used for in vivo human brain imaging at 4 T [51, 135].

MRI relaxation time parameters have different sensitivities to the sample orientation with respect to the main magnetic field. In the present work, specimens were placed consistently in each study such that differences between samples due to the orientation were minimized. $T_2$ relaxation time is known to be susceptible to the magic angle effect, i.e. sensitive to the orientation of the collagen network with respect to the $B_0$ magnetic field. In addition, the RFR parameters have also been reported to be sensitive to the magic angle effect in articular cartilage. In initial studies, $T_{1\rho}$ has been shown to be less sensitive to sample orientation than $T_2$, with adiabatic $T_{1\rho}$ being even less sensitive than CW-$T_{1\rho}$ [129–131].

In summary, the systematic comparison of different quantitative MRI relaxation techniques revealed that rotating frame MRI relaxation parameters, namely $T_{1\rho}$ and $T_{2\rho}$, which are sensitive to slow macromolecular motion, efficiently detected degeneration in a rabbit model of early OA and in spontaneously degenerated human articular cartilage.
Furthermore, $T_{1\rho}$, $T_{2\rho}$ and also inversion prepared MT and RAFF were highly correlated to the histopathological grade and biomechanical status of the tissue in study II. Although RAFF was not sensitive to degeneration in the ACLT rabbit model, the technique can be implemented in a number of different configurations and higher rank rotating frames [53]. After optimizing the technique it may become a potential biomarker for cartilage degeneration, especially owing to its low SAR properties compared to the other RFR techniques. Furthermore, the method has already been implemented for imaging the human brain at 4 T with promising results [51, 52]. However, banding artifacts most likely caused by $B_0$ inhomogeneities in study I in CW-$T_{1\rho}$ as well as in RAFF maps [187] were absent in the adiabatic $T_{1\rho}$ or adiabatic $T_{2\rho}$ data, demonstrating the advantages of using adiabatic pulses that are less sensitive to $B_1$ and $B_0$ field variations. The present findings are also in agreement with another recent investigation in which the adiabatic $T_{1\rho}$ was found to be the most sensitive parameter to trypsin-induced changes in bovine articular cartilage structure [107]. The adiabatic RF pulses cover a broad range of resonance frequencies [48, 49], which may be the reason for improved sensitivity of the adiabatic $T_{1\rho}$ method as compared to the CW preparation. In addition, $T_{1\rho}$ in general has been reported to be a more sensitive biomarker for cartilage degeneration and less sensitive to the magic angle effect [129, 130] than $T_2$. In the future, clinical studies with RFR techniques employing adiabatic RF pulses may be preferable due to increased flexibility for dealing with the SAR constraints and reduced $B_0$ and $B_1$ inhomogeneity issues.

7.2 MRI of short $T_2$ components

In the present study, the feasibility of the SWIFT MRI technique for assessment of spontaneous osteochondral repair in the equine model and osteoarthritic changes in the human cartilage–bone interface was investigated. Assessment of tissues with very short $T_2$ relaxation time such as subchondral bone, or the zone of calcified cartilage require an MRI method, that is capable of capturing $T_2$ species in the sub-millisecond range. The SWIFT method utilizes interleaved excitation and signal acquisition [20, 21], capturing virtually all of the signals which are undetectable with conventional SE or GRE based sequences [18, 19].

The feasibility of SWIFT for evaluating features of articular cartilage and subchondral bone was demonstrated in study III. More specifically, the healing outcomes of the equine specimens were detected using SWIFT with fat and water suppression schemes.
Spontaneous cartilage repair was more successful in osteochondral lesions as compared to chondral lesions, which was also shown in the SWIFT images. Osteochondral lesions penetrate into subchondral bone, which releases stem cells that are capable of differentiation and production of new cartilaginous tissue and bone while spontaneous repair of chondral-only lesions is much more inefficient [4, 188–191]. With the fat and water suppression schemes of SWIFT, fatty bone marrow could be separated from fibrous tissue that infiltrated into the subchondral bone. In both equine and human cartilage, SWIFT without any contrast modifications demonstrated constant signal intensities in non-calcified cartilage in both degenerated tissue and intact cartilage, making the technique useful, for example, in morphological studies. Magnetization preparation schemes in SWIFT could also be used for relaxation time mapping ($T_1$, $T_{1p}$, $T_{RAFF}$) [64, 65, 67, 68], which provides a new perspective in the quantitative assessment of articular cartilage and bone.

In SWIFT difference images of relatively intact human cartilage (unpublished data), a band of high intensity signal was detected at the cartilage-bone interface, in the zone of calcified cartilage. A similar high-intensity signal at the osteochondral junction has also been found in UTE studies that utilize subtraction images of different TEs [159–161]. However, the same feature was not seen in equine cartilage (study III), possibly due to resolution as equine cartilage was much thinner than human cartilage in the present study, or due to actual physiologic differences.

With the advancement of cartilage degeneration, SWIFT difference images demonstrated loss or thinning as well as diffuse thickening of the high intensity signal at the cartilage–bone interface, which were associated with sclerosis of the subchondral bone, thickening of calcified cartilage and duplication of tidemarks, which are typical features in advanced OA [4, 9, 38]. The changes in the high intensity signal band are in agreement with recent UTE studies [161] and have previously been associated with increased vascular invasion of cartilage due to degeneration [162]. The source of the high intensity signal seems to correspond to the zone of calcified cartilage and/or deep regions in articular cartilage. Furthermore, the signal intensity changes likely reflect OA in some way, although the mechanisms are not fully understood.

Structural bone parameters derived from SWIFT combination images (fat + water image) correlated highly with those derived from micro-CT reference measurements, demonstrating the advantage of SWIFT in the imaging of calcified structures with a very short transverse relaxation time. In previous studies with high-resolution MRI, high correlations have also been found between MRI and micro-CT derived structural
bone parameters \[81, 152, 153, 155\]. It has been reported that the voxel size should be less than \(100^3 \ \mu m^3\) for a reliable estimation of the bone parameters and detection of osteoporotic changes \[175\]. Although SWIFT overestimated the absolute bone parameter values, the findings are in agreement with previous studies \[153, 156\].

In study III, the SNRs of SWIFT, GRE and FSE images were compared. Due to the superior \(T_2\) sensitivity of SWIFT in comparison to GRE and FSE techniques, the SNR was highest for SWIFT throughout the osteochondral specimens, especially in the subchondral cortical bone. Similar findings were observed in the human specimens although absolute SNRs were not calculated. As deep layers of cartilage have a short \(T_2\) relaxation time, intact cartilage appeared thicker in the SWIFT images in comparison to FSE imaging. This is because the longer TE of the FSE sequence results in poorer visibility of the deep cartilage layers and especially of bone. At lesion sites, the differences in cartilage thicknesses were minor, probably due to increased water content and resulting longer \(T_2\) relaxation times.

Previously, the SWIFT technique has been applied to the imaging of musculoskeletal tissues in only a few studies \[20, 65, 148, 157\]. The present findings indicate that SWIFT could be applied in the three-dimensional morphometric assessment of articular cartilage and subchondral bone, thereby not being limited to only monitoring osteoarthritic changes. With a further reduction in imaging time, three-dimensional relaxation time mapping with SWIFT may become useful for evaluating the status of the whole joint. Besides bone and cartilage, SWIFT could also be applied to other joint component with short \(T_2\)s such as menisci, ligaments or tendons. Furthermore, evaluation of the osteochondral junction could be useful in verifying the existence of calcified cartilage after cartilage repair. However, further in vitro and in vivo studies are required for testing its clinical applicability. Although SWIFT has moderate requirements on peak RF power, the interleaved excitation and signal acquisition scheme requires very fast transmit/receive circuits to yield practically zero effective TE. In terms of achievable echo times and scanning times, SWIFT is comparable to UTE and ZTE sequences \[57\]. One important advantage of SWIFT regarding the clinical implementation is the nearly silent MRI scans \[20, 148\], due to incremental changes in gradients, which are more comfortable for patients. In summary, SWIFT enabled the assessment of spontaneous osteochondral repair in an equine model and was sensitive to osteoarthritic changes in the human cartilage–bone interface.
7.3 Future prospects

In future, the intention is to perform clinical measurements using quantitative MRI methods, particularly with adiabatic $T_{1\rho}$ and adiabatic $T_{2\rho}$ to verify the potential indicated by the ex vivo results. In vivo studies with RFR techniques employing adiabatic RF pulses are preferable due to SAR properties and insensitivity to $B_0$ and $B_1$ field inhomogeneities. Adiabatic $T_{1\rho}$ dispersion in cartilage could be studied by modifying the contrast using different modulation functions with HS$n$ pulses. In addition, RFR techniques should be compared to conventional $T_2$ measurements. Development of magnetization preparation schemes for SWIFT may provide new aspects for quantitative MRI studies.

As all joint compartments are affected in OA, SWIFT should be applied to other short $T_2$ tissues and their pathologies such as menisci, tendons and ligaments. Furthermore, signal changes in subchondral bone and marrow with pathologies are to be quantified and investigated. Future studies should also compare SWIFT with UTE and ZTE sequences, for example, in terms of SNR and $T_2$ sensitivity. Investigation of the origin of the high intensity signal at the cartilage–bone interface in SWIFT images and the mechanisms of signal change in OA could potentially advance knowledge about the disease pathology.
8 Summary and conclusions

This thesis can be summarized into the following findings:

1. Adiabatic $T_{1\rho}$ and $T_{2\rho}$ relaxation time parameters were the most sensitive to both very early degenerative changes in a rabbit model of early cartilage degeneration and degeneration in human cartilage.

2. Rotating frame relaxation parameters and an inversion-prepared MT experiment were highly correlated with biomechanical parameters and the histopathological OARSI grade of degenerated human cartilage specimens.

3. The SWIFT MRI technique enabled three-dimensional assessment of the structural properties of subchondral bone as well as the assessment of the outcome of spontaneous osteochondral repair.

4. SWIFT MRI was sensitive to osteoarthritic tissue changes in the zone of calcified cartilage and subchondral bone, which were associated with the degree of OA.
References


comparing magnetic resonance imaging at 3 tesla with high-resolution peripheral quantitative computed tomography ex vivo and in vivo.


Original publications


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NOVEL MAGNETIC RESONANCE IMAGING TECHNIQUES FOR ARTICULAR CARTILAGE AND SUBCHONDRAL BONE

STUDIES ON MRI RELAXOMETRY AND SHORT ECHO TIME IMAGING