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Novel Quantitative Methods for the Diagnosis of Cartilage Degeneration

Doctoral dissertation

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ABSTRACT

Osteoarthritis (OA) is the most common joint disease. Its prevalence increases with age and virtually everyone over 65 years of age displays radiologic evidence of the disease. Over one-third of elderly population experiences symptoms from OA, causing pain and reducing the quality of life. In addition to the suffering caused to individual persons, substantial costs to the society arise due to this disease.

Current methods do not enable the diagnosis of the earliest OA changes in joints at a stage where spontaneous regeneration of the joint would be still possible. Quantitative and sensitive methods to detect early OA changes are needed if one wishes to study the efficacy of disease modifying osteoarthritis drugs (DMOADs) and to target their use optimally. Several biophysical methods for this purpose have been proposed, but little is known about their diagnostic performance and no objective comparison between these methods has been performed. Moreover, the effect of site-dependent variation of cartilage properties on diagnostic methods has remained unclear.

The present thesis work examined the effect of cartilage collagen network on its mechanical properties, especially on the Poisson's ratio. Moreover, the sensitivity and specificity of several quantitative biophysical diagnostic methods were determined and compared, including indentation methods, quantitative MRI (T_2 mapping and dGEMRIC) and several ultrasound parameters (R, IRC, URI). Further, we assessed the degree of spatial variation of mechanical, compositional and acoustic properties within one cartilage surface.

The results revealed that several of the novel quantitative diagnostic methods possessed both good sensitivity and specificity for detecting OA changes in cartilage. The measurement results were significantly correlated with the biomechanical properties and composition of cartilage. The indentation measurements could be used to quantify the mechanical properties of the tissue. However, if one wishes to diagnose tissue normality or pathology, the results should be compared with site-matched reference values. Importantly, the results suggest that in healthy tissue, no statistically significant topographical variation exists in the values of ultrasound reflection from the cartilage surface (p=0.61), and therefore no site-matched reference values are needed with this method. Further, the collagen network structure was found to be the primary determinant of the Poisson's ratio of cartilage.

In conclusion, the mechanical, ultrasound and MRI parameters show potential for accurate diagnostic tools for determining cartilage integrity and could be considered when determining the efficacy of DMOADs and the results of cartilage repair techniques. After effective DMOADs have been developed, these methods may well be suitable to screen individuals at risk for OA in order to detect those who might benefit from the medication.

To Anu and Sakari

Acknowledgements

Abbreviations

2D	2-dimensional
AUC	area under the curve
BMI	body mass index
COX	cyclooxygenase
CRP	C-reactive protein
CT	computed tomography
dGEMRIC	delayed gadolinium-enhanced magnetic resonance imaging of cartilage
DMOAD	disease-modifying osteoarthritis drug
ECM	extracellular matrix
ePLM	enhanced polarized light microscopy
FE	finite element
FTIRI	Fourier transform infrared imaging
GAG	glycosaminoglycan
Gd-DTPA^{2-}	gadolinium diethylene triamine pentaacetic acid
HHGS	histological-histochemical grading system
HUM	humeral head
ICRS	International Cartilage Repair Society
IL	interleukin
iNOS	inducible nitric oxide synthase
JSN	joint space narrowing
MFC	medial femoral condyle
MMP	matrix metalloproteinase
MRI	magnetic resonance imaging
MTP	medial tibial plateau
NEG	non-enzymatic glycation
NIH	National Institutes of Health
NSAID	non-steroidal anti inflammatory drug
PAT	patella

PG	proteoglycan
OA	osteoarthritis
OARSI	Osteoarthritis Research Society International
OATS	osteochondral autograft transfer system
OCT	optical coherence tomography
OD	optical density
RA	rheumatoid arthritis
ROC	receiver operating characteristic
SD	standard deviation
WOMAC	Western Ontario and McMaster Universities index for osteoarthritis

Symbols

α	attenuation
E_{DYN}	dynamic modulus of cartilage
E_{EQ}	equilibrium modulus of cartilage
E_{US}	ultrasound indentation modulus of cartilage
F_{IND}	indentation stiffness of cartilage
IRC	integrated ultrasound reflection coefficient
n	number of samples
p	p-value
r	correlation coefficient
R_{US}	ultrasound reflection coefficient from cartilage surface
SOS	speed of sound
T	tesla
T_1	T_1 relaxation time of cartilage
T_2	T_2 relaxation time of cartilage
URI	ultrasound roughness index

List of the original publications

This thesis is based on the following original articles referred to in the text by their Roman numerals:

I Kiviranta P., Rieppo J., Korhonen R.K., Julkunen P., Töyräs J., Jurvelin J.S.: Collagen network primarily controls Poisson's ratio of bovine articular cartilage in compression. Journal of Orthopaedic Research 2006; 24: 690-9; doi 10.1002/jor.20107

II Kiviranta P., Töyräs J., Nieminen M.T., Laasanen M.S., Saarakkala, S, Nieminen H.J., Nissi M.J., Jurvelin J.S.: Comparison of novel clinically applicable methodology for sensitive diagnostics of cartilage degeneration. European Cells and Materials 2007; 13: 46-55

III Kiviranta P., Lammentausta E., Töyräs J., Kiviranta I., Jurvelin J.S.: Indentation diagnostics of cartilage degeneration. Osteoarthritis and Cartilage. 2008; 16:796-804 (2008); doi:10.1016/j.joca.2007.10.016

IV Kiviranta P., Lammentausta E., Töyräs J., Nieminen H.J., Julkunen P., Kiviranta I., Jurvelin J.S.: Differences in acoustic properties of intact and degenerated human patellar cartilage during compression. Ultrasound in Medicine and Biology; 35:1367-1375 (2009). doi:10.1016/j.ultrasmedbio.2009.03.003

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1 Introduction

Osteoarthritis (OA) is the most common degenerative joint disease and it is responsible for major costs to modern society. It has been estimated that 20-40% of the elderly population suffers from symptomatic OA (Felson 1988). Radiographic evidence of OA changes in at least one joint has been reported in virtually all the population over the age of 65 years (Lawrence et al. 1966). The condition causes a major economic burden to the society through lost working time as well as social and medical costs. Musculoskeletal conditions, of which OA is the most common, have been estimated to consume up to 2.5% of the gross national budget of developed countries (March et al. 1997, Yelin et al. 1995). Nonetheless, the impact of the disease on the affected individuals and their relatives and friends is even greater, as OA causes pain, reduces the ability to perform activities of daily living and impairs the quality of life (Brooks 2002).

In OA, the normal functionality of the joint is disturbed due to degenerative processes that affect all components of the joint, in particular articular cartilage. Since the cartilage tissue lacks innervation, the early OA changes do not usually evoke any symptoms. After the process has advanced to a point where spontaneous regeneration of the joint is no longer possible, the first symptoms may be detected. At this stage, OA can often be diagnosed with current diagnostic modalities, such as plain radiographs. Interestingly, the exact origin of the symptoms in OA is still unclear (Dieppe et al. 2005).

There is currently no cure for primary OA, and the etiology of this disease remains still unresolved. However, active research is being conducted to reveal the mechanisms associated with the early OA changes so that subsequent degenerative OA processes could be halted or even reversed *e.g.* with drugs. It is evident that sensitive quantitative methods are needed to demonstrate the efficacy of these medications (Qvist et al. 2008). It is critical to detect OA early enough in order to efficiently target the use of these novel therapeutic approaches. Furthermore, the detection

of early degenerative changes could help to motivate patients to changes in their life-styles, e.g. to effective weight control or changes in working conditions. These are known risk factors for OA and can threaten the joint health (Cimmino et al. 2005). This could help in preventing the inexorable rise in the number of new OA cases (Felson et al. 1998). Furthermore, the development of cartilage repair techniques has created a demand for quantitative assessment of the results obtained with these procedures.

In the past two decades, several indentation techniques, acoustic methods and magnetic resonance imaging (MRI) methods have been developed to characterize and quantify cartilage properties. These techniques are mostly based on the determination of the properties of articular cartilage, which are known to change during the OA process. *I.e.* there can be changes in cartilage composition, the mechanical properties of the tissue and surface integrity. However, there are no comparative studies of these methods.

In this thesis work, we compared several novel quantitative methods based on magnetic resonance imaging, mechanical and acoustic determination of the cartilage properties. The methods are potentially suitable for clinical use and in the future they may assist in diagnosing early degenerative changes in cartilage. Furthermore, we characterized the structure-function relationships in normal and pathological cartilage to better understand the mechanical functioning of cartilage. In addition, we clarified the uncertainties induced by the potential change in cartilage ultrasound speed during mechano-acoustic measurements of cartilage integrity.

2 Articular cartilage

2.1 Structure and composition

Articular cartilage is highly specialized connective tissue which covers the ends of articulating bones in the diarthrodial joints. These joints consist of multiple components such as ligaments, supporting muscles and synovial membrane lining the synovial capsule (Wooley et al. 2005). The unique composition and structure of cartilage tissue enable the articulating surfaces to glide over each other with nearly no friction (Forster et al. 1996, Mow et al. 1992). Cartilage also acts as a wear-resistant surface during locomotion, as it absorbs mechanical energy during movement and distributes the loads evenly to the underlying bones with the help of other components of the joint, such as the menisci (Ahmed et al. 1983, Wooley et al. 2005).

Inspired by an illustration of Nucleus Inc (nucleusinc.com)
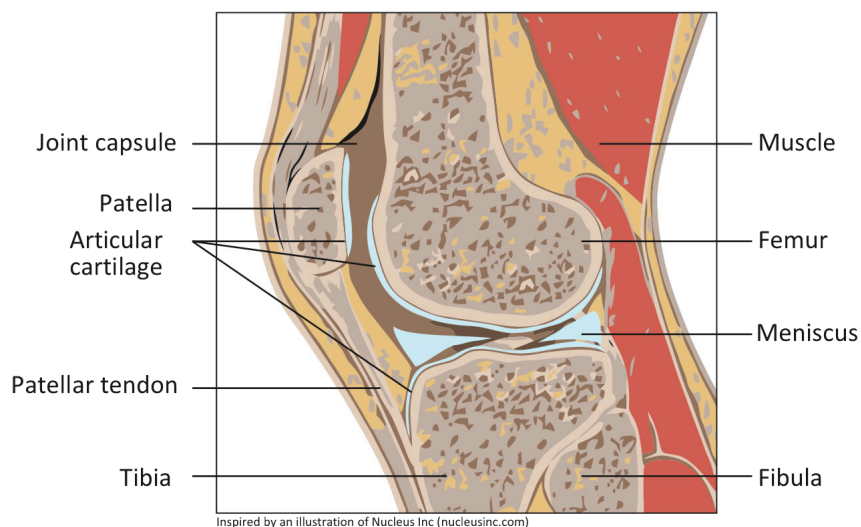
Figure 2.1 A sagittal section of the structure of the knee joint. Cartilage lines the articulating bone ends. The menisci are located between the tibia and femur and help to distribute the loads more evenly.

Cartilage tissue differs from most other soft tissues. As it lacks a vascular system, the nutrients for the cartilage cells, chondrocytes, predominantly diffuse from the synovial fluid. Furthermore, cartilage tissue has no innervation and therefore its early degenerative changes and minor lesions may be asymptomatic. Chondrocytes occupy only 1-2% of the total tissue volume (Hunziker et al. 2002, Stockwell 1967), with the rest of the tissue being composed of the extracellular matrix (ECM). The ECM produced by the chondrocytes consists mostly of proteoglycans (PGs) and a collagen fibril network (Huber et al. 2000).

Chondrocytes are cells of mesenchymal origin. They start their differentiation early during embryonic development (Corvol 2000). Chondrocytes are the only cell type present in articular cartilage tissue and therefore they play key role in determining cartilage health. Although a proliferative cohort of chondroprogenitor cells has been detected in young and fetal animal cartilage tissue (Dowthwaite et al. 2004, Koyama et al. 2008) and adult human chondrocytes have been shown to exhibit a low proliferative activity in OA cartilage (Aigner et al. 2001, Mankin et al. 1971), no actual chondroprogenitor cells have been observed in mature articular cartilage. This may impair the ability of adult articular cartilage to undergo repair after injury. In normal tissue, the chondrocytes maintain the cartilage homeostasis by controlling the anabolic and catabolic activity within the tissue. Then, the slow ECM component synthesis is in balance with the minimal and controlled activity of degrading enzymes, released to the surrounding ECM by the chondrocytes (Goldring 2000).

The synovial fluid in the joint cavity lubricates the contacting articular surfaces, contributing to the very low friction properties of the opposing cartilage surfaces. This viscous fluid secreted by the cells in the synovium contains mainly water, nutrients for chondrocytes, proteins and electrolytes (Fam et al. 2007).

Once articular cartilage has matured after the age of 20 years, its components have a very long lifetime and a slow rate of turnover (Bank et

al. 1998). In ECM, collagen has been evaluated to have a half-life of over 100 years (Verzijl et al. 2000). Therefore, in healthy cartilage, the turnover rate of proteins is very slow and the enzymatic degradation of ECM is minimal. The slow rate of turnover restricts the capacity of spontaneous regeneration after cartilage damage and this makes cartilage vulnerable to degeneration. The half-life of PGs is faster than that of collagens, varying from 3 to 25 years between different components of PGs (Maroudas et al. 1998).

2.2 Collagen fibril network

Collagen is the main protein in cartilage and in many other connective tissues. In cartilage, most collagen is type II, although small amounts of other types of collagen (III, IX, X, XI) have also been identified (Eyre 2002, Eyre et al. 2006). The variable types of collagens in articular cartilage play different roles in creating the typical fibrillar structure within the tissue (Eyre 2004). Type II collagen is synthesized by chondrocytes as procollagen, which consists of three alpha (II) chains (Prockop 1979). The procollagen molecules are secreted into ECM, where the N- and C-terminal propeptides of the molecules are enzymatically removed. The new collagen molecules exhibit a unique triple helical structure and they spontaneously assemble to form thin collagen fibrils. In tissues, collagen fibrils are packed together into more organized fibers which are responsible for the high tensile strength and dynamic compressive modulus of cartilage (Bader et al. 1981, Bader et al. 1992, Bader et al. 1994). The type II procollagen synthesis is most active during embryonic development (Nelson et al. 1998). The development of randomly oriented collagen fibrils has been suggested to create a scaffold, which develops into the typical three-dimensional highly specialized zonal

Superficial zone

Transitional zone
(middle zone)

Radial zone
(deep zone)

Tidemark
Calcified cartilage

Subchondral bone

Trabecular bone

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Collection of Medical Illustrations
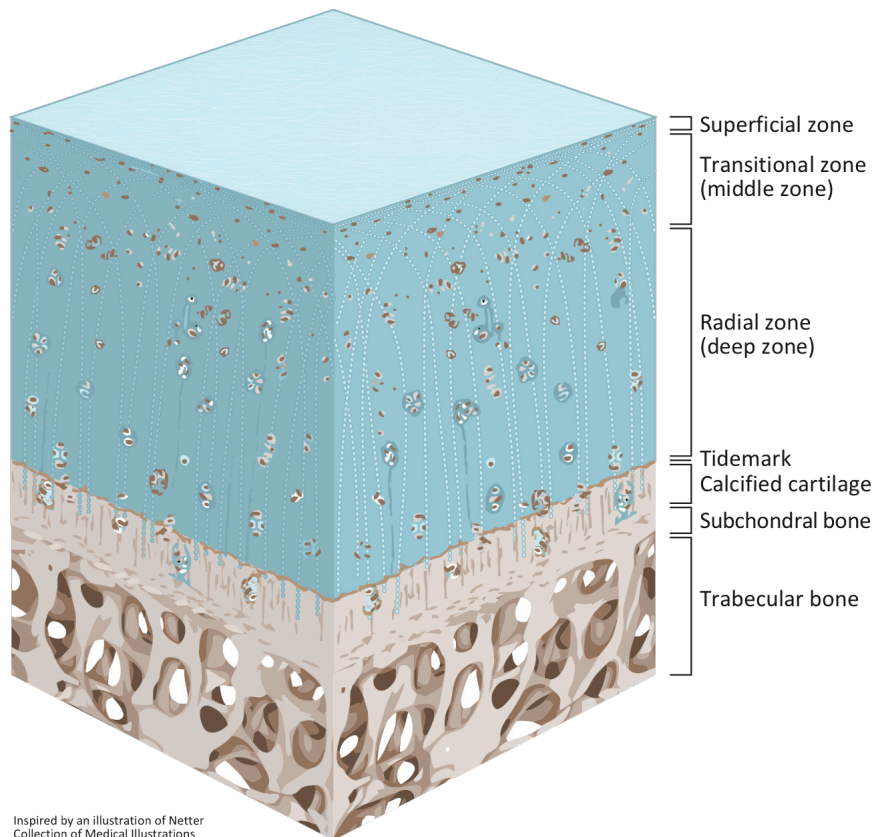
Figure 2.2 Structure of articular cartilage. The collagen fibrils in the superficial zone form a dense network that can resist high tensile forces. In the transitional zone, the orientation of the fibrils shows transition in their alignment. In the deep zone, the fibrils are aligned perpendicular to cartilage surface. The interface between the deep cartilage and the calcified cartilage is called the tidemark. The dense subchondral bone plate lies below the calcified cartilage layer. Under this layer, the bone transforms into trabecular bone.

architecture. This process may be activated by external loads, e.g. with the help of exercise (Helminen et al. 2000).In mature articular cartilage, the collagen fibers are arranged in a tri-laminar structure (Benninghoff 1925), as presented in **Figure 2.2**. The collagen fibers in the superficial zone are oriented in parallel to the cartilage surface, forming a wear-resistant

meshwork that resists tensile and dynamic compressive loads during movement. In the layer below the superficial one, the transitional zone, the collagen fibers descend towards the deep cartilage and the orientation of the fibers is more random. In the deep or radial zone, the collagen fibers run perpendicular to the cartilage surface and are linked to the subchondral bone. The deepest cartilage is calcified and is separated from the uncalcified cartilage by the thin tidemark layer.

2.3 Proteoglycans

Proteoglycans (PGs) are macromolecules composed of a core protein to which the glycosaminoglycan (GAG) chains are attached (Carney et al. 1988, Hardingham et al. 1992). The numerous negatively charged GAG side chains in PGs repel each other and this creates an osmotic imbalance, which is partially balanced by the presence of positively charged cations and influx of water into the cartilage tissue. Up to 90% of the total PG mass of cartilage is composed of large aggregating PGs (Huber et al. 2000). The PGs and hyaluronan associate to form large PG aggregates. Link proteins serve to stabilize PG aggregates and increase the size of the aggregates (Hardingham et al. 1974). The large size of the PG aggregates prevents the free redistribution of PGs despite the osmotic imbalance, and this leads to an influx of water into the cartilage and as a consequence to the expansion of the tissue matrix. This interaction between PGs, water and cations creates a positive swelling pressure, which is restricted by the stiff collagen network (Maroudas 1976, Mow et al. 1992).

2.4 Structure-function relationships in normal and degenerated cartilage

Articular cartilage is a permeable viscoelastic tissue with unique mechanical properties. The mechanical properties of the tissue can be characterized by determining *e.g.* the dynamic and equilibrium moduli of the tissue. Further, the lateral expansion of tissue under compression is described by Poisson's ratio of the tissue, *i.e.* the lateral to axial strain

ratio at equilibrium. These mechanical parameters and thus the function of the tissue are controlled by the composition and interactions between the main components of the cartilage, i.e. the solid phase with collagen network and inbound PGs, interstitial water and the ion phase with mostly positively charged cations (Kempson et al. 1970). In normal cartilage tissue, loading of the cartilage increases its internal pressure. Collagen fibrils resist the change in the shape of the tissue, and therefore in the presence of a persistent loading, the increased pressure causes a flux of water out of the cartilage tissue until a mechanical equilibrium is achieved. This balance during static loading is mainly controlled by the PG content of the tissue (Kempson et al. 1970). Therefore, the loss of PGs usually results in a decrease of the equilibrium compressive modulus of the tissue. The flow of the interstitial fluid is reflected by the permeability properties of the tissue. On the other hand, if the collagen network of the cartilage is damaged or the collagen content is reduced, a decrease in the dynamic modulus of the tissue can typically be observed.

Changes in the mechanical properties of the tissue also alter the functional capacity of cartilage to withstand normal loading. Therefore unfavorable changes in the mechanical properties may make cartilage more sensitive to further injuries.

The structure-function relationships have been intensively studied in articular cartilage with and without spontaneous OA changes as well as with cartilage samples that have been enzymatically treated to degrade either tissue collagen or proteoglycans (Bader et al. 1992, Bader et al. 1994, Rieppo et al. 2003). Typically, the dynamic or equilibrium response of cartilage could not be fully explained by the cartilage composition. This emphasizes the fact that cartilage is not simply a sum of its primary components and that the mechanical response of cartilage depends on the structure of the collagen network as well as on the interactions between its different components.

Several theoretical models have been developed to better understand the mechanical functionality of cartilage. The first mechanical model for

indentation of cartilage was the elastic single phasic model (Hayes et al.
1972). Later studies have incorporated the viscoelastic (Parsons et al.
1977) nature of cartilage and poroelasticity of the ECM into the biphasic
model (Mak et al. 1987, Mow et al. 1980). In the most recent mechanical
models, cartilage is modeled as poroviscoelastic material with complex,
inhomogenous intrinsic structure. The effect of both PGs and collagen
network on the mechanical behavior of cartilage is also incorporated.
These models have been shown to predict accurately the mechanical
behavior of cartilage during loading (Julkunen et al. 2008).

3 Osteoarthritis

3.1 Etiology and pathogenesis of osteoarthritis

Normal joint and articular surface
Smooth cartilage surface

Early degenerative changes
Superficial fibrillation of
cartilage surface
Slight increase in subchondral
bone density
Loss of PGs and reduction of
cartilage stiffness

Advanced degenerative changes
Progressive cartilage fissuration
Excavation of superficial and
mid-zone cartilage
Joint space narrowing
Marginal osteophyte formation
Development of bone cysts

End-stage degenerative changes
Cartilage denudation
Increased bone cysts
Deformation of the joint

Figure 3.1 Characteristic changes that take place during the OA process include fibrillation and cleft formation and alterations in mechanical properties of cartilage. Osteophyte formation, subchondral bone sclerosis and cyst formation are typical changes in bone associated with OA.

Osteoarthritis is the most common joint disease. It has been described to affect most joints of the human body; however, in clinical practice most important and most prevalent are knee, hip, hand, foot and spine OA. The disease is a continuum, where the earliest detectable degenerative changes include loss of PGs in the superficial layer of cartilage. An important step in OA is the initial fibrillation of the superficial collagen network (Buckwalter et al. 1997, Dodge et al. 1989, Mow et al. 1992). The

deterioration of the supporting collagenous meshwork leads to influx of water leading to a decrease in PG content of superficial cartilage. The initial loss of PGs from the superficial cartilage induces a steeper swelling pressure gradient in the deeper cartilage (Maroudas 1976). Therefore, a greater stress on the collagen network in deeper cartilage may occur, which exposes the cartilage to create the formation of deeper fissures, clefts, first in the transitional zone and these then progress into the radial zone and this worsens the damage to articular cartilage. As PGs and collagen are responsible for providing the structural and mechanical integrity of the cartilage tissue, the mechanical properties of cartilage are also typically impaired at an early stage (Knecht et al. 2006).

Healthy cartilage tissue maintains its integrity during a very slow renewing process. This seems to be most active around the chondrocytes and at the bone-calcified cartilage junction. The synthesis of matrix components is in equilibrium with the slow actions of enzymes that degrade the extracellular matrix. The metabolic activity of chondrocytes has been reported to increase in OA cartilage, perhaps the cells attempt to combat the tissue degeneration (Mankin et al. 1970). Several enzymes that degrade the components of articular cartilage tissue have been characterized (Cawston et al. 2006). Matrix metalloproteinases (MMPs) are regarded as the most important group of these enzymes, and an imbalance between the degrading enzymes and their inhibitors has been detected in OA (Dean et al. 1989). Other degrading enzymes, such as aggrecanases, have also been detected and their role in the development of OA is being studied (Burrage et al. 2007). Doubtlessly, OA process involves disruption of the strictly controlled homeostasis in cartilage. Indeed, OA has been demonstrated to induce increased release of collagen degradation products into synovial fluid (Lohmander et al. 2003) and serum (Christgau et al. 2004). The detection of these degradation products may help in diagnosing the earliest OA changes.

Since adult articular cartilage has a very limited capacity for spontaneous repair, originally small lesions may potentially progress to

OA if the degradative processes continue and overwhelm repair strategies (Huber et al. 2000). The cartilage defects in symptomatic OA tend to progress (Davies-Tuck et al. 2008). Therefore, once the patient develops symptomatic OA, the disease severity typically increases gradually with time (Felson et al. 1995). Therefore advanced OA is typically characterized by a progressive erosion of the articular surface followed by gradual denudation of the joint surface.

During the OA process, the subchondral bone is also altered. Cartilage and bone changes have been suggested to interrelate, at least to some extent (Felson et al. 2004b, Karsdal et al. 2008). Subchondral sclerosis and formation of the osteophytes are typical features of OA, and subchondral bone cysts are relatively frequently observed in advanced disease (Hayes et al. 2005). It has also been suggested that the subchondral bone sclerosis and stiffening would be one initiator of the OA process. This would cause an increase in the loading applied to cartilage (Radin 1976), with an associated secondary change of its structure. According to this hypothesis, the changes in cartilage would be secondary and take place subsequent to alterations in subchondral bone.

Several causes of secondary osteoarthritis have been identified and include factors such as high intensity impact joint loading, intra-articular fractures, ligament injuries and several metabolic disorders (Buckwalter et al. 1997, Buckwalter 2002, Buckwalter et al. 2006, Mitchell et al. 1977). In secondary OA, the degenerative changes in cartilage occur as a consequence of some trigger which endangers the mechanical integrity of the tissue, typically by damaging the collagen network of cartilage. However, in most cases, no such cause can be identified and the disease is termed primary OA. The etiology of this disease is still unclear (Buckwalter et al. 2004). Although primary OA is very strongly associated with increasing age (Crepaldi et al. 2003, Kirkwood 1997), it should not be regarded as "normal wear and tear" (Buckwalter et al. 1997).

Several risk factors for primary OA have been identified in large-scale follow-up studies (Arden et al. 2006, Buckwalter et al. 2004, Cimmino et

al. 2005, Felson et al. 1997, Felson et al. 1998, Felson et al. 2000, Felson 2004, Sangha 2000). The prevalence of OA has long been known to be more common in women (Buckwalter et al. 2000), and therefore the involvement of sex hormones has been proposed. Another factor elevating the risk for OA is increased body weight, *i.e.* body mass index (BMI) (Felson et al. 1988, Schouten et al. 1992, Spector et al. 1994), especially when it is combined with malalignment of the joint (Felson et al. 2004a, Sharma et al. 2000). Earlier knee trauma is a risk factor for later knee OA (Gelber et al. 2000), particularly if it involves either partial or total meniscectomy (Roos et al. 1998). This arises due to the critical role of the meniscus in distributing the loads evenly between the femoral and tibial joint surfaces and the removal alters the load distribution. Both direct injury and meniscectomy may expose cartilage to mechanical overloading, which can damage the cartilage and lead to OA (Kurz et al. 2005, Mankin 1982). Further, in animal models, strenuous running exercise has induced alterations in the cartilage collagen network, which may be indicative of degenerative changes and possible future cartilage degeneration (Arokoski et al. 1996, Brama et al. 2000). However, the effect of exercise as a risk factor in humans is more unclear (Urquhart et al. 2008). It appears that large individual variation exists in the ability of cartilage to withstand loading. Possible explanations for this could be variations in the PG content of cartilage, as it has been shown in animal studies that the PG content of cartilage decreases during immobilization (Kiviranta et al. 1987). After the PGs have been depleted, strenuous loading of the joint may cause irreversible damage by disrupting the reversal of the atrophic changes (Palmoski et al. 1981) and this can initiate the OA process in the joint (Buckwalter 1995). It is evident, however, that participation in strenuous types of sports may jeopardize the health of load bearing joints (Lane et al. 1999, Marti et al. 1989).

Overall, it has been suggested that clinical OA could represent a cluster of conditions of different origins leading to the same endpoint (Hart et al. 1995, Mitchell et al. 1977). In addition to articular cartilage,

OA affects all components of the joint, including synovium, subchondral bone and ligaments (Aigner et al. 2006, Brandt et al. 2006).

OA is often clustered in families. This has led to thoughts that genetic factors predispose to this disease (Cicuttini et al. 1996, Kellgren et al. 1963). This was confirmed by results from a twin study, where the heritability of OA was estimated to range from 39% to 65% (Spector et al. 1996). However, even in the early studies of OA genetics, it was noted that primary OA does not follow a simple Mendelian heritability (Kellgren et al. 1963). This suggests that the disease is not caused by a single gene mutation and, to date, no single gene mutation to account for all OA cases has been identified. Therefore the genetic predisposition to OA has been interpreted as signifying a multifactorial disease (Bateman 2005) and several genetic loci for OA susceptibility genes have been identified (Aigner et al. 2003, Peach et al. 2005).

3.2 Prevalence of osteoarthritis

The development of radiological criteria for OA in the 1950s by Kellgren and Lawrence made it possible to conduct studies to determine the prevalence of radiographic OA (Kellgren et al. 1957). From the 1980s, extensive population based studies have been carried out to examine the prevalence of radiological and symptomatic OA. The studies have mostly evaluated OA in knee joints or hands. These studies have revealed that prevalence of OA increases with age. Further, women are more often affected by this disease. Since the percentage of population over 65 years of age is on the increase, the total number of patients suffering from this disease has been estimated to increase in the near future. However, the incidence of knee OA has been reported to decline in women under 75 years, but to increase in men over 85 years of age in a Finnish population based study (Heliövaara et al. 2007).

OA is a very common musculoskeletal disorder (Lawrence et al. 2008, Petersson 1996, van Saase et al. 1989) It is estimated to be the most common chronic condition that causes limitation in activity in population

over 45 years of age (Verbrugge et al. 1995). In large-scale studies using radiographic imaging, evidence of OA changes has been detected in practically all individuals older than 65 years of age (Lawrence et al. 1966). The lifetime risk of symptomatic knee OA has been estimated to be 45% in the general population, and it has been calculated to affect nearly 2 out of 3 persons with an elevated BMI (Murphy et al. 2008). The reported incidence of OA in population studies varies extensively (**Table 3.1**). The discrepancy between the results is partially explained by the differences in methodology, *i.e.* some studies included only radiographic evaluation of certain joints, others have included also extensive physical examination together with radiographic imaging. Furthermore, the prevalence between different geographic areas and populations seem to explain part of the variation (Corti et al. 2003). In studies conducted in the USA, the prevalence of radiographic knee OA has been found to reach 55.6% in women and 40.7% in men over 80 years of age (Dillon et al. 2006). When symptomatic OA is defined as a disease in which the patient experiences frequent pain in a joint with radiographic evidence of OA, the prevalence of symptomatic knee OA has been estimated to achieve values up to 18.7% and 13.2% for female and male populations over 45 years of age, respectively (Jordan et al. 2007). A recent extensive Finnish population study evaluated the prevalence of knee OA to be higher than that of American population (Arokoski et al. 2007). However, in that study, the evaluation did not systematically include X-ray imaging of the joints, and thus the diagnosis was based on a thorough clinical examination supported by earlier medical history and imaging studies. A subgroup analysis of this study revealed also high rates for radiologic OA in Finnish population as well as a moderate agreement between the clinical and radiological diagnosis (Toivanen et al. 2007).

Hand OA is even more common than knee or hip OA. Typically, radiographic OA changes in the small joints of the hand can be detected in virtually everyone over 70 years of age (Hochberg et al. 1993, Lawrence

et al. 1966), but only about one-fourth of them experiences a symptomatic disease (Zhang et al. 2002).

The high prevalence of OA explains partially the massive burden of this disease on individuals and societies. Furthermore, the burden is increased by the chronic nature of the disease, *i.e.* once individuals develop symptomatic OA, they will suffer from the disease for the rest of their lives (Buckwalter et al. 2004). The disease hinders the functional capacity of the individuals, restricts their social activities and well-being (Brooks 2002, Carr 1999). Furthermore, costs are generated directly and indirectly as medical and social costs and absence from work. Indeed, the total burden of all musculoskeletal disorders has been estimated to be equivalent to between 1-2.5% of the total gross national product (March et al. 1997, Yelin et al. 1995).

Table 3.1 Prevalence of OA.

Reference, number of subjects	Diagnostic method	Age (years)	Prevalence (%) Male	Female	Total
Knee OA					
The Framingham Study, n=1424 (Felson et al. 1987)	Radiographic	63-94	30.9	34.4	33.0
		63-69	30.4	25.1	27.4
		70-79	30.7	36.2	34.1
		80-	32.6	52.6	43.7
Johnston County OA project, n=3018,	Radiographic	45-	23.7	31.0	27.8
(Jordan et al. 2007)	Symptomatic*	45-	13.5	18.7	16.4
NHANES III, n=2415 (Dillon et al. 2006)	Radiographic	60-			37.4
		60-69	27.4	35.2	
		70-79	33.5	44.6	
		80-	40.7	55.6	
	Symptomatic*	60-			12.1
		60-69	7.5	10.6	
		70-79	12.9	15.3	
		80-	12.7	18.2	
Kuopio OA 2000 Study, n=130, (Toivanen et al. 2007)	Radiographic	45-82	26.8	23.6	24.6
Health 2000 Survey, Finland n=6354,	Clinical	30-44	0.3	0.4	
(Kaila-Kangas 2007)		45-54	2.7	2.2	
		55-64	9.1	8.2	
		65-74	10.8	18.2	
		75-84	15.6	32.1	
		85-	44.2	35.6	
Hip OA					
(Lawrence et al. 1966) ** n=567	Radiographic	55-	18.7	9.2	13.4
Health 2000 Survey, Finland, (Kaila-Kangas	Clinical	30-44	0.5	0.4	
2007)		45-54	1.8	0.8	
n=6354		55-64	5.2	3.1	
		65-74	12.2	11.5	
		75-84	20.4	20.4	
		85-	39.8	24.5	
Hand OA					
The Framingham Study, n=1041, (Zhang et al. 2002)	Symptomatic*	71-74	16.4	27.2	
		75-79	11.9	26.1	
		80-	13.5	26.0	
n=317, (Hochberg et al. 1993)	Radiographic	40-49		21.4	
		50-59		58.8	
		60-69		83.5	
		70-79		96.0	
		80-		100.0	

*Symptomatic OA (Presence of joint symptoms in at least one joint with radiographic evidence of OA in the same joint)
**Data adapted from Lawrence 1966 by calculating the percentage of all studied people over 55 years of age with radiographic hip OA despite the presence or absence of RA.

3.3 Treatment and prevention of osteoarthritis

Several global organizations and authors have published guidelines for good treatment of OA (Altman et al. 2000, Conaghan et al. 2008, Working group established by the Finnish Medical Society Duodecim and the Finnish Orthopaedic Association 2007). Currently, there is no effective treatment to slow down or stop the OA process. Therefore, the treatment mainly emphasizes the alleviation of the symptoms and pain management. Further, it would be optimal if the treatment could improve and maintain the functional capacity of the patient. It has been recognized that the treatment of OA should be tailored individually, and if applicable, it should always include non-pharmacological treatments such as patient guidance, exercises and weight reduction (Dieppe et al. 2005, Felson et al. 1992, Jordan et al. 2003, Roddy et al. 2006). Exercise has been shown to have beneficial effects in patients with OA (Bennell et al. 2005, Ettinger et al. 1997). Although the effect of this approach is usually limited (Fransen et al. 2008), it should be regarded as a first line treatment together with the empowerment of the patient.

3.3.1 Pharmacological pain control strategies

Most therapeutic recommendations agree that when the non-pharmacological intervention is not sufficient in treating pain, the pharmacological treatment should begin with paracetamol because of its favorable efficacy-safety profile (Bannwarth 2006, Jordan et al. 2003). Topical analgesics or peroral non-steroidal anti-inflammatory drugs (NSAIDs) should be considered for those patients, who are unresponsive to paracetamol. However, since NSAIDS may be superior to paracetamol (Towheed et al. 2006), some experts consider that NSAIDs should be used as the first-line oral analgesics. The gastrointestinal problems associated with NSAIDs must be taken into account for individuals at risk and the cyclooxygenase 2 (COX-2) selective NSAIDs can be used for patients who exhibit a higher risk for suffering gastrointestinal side effects. Lately, the cardiovascular safety of COX-2 selective and other NSAIDs has been

debated. Currently, COX-2 selective medications are contraindicated for people with ischemic heart disease and hypertension if the blood pressure is over target values. Opioid analgesics, such as codeine and tramadol can be used as such or combined with paracetamol in patients for whom NSAIDs are contraindicated, ineffective or poorly tolerated (Jordan et al. 2003). Furthermore, novel approaches in treating pain in patients with OA are being developed and include modulation of the sensory protein response at the nociceptive nerve endings (Schaible et al. 2006).

Intra-articular treatment with hyaluronan or glucocorticoid injections has also been utilized (Gerwin et al. 2006). These therapies seem to provide temporary relief of pain in certain patients, but no convincing evidence for disease-modifying effects has been demonstrated (Ayral 2001, Gossec et al. 2006).

3.3.2 Quest for disease-modifying osteoarthritis drugs

It has been proposed that the initial degenerative changes are still reversible in the very first stage of the OA process. This is why the early detection of the cartilage impairment is so crucial. Currently, intensive research is being conducted to find ways to stop or reverse the degenerative processes in cartilage (Pelletier et al. 2007, Steinmeyer et al. 2006). Commonly, these drugs are called disease-modifying osteoarthritis drugs (DMOADs) (Goldring 2006) or structure-/disease-modifying agents for osteoarthritis (Altman 2005). The quest for these drugs by the pharmaceutical industry has been continuing for a long time without any major breakthrough (Qvist et al. 2008).

Several studies for pharmacological intervention aimed at slowing down, stopping or even reversing the OA process are being conducted (Krasnokutsky et al. 2007, Pelletier et al. 2005, Pelletier et al. 2007). Since OA is recognized as affecting all components of the joint, it has been suggested that the medical treatment should also target all of these components (Goldring 2006). Increased knowledge about signaling pathways within the chondrocyte might be one way to find solutions to

influence the reparative, catabolic and inflammatory reactions (Goldring et al. 2004, Malemud et al. 2003), and the ultimate goal is to cease or even reverse the degenerative processes within the joint. Attempts have been made to alter the matrix metalloproteinase (MMP) response within the joint using MMP-inhibitors (Burrage et al. 2007, Cawston et al. 2006, Murphy et al. 2005) or by inhibition of MMP gene expression (Mix et al. 2004) and by modifying the cytokine cascade with interleukin (IL) 1b-inhibitors (Braddock et al. 2004). Furthermore, it has been suggested that the OA process might be modulated by targeting the processes associated with subchondral bone turnover (Karsdal et al. 2008). Therefore, drugs originally intended for treating osteoporosis, such as the bisphosponates (Bingham et al. 2006, Spector et al. 2005) and calcitonin (Bagger et al. 2005, Karsdal et al. 2007, Manicourt et al. 2006) have been proposed for the treatment of OA. These compounds have been shown to induce a significant reduction in biochemical cartilage degradation markers (Bagger et al. 2005). However, clinical trials with these pharmaceuticals have not been able to reveal any effect on the progression of the disease (Qvist et al. 2008). Doxycycline (Brandt et al. 2005) and diacerein (Dougados et al. 2001) have also been suggested to function as DMOADs, and studies are continuing with substances affecting the inducible nitric oxide synthase (iNOS) (Adis R&D Profile 2007). Furthermore, some attempts have been made to exploit gene therapy for the treatment of osteoarthritis (Evans et al. 2004, Evans et al. 2006, Frisbie et al. 2002, Goldring 2006). Regulation of the expression of genes responsible for producing ECM has been proposed as a potential way of reversing the OA process (Okazaki et al. 2004).

In some countries, glucosamine and chondroitin sulphate are labeled as dietary supplements, whereas in other countries they require a prescription. Their mechanism of action is unknown, but in some studies they have been claimed to confer a chondroprotective action, slowing the progression of the disease (Reginster et al. 2001). However, convincing evidence of their effect is still lacking. They are known to be partially

absorbed in the gastrointestinal tract (Ronca et al. 1998), but their actions within the joint are still unclear. In all studies, both glucosamine and chondroitin sulphate have been shown to be tolerated as well as placebo. Several studies supported or performed by the manufacturer of these agents have claimed to demonstrate a pain-relieving effect of these agents (McAlindon et al. 2000). However, because of concerns about the quality of the trials, meta-analyses were performed and no statistically significant benefit of glucosamine for pain or functionality was found (Towheed et al. 2005). Furthermore, an independent double-blinded study, funded by the National Institutes of Health (NIH) with the same aim, failed to confirm the efficacy of glucosamine, chondroitin sulphate or their combination (Clegg et al. 2006).

Several promising molecules are in pre-clinical trials, and some have advanced to clinical trials for OA treatment (Goldring 2006, Qvist et al. 2008). One major obstacle is the difficulty in developing diagnostic methods for early cartilage degeneration, and subsequently for monitoring the effect of these novel pharmaceuticals (Goldring 2006, Qvist et al. 2008). In order to determine the efficacy of these novel medications, it is crucial to develop novel methods which are capable of sensitively detecting the effects of each treatment.

3.3.3 Surgical treatments

Operative treatment is often considered in advanced OA when medical treatment fails to alleviate pain sufficiently (Jordan et al. 2003). Arthroscopic lavage and débridement are typical arthroscopic procedures used to treat patients who are unresponsive to pharmacological therapy (Frizziero et al. 2005, Segal et al. 2006). In uncontrolled studies these procedures have had a limited effect on OA symptoms, in particular it has proved difficult to select those patients who would benefit most (Dervin et al. 2003). However, in blinded and randomized studies, no difference between the groups that had gone through actual or sham procedures has been noted (Bradley et al. 2002, Moseley et al. 2002). High tibial

osteotomy has been used to treat the medial compartment OA of the knee by surgical realignment of the loading axis of the limb. Once again, the results with these procedures are somewhat contradictory (Brouwer et al. 2005).

In progressive OA, only arthroplastic surgery can alleviate the pain and improve the functionality of the joints (Franceschini et al. 2005, Katz 2006). Although the operation is costly, it has been evaluated as being cost-effective (Chang et al. 1996). The annual rate of total joint replacement has increased (Katz 2006), and OA is the most common indication for total knee replacement. In Finland, the incidence of primary total hip replacements in the year 1999 was 93/100,000 (Puolakka et al. 2001), whereas the incidence for primary total knee replacements in Great Britain was 62/100,000 and 70/100,000 for men and women, respectively (Dixon et al. 2004).

3.3.4 Cartilage repair techniques

Several cartilage repair techniques have been developed and several of these are in clinical use. The microfracture technique, osteochondral transplants (Mosaicplasty or osteochondral autograft transfer system [OATS]) and autologous chondrocyte implantation are techniques used to repair articular cartilage lesions. The first clinical applications of artificial engineered cartilage have been introduced lately and intensive research is being conducted to develop even more efficient techniques and materials for cartilage repair. Currently, these techniques are used for treatment of localized cartilage lesions, mostly of traumatic origin. Generalized OA is not an indication for these procedures. However, attempts have also been made to treat primary OA using tissue-engineering techniques. Biologic solutions, manufactured using tissue-engineering techniques are also being sought to replace metal prostheses with viable biological materials (Nesic et al. 2006, Schurman et al. 2004). Also then, improved diagnostic techniques are needed to quantitatively assess the benefits of cartilage repair techniques.

3.3.5 Prevention of osteoarthritis

As in any disease, the prevention of OA should be practiced at all levels to minimize the incidence and total burden of the disease (Felson et al. 1998). Measures should be targeted at modifiable risk factors. The most important targets include reduction of obesity, prevention of knee injuries and modification of activities, particularly those involving lifting heavy loads or bending on knees. If it were possible to achieve these targets, then it has been estimated that over half of all new OA cases could be prevented (Felson et al. 1998).

4 Clinical methods for diagnosis of osteoarthritis and cartilage degeneration

4.1 Current methods and criteria for OA diagnosis

In clinical practice, patient history and clinical examination are the two first steps in diagnosing any joint disease (Dieppe et al. 2005). The patient is asked about the development, duration and type of symptoms. Joint stiffness is often present, and pain typically increases during loading and alleviates at rest, but in advanced OA, also pain at rest and night pain may be present (Dieppe et al. 2005, Hunter et al. 2006).

The physical examination includes careful inspection of the affected joint *e.g.* for signs of inflammation or deformation. The joint is also palpated, whereby deformation and tenderness of the joint can be further evaluated. The range of movement of the joint and the stability of the ligaments around the affected joint are also tested (Hunter et al. 2006). Effusion of the joint can be present. If needed, further tests or imaging procedures can be undertaken to support the clinical diagnosis of OA or to rule out other diseases. This all aims at achieving a comprehensive evaluation of the affected joint.

Several diagnostic criteria have been presented for hand, knee and hip OA, but most commonly used are those published by the American College of Rheumatology (Altman et al. 1986, Altman et al. 1990, Altman et al. 1991). However, all diagnostic strategies have certain limitations and none of these methods has gained as universal approval as the Kellgren-Lawrence classification for OA features in radiographs (Peat et al. 2006, Sangha 2000). The clinically defined OA diagnosis can be set based on the symptoms of the patient and findings from physical examination of the patient (Felson 2006, Lane 2007). The subjective symptoms of the patients can be assessed with different questionnaires, such as Western Ontario and McMaster Universities index for Osteoarthritis (WOMAC) (McConnell et al. 2001), but these are not used routinely in clinical

practice (Fioravanti et al. 2005). The clinical-radiological criteria require that the patient has frequent pain in a joint with radiographic OA changes. The most often utilized imaging technique in OA diagnostics is X-ray imaging.

4.1.1 Radiography

The most widely used radiographic classification system for OA was introduced by Kellgren and Lawrence in 1957 (Kellgren et al. 1957). The criteria are based on visual evaluation of the joint space, subchondral sclerosis and detection of osteophytes (**Figure 4.1**). The grading system was a major breakthrough, as it enabled the study of prevalence and advancement of OA. Later, the inter- and intra-rater reliabilities of the technique have been evaluated to be sufficient (Gunther et al. 1999). The criteria for radiographically verified OA by Kellgren and Lawrence have been utilized in several studies which aimed to determine the prevalence of OA and its risk factors (Felson et al. 1987, Kellgren 1961).

Plain radiography has long been the primary diagnostic modality together with the clinical examination. When judging the radiographs, the width of the joint space is evaluated to detect any possible narrowing. Furthermore, the structure of articulating bone ends is assessed in order to detect the presence of osteophytes and deformation or other changes in the subchondral bone, such as sclerosis.

The Kellgren-Lawrence system for classifying radiographic OA.
0 Normal joint structure.
1 Doubtful OA: Possible marginal osteophyte, possible joint space narrowing.
2 Minimal OA: Definite joint space narrowing and osteophytes.
3 Moderate OA: Several moderate osteophytes, advanced joint space narrowing, possible deformation of bone ends, mild subchondral sclerosis.
4 Severe OA:Several large osteophytes, notable joint space narrowing, deformation of bone ends, subchondral sclerosis.

Figure 4.1 Radiologic changes in OA process from normal joint to one with severe OA changes according to Kellgren and Lawrence. Radiographs by courtesy of Dr. Marja Pitkänen.

A serious problem with these commonly used diagnostic methods is their low sensitivity for detecting changes associated with early cartilage degeneration. As articular cartilage is radiolucent in conventional X-ray imaging, cartilage changes can be assessed only indirectly via the evaluation of joint space narrowing (JSN). Furthermore, the earliest degenerative changes cannot be detected in the physical examination, and they are often asymptomatic, as cartilage tissue has no innervation (Wooley et al. 2005). The role of radiographs has been questioned, but they still have important value in determining the severity of the progression of the disease (Cibere 2006). Furthermore, major discordance can exist between the radiographic OA changes and the symptoms of the patients (Hannan et al. 2000). No single clinical symptom or a combination of symptoms has been able to reliably predict radiographic knee OA (Claessens et al. 1990). Therefore, the plain radiographs have maintained their position in clinical practice and research and are useful when deciding about the mode of treatment.

4.1.2 MRI

The development of imaging modalities, such as magnetic resonance imaging (MRI) and computed tomography (CT) has improved the diagnostics of many diseases, including OA. When compared to radiographs, MRI permits the visualization of all components of the joint, including soft tissues such as articular cartilage. Furthermore, changes and degeneration in subchondral bone, ligaments and menisci can be detected. With novel high resolution MRI devices, it is possible to detect also minor cartilage lesions as well as alterations in other joint components. In clinical practice, MRI is not the primary imaging modality when diagnosing OA due to the associated high cost and low availability. However, when it is applied, typically the morphology of the cartilage is evaluated visually (Hayes et al. 2005). The quantitative parameters of cartilage MRI, which are discussed later, are mostly limited to research purposes.

4.1.3 Arthroscopy

Articular cartilage lesions can be visually evaluated in arthroscopy. In an attempt to standardize the visual classification of cartilage injury, the International Cartilage Repair Society (ICRS) has created a visual classification system for grading cartilage damage during normal arthroscopy (ICRS Hyaline Cartilage Lesion Classification System) (Brittberg et al. 2003). This system classifies the damage into four grades depending on the lesion depth (0 Normal, 1 Nearly normal, 2 Abnormal, 3-4 Severely abnormal) with each grade having one to four subgroups (**Figure 4.2**). An estimate of the damaged area size can also be judged. In addition, a manual tool is often used for subjective palpation of cartilage stiffness and tissue integrity. However, these measures are subjective and only semiquantitative at their best. Furthermore, the estimates of damaged area size have proven to be rather inaccurate (Oakley et al. 2003).

4.1.4 Ultrasonography

Ultrasound has a long history as an imaging modality when studying soft tissues. Ultrasound imaging of joint structures is relatively often used in the treatment of patients with rheumatoid arthritis (RA). In patients with RA, ultrasound imaging can be used to screen joints for signs of disease activity, such as effusion and thickening of the synovium (Cimmino et al. 2008). With modern ultrasonographic appliances, also the small joints of the hand and feet can be evaluated (Cimmino et al. 2008). Visual evaluation of ultrasonographic 2D-images can be used to detect some typical visual features of OA (Aisen et al. 1984, Disler et al. 2000), and even transcutaneously *in vivo* (Conaghan et al. 2005, D'Agostino et al. 2005, Grassi et al. 2005). Again, there is no consensus about the usability of the method in clinical practice.
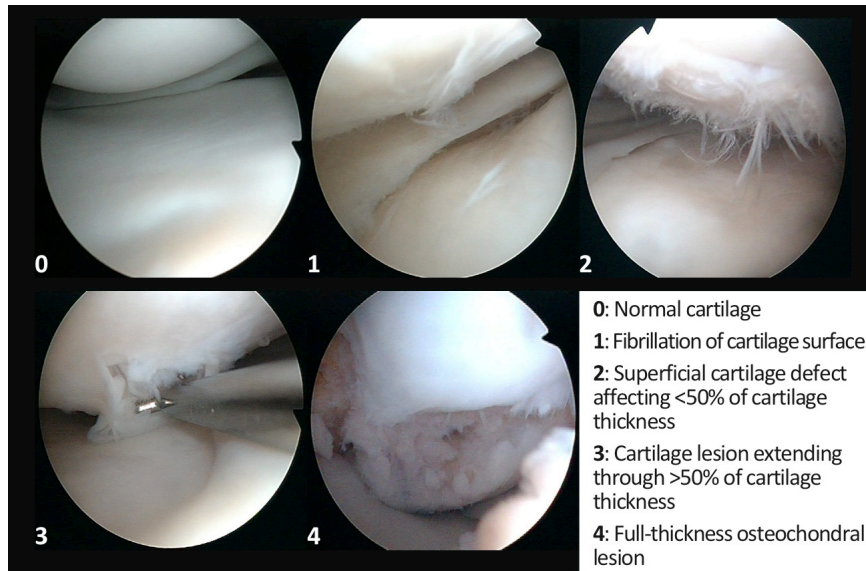
Figure 4.2 Images from human knee arthroscopy. **0)** Normal cartilage tissue with smooth, intact surface (ICRS 0). **1)** Cartilage with fibrillation and superficial lacerations (ICRS 1) **2)** Abnormal cartilage sample, where the lesion extends deeper than the superficial layer, but less than 50% of total cartilage thickness. **3)** Cartilage lesion, which extends through >50% of total cartilage thickness. In this figure, an arthroscopic probe is visible and is being used to test the lesion depth. **4)** Full-thickness osteochondral lesion, where subchondral bone is also affected (ICRS 4). Arthroscopic images by courtesy of Dr. Heikki Nurmi.

5 Novel methods for detection of early signs of cartilage degeneration

5.1 Need for early OA diagnosis

A serious hindrance for the development of early OA diagnostics is the fact that there is currently no curative treatment for the condition. In the case early OA changes could be detected, there is a lack of treatment options to prevent the progression of the disease. To motivate the development of more sensitive diagnostic tools for early OA detection, efficient treatment options for prevention of OA progress would also be needed. As described earlier, active research is being conducted *e.g.* to find pharmaceutical treatments for these indications. However, this research is at least in part restricted by the fact that there are no generally accepted methods for detection of early cartilage degeneration (Qvist et al. 2008).

5.2 Early degenerative changes

The earliest changes in the degenerative processes of cartilage include fibrillation of the superficial tissue and impairment of its mechanical properties. Fibrillation is a process, where the superficial collagen network is damaged and the integrity of superficial tissue is impaired. A decrease of the PG content in the superficial cartilage has been suggested to be one of the first detectable changes in the OA process (Buckwalter et al. 1998) and this leads to a decrease in the compressive stiffness. The altered mechanical properties make the tissue more vulnerable to further damage. The disruption of the superficial collagen network creates minor cracks in the cartilage surface, which increases the friction between the articulating joint surfaces.

5.3 Indentation techniques

The functionality of articular cartilage depends on its mechanical properties. Therefore, by determining these properties, important

information on cartilage functionality can be obtained (Knecht et al. 2006). Several methods have been applied to determine mechanical properties of cartilage tissue.

Indentation methods have been developed for the quantitative assessment of the mechanical properties of cartilage. For *in vivo* assessment of cartilage with these methods, at least the arthroscopic approach is required. Most equipment developed for this purpose is based on applying a predefined compression of cartilage surface mechanically with an instrument and then making a simultaneous measurement of the applied forces (Appleyard et al. 2001, Lyyra et al. 1995, Niederauer et al. 2004). The force by which cartilage resists deformation is regarded as an index for structural stiffness. The thickness of cartilage cannot be evaluated with conventional indentation devices even though the thickness is known to affect the indentation response of cartilage. Therefore, the determined stiffness values include some error, as the variation in tissue thickness is not taken into account (Hayes et al. 1972). Furthermore, it is considered that these methods gather information mostly from the superficial layer of cartilage (Korhonen et al. 2002b). Challenges in obtaining reproducible localization and orientation of the indentation have also restricted the spread of the method into wider clinical use.

5.4 Ultrasound and mechano-acoustic methods

To overcome the restrictions of plain mechanical indentation, our research group has further developed a commercial indentation instrument (Artscan 200, Artscan Oy, Helsinki, Finland) by equipping it with a miniature ultrasound transducer. In the ultrasound indentation, the cartilage surface is compressed with a flat miniature ultrasound transducer. Ultrasound signal is reflected from the bone-cartilage interface and by determining the time that the ultrasound signal takes to travel through-and-back out of the cartilage during indentation testing, it is possible to determine the original cartilage thickness as well as the applied

strain. The in-built force gauge is used to determine the applied compressive force. In response to dynamic loading, *i.e.* under high rate and small strain compression, cartilage can be modeled as a linearly elastic material. Therefore, it is possible to calculate the elastic modulus for the cartilage (Hayes et al. 1972). Different approaches, such as a water-jet system, have also been introduced for the same purpose (Duda et al. 2004, Lu et al. 2005, Lu et al. 2009). In these techniques, the tissue thickness and deformation under load are determined by ultrasound time-of-flight technique. However, recent publications suggest that ultrasound speed in cartilage may change during compression (Nieminen et al. 2006). This can induce significant errors in the determined thickness, deformation and mechanical modulus values (Nieminen et al. 2007).

5.5 Ultrasonography and quantitative ultrasound parameters

Ultrasound speed has been suggested to be a sensitive measure for assessing cartilage pathology, and its measurement might help in diagnosing early OA changes (Suh et al. 2001, Töyräs et al. 2003). The ultrasound echo from the cartilage surface reflects surface roughness and tissue integrity (Adler et al. 1992, Cherin et al. 1998, Chiang et al. 1994, Disler et al. 2000, Nieminen et al. 2002, Saied et al. 1997, Töyräs et al. 1999). Later, quantitative methods for the surface analysis have been developed (Saarakkala et al. 2004). In the simplest approach, the ultrasound reflection from the cartilage surface can be quantified during point-like measurements (Chérin et al. 1998, Saarakkala et al. 2004). 2D-imaging of cartilage surface makes it possible to calculate quantitative parameters describing the roughness of the cartilage surface (Saarakkala et al. 2004). As ultrasound is able to penetrate the cartilage tissue, tissue thickness can also be determined and alterations in subchondral bone and in the internal structure of cartilage may be detected (Laasanen et al. 2003, Laasanen et al. 2006, Saarakkala et al. 2006). The presented methods require that the ultrasound transducer is in close contact with the cartilage surface and therefore at least a minimally invasive approach

is required, *e.g.* during arthroscopic surgery. The reason for this is that high-frequency ultrasound has limited penetration properties in tissues. Although some of the presented methods might also be suitable for a transcutaneous approach, the anatomy of the knee joint does not allow imaging of all joint surfaces. A novel method for 2D-imaging of intra-articular structures is the application of minimally invasive intra articular-ultrasound imaging of articular cartilage, which is analogous to intravenous ultrasound imaging (Viren et al. 2009).

5.6 Quantitative MRI

Today, MRI is typically used for imaging purposes, however, modern MRI appliances for both research and clinical use are capable of determining tissue-specific quantitative parameters. In the quantitative analysis of cartilage, several magnetic resonance parameters have been developed (Blumenkrantz et al. 2007, Burstein et al. 2003, Conaghan 2006, Gray et al. 2004). The T_2 relaxation time of cartilage has been shown to reflect the structure and integrity of collagen fibril architecture of articular cartilage and the OA severity (Dunn et al. 2004, Nieminen et al. 2000). The depth-wise differences in T_2 values of cartilage have been attributed to the properties of intrinsic water in the tissue and the interactions of water molecules with the collagen network of the tissue (Rubenstein et al. 1993). Furthermore, the technique of measuring T_1 relaxation of cartilage in the presence of a gadolinium contrast agent (delayed gadolinium enhanced magnetic resonance imaging of cartilage, dGEMRIC) is used for determining the relative proteoglycan content of cartilage. The paramagnetic contrast agent gadolinium strongly shortens the T_1 relaxation of cartilage. The negatively charged Gd-DTPA^{2-} is thought to distribute in cartilage in an inverse manner to the proteoglycan concentration of the tissue. Therefore, the T_1 relaxation in the presence of gadolinium has been shown to associate with the PG content of the tissue *in vitro* (Bashir et al. 1996, Bashir et al. 1999, Blumenkrantz et al. 2007, Gray et al. 2001). Other MRI techniques have also been presented for

quantitative assessment of articular cartilage. The T_{1rho} relaxation time has also been shown to reflect the PG content and distribution within cartilage (Akella et al. 2001). Diffusion imaging (Filidoro et al. 2005), magnetization transfer (Gray et al. 1995) and ^{23}Na-MRI techniques (Insko et al. 1999) have also been claimed to offer non-invasive assessment of articular cartilage. Novel GAG-targeted contrast agents are also being developed to improve cartilage evaluation (Winalski et al. 2008). Furthermore, the magnetic resonance imaging can be used to quantify cartilage morphology, such as cartilage volume or thickness (Eckstein et al. 1994, Eckstein et al. 2005, Peterfy et al. 1994), and therefore repetitive scans can be used to monitor progression of the disease (Raynauld et al. 2004). However, a recent study on volumetric evaluation of cartilage using MRI suggests that the method is not sensitive enough to identify early OA changes (Reichenbach et al. 2009).

5.7 Other methods

Several other biophysical methods have been developed for detecting OA changes in cartilage. These include optical coherence tomography (OCT) (Adams et al. 2006, Herrmann et al. 1999, Pan et al. 2003, Xie et al. 2006, Xie et al. 2008), contrast agent enhanced computerized tomography (CT) (Kallioniemi et al. 2007, Piscaer et al. 2008, Silvast et al. 2009a, Silvast et al. 2009b, Xie et al. 2009), atomic force microscopy (Stolz et al. 2004), confocal microscopy (Chiang et al. 1997), contact and laser profilometry (Forster et al. 1999), spectroscopy (Spahn et al. 2007), and determination of streaming potentials within cartilage tissue during arthoscopy (Garon et al. 2002, Legare et al. 2002). Even radiographic techniques, using synchrotron sources of X-rays, have been able to visualize articular cartilage, thus potentially providing valuable information for evaluating also early OA changes in cartilage (Muehleman et al. 2004a, Muehleman et al. 2004b).

Several biochemical biomarkers or their combinations have also been proposed to serve as diagnostic tools for OA (Davis et al. 2007). Some

biomarkers have been suggested to serve as prognostic tools (Garnero 2002). The basic concept behind most biomarkers is that the metabolites resulting from the cartilage degradation, attempts at cartilage repair, increased cartilage or bone remodeling and turnover or synovial inflammation, can be detected from serum, urine or synovial fluid (Davis et al. 2007, Garnero et al. 2000, Lohmander et al. 2003). It has been suggested that as the biomarkers arise from a variety of sources, it would be useful to clarify the sources of the biomarkers. Different categories of biomarkers could be used as prognostic, diagnostic or investigative tools. Furthermore, the efficacy of therapeutic intervention and total burden of the disease could be assessed by monitoring changes in specific biomarker patterns (Bauer et al. 2006). However, the currently available biomarkers are not sensitive enough to be used as disease-activity or progression markers of OA (Kraus 2006).

6 Aims of the present study

The function of cartilage tissue is dependent on the composition and structure of the tissue. Therefore, understanding the structure-function relationships is essential for the development of efficient diagnostics of OA. Furthermore, several quantitative biophysical methods have been introduced for the detection of cartilage degeneration, but comparative data between these methods is scarce. The specific aims of this study were:

1. to characterize the structural components that play key roles in determining the Poisson's ratio of cartilage.

2. to compare MRI, ultrasound and mechanical methods in evaluating degenerative changes in cartilage.

3. to clarify the extent of site-dependent variations in the mechanical, compositional and structural properties of the patellar cartilage surface and how these properties are affected by the OA process.

4. to investigate the effect of the spatial variation of tissue properties on the early OA diagnostics and to compare arthroscopically applicable methods in this regard.

5. to investigate how changes in the ultrasound speed in cartilage during compression influence the reliability of ultrasound indentation measurements.

7 Materials and methods

7.1 Tissue specimens and sample preparation

This study was carried out *in situ* and *in vitro*. A brief overview of the materials and methods of the studies I-IV is presented in **Table 7.1**. Bovine tissue was used in studies I and II, whereas human cartilage tissue was investigated in studies III and IV. Bovine tissue samples were obtained from a local slaughterhouse. Knee and shoulder joints with intact joint capsules were dissected and delivered within a few hours *post mortem*. In study I, osteochondral samples were prepared from the patella, medial femoral condyle, medial tibial plateau and humeral head. Only visually healthy tissue was utilized in this study. In study II, the samples were prepared from the lateral facets of bovine patellae. In this study, the material included samples with visual signs of degeneration, in addition to samples with a healthy appearance. In both studies, the samples were frozen after initial preparation and thawed before the measurements.

Human patellae for studies III and IV were collected from right knee joints of 14 cadavers at autopsy in Jyväskylä Central Hospital within 48 hours postmortem. The collection and use of human tissue was conducted with the permission from the National Agency of Medicolegal Affairs in Finland (Permission n:o 1781/32/200/01). Knee joints were opened, the patellae were dissected free and frozen separately for later use. From the patellar cartilage surface, six anatomically defined measurement locations were identified on the patellar cartilage surface using a marker without harming the points of interest.

Table 7.1 Materials and methods utilized in the thesis work

Study #	I	II	III	IV
n	30	32	84/75/73	73
Species	Bovine	Bovine	Human	Human
Location	HUM, MFC, MTP, PAT	PAT	PAT	PAT
Testing method	Mechanical testing -E_{EQ} -Poisson's ratio	MRI -$dGEMRIC$ -T_2 US indentation -E_{US} -R_{US}, Ultrasound -URI -IRC -speed -attenuation	Indentation -F_{IND} US indentation -E_{US} -R_{US},	Ultrasound -speed -attenuation -changes during compression
Reference methods	Microscopy -ePLM -OD -PGs -FTIRI -Collagen -Mankin score	Microscopy -Mankin score Water content Biochemistry -Collagen -PGs Mechanical testing -E_{EQ} -E_{DYN}	Microscopy -OD -PGs -FTIRI -Collagen -OA grade Mechanical testing -E_{EQ} -E_{DYN}	Microscopy -OD -PGs -FTIRI -Collagen -OA grade Mechanical testing -E_{EQ} -E_{DYN} Water content

PAT: patella; MFC: medial femoral condyle; HUM: humeral head; MTP: medial tibial plateau.

7.2 Novel diagnostic techniques

7.2.1 Indentation techniques

In study III, a modification of commericially available indentation instrument (Artscan 200, Artscan Oy, Helsinki, Finland) was used for testing of human patellar cartilage *in situ* (**Figure 7.2** a). Six measurement locations were selected on the patellar cartilage surface (**Figure 7.1**) and the indentation measurements were conducted using

the instrument with a small semi-spherical indenter (diameter 0.5 mm) (Töyräs et al. 2001). The force by which the cartilage resists the compression was used as an index for cartilage stiffness. Two independent sets of compressions were performed and four compressions from each set were averaged for analysis.

7.2.2 Mechano-acoustic techniques

The mechano-acoustic indentation device is the result of further development of the mechanical indentation instrument (Laasanen et al. 2002). It has a miniature ultrasound transducer (ø=3mm, f=10MHz) mounted on the tip of the instrument. This flat transducer is used to compress articular cartilage (**Figure 7.2** b). The reflected ultrasound signal from the cartilage-bone interface is measured to determine tissue thickness using the time-of-flight method. Cartilage tissue was compressed with the instrument at six measurement locations on the patellar cartilage (**Figure 7.1**). The measurement device was first placed on the cartilage surface. In order to determine cartilage thickness reliably, it was important to keep the indenter perpendicular to the cartilage surface and the underlying subchondral bone, from where the ultrasound echo is reflected. This was ensured by following the ultrasound signal during the compression test. By using a predefined contact stress, the cartilage thickness was determined by using the time-of-flight method. The controller software calculated the cartilage thickness during the compression test, and compressions of 5% of the original thickness of the tissue were performed. During testing, the applied strain and the reaction force were determined. These parameters were then used to calculate the dynamic tissue modulus. Furthermore, the same instrument was used to determine the ultrasound reflection coefficient of cartilage surface. First, a plastic sleeve was attached to the miniature ultrasound transducer of the device to maintain a constant distance between the cartilage surface and the ultrasound transducer (**Figure 7.2** c). Then, the device was placed on the cartilage, and the maximum peak-to-peak echo amplitude was

measured for calculation of the ultrasound reflection coefficient of the cartilage surface (R_{US}). The mechanical measurements were performed without the external sleeve.
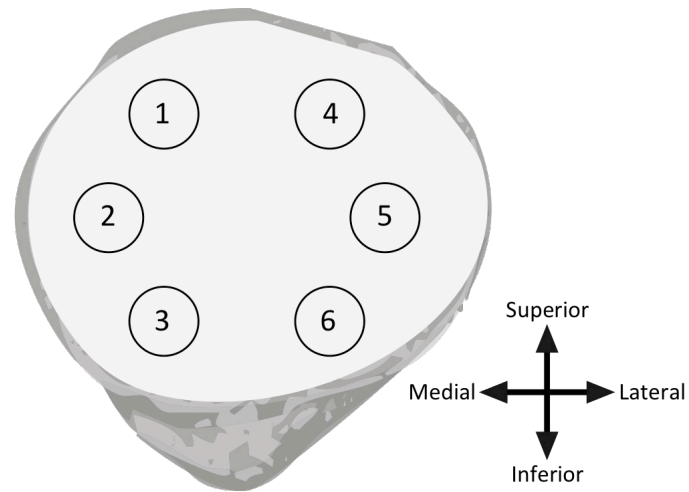
Figure 7.1 Measurement locations on human patellar cartilage surface in studies III and IV. 1 Superomedial, 2 Central medial, 3 Inferomedial, 4 Superolateral, 5 Central lateral and 6 Inferolateral
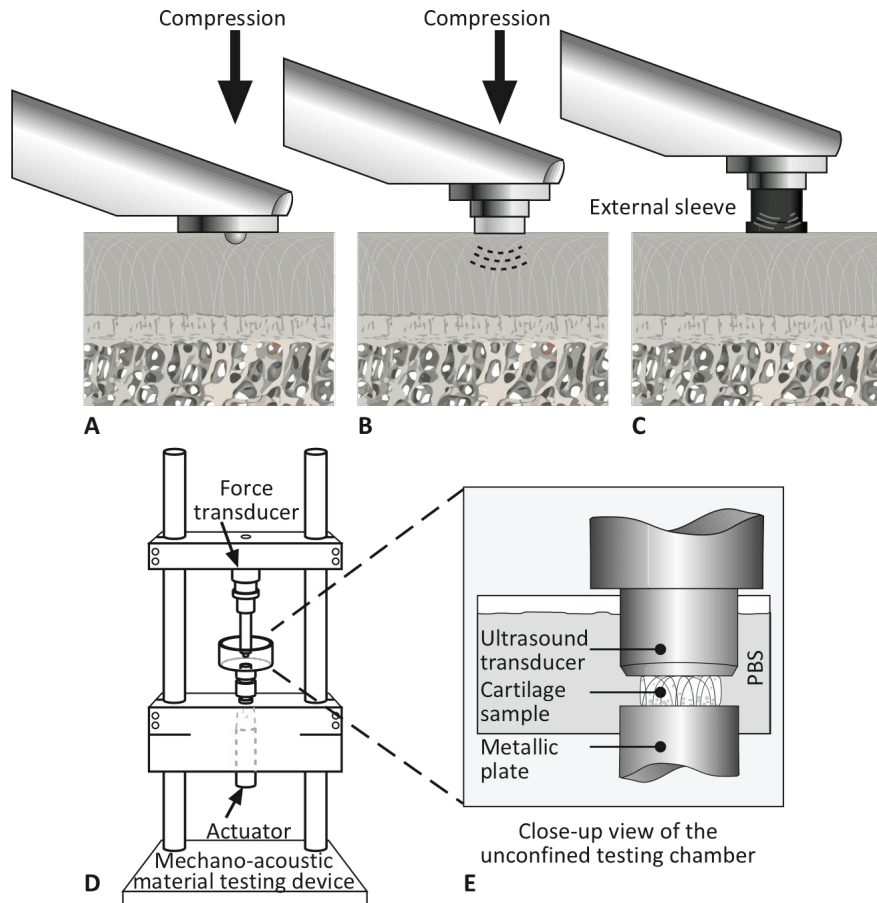
Figure 7.2 Schematic presentation of the mechanical indentation measurement (A), ultrasound indentation measurement (B) and measurement of R_{US}(C). The mechanical reference measurements were carried out with a mechano-acoustic material testing device in unconfined geometry in all studies (D). Ultrasound speed and attenuation were determined during mechanical reference testing in studies II and IV (E).

7.2.3 Ultrasound techniques

The measurements of *IRC*, *URI* and *R* in study II were performed by scanning the samples with a commercially available quantitative ultrasound-imaging unit (Dermascan-C, Cortex Technology, Hadsund,

Denmark) (Saarakkala et al. 2006). Briefly, the ultrasound signal was recorded for analysis of reflection coefficient in the time-domain (R, %) and frequency-dependent integrated reflection coefficient in the frequency domain (IRC, dB). The ultrasound roughness index (URI, µm) was determined from the ultrasonically determined surface contour profile.

7.2.4 MRI techniques

In study II, quantitative MRI was performed on bovine samples (Nissi et al. 2004). The measurements were conducted using a 9.4 T research MRI device (Oxford Instruments Plc, Witney, UK). In study II, for comparison with other parameters, we determined the T_2 relaxation times of superficial cartilage and the bulk dGEMRIC value (T_1 relaxation time in presence of Gd-DTPA^{2-} contrast agent.

7.3 Reference methods

7.3.1 Mechano-acoustic measurements

Biomechanical testing of cartilage samples was carried out in all studies. The testing was conducted using a custom-built mechano-acoustic high-resolution material testing device (**Figure 7.2** d) (Laasanen et al. 2002). The cartilage discs were tested in unconfined compression geometry (**Figure 7.2** e). During the measurements, the ultrasound signal was collected and analyzed (studies II and IV). The ultrasound speed was analyzed with the time-of-flight method. Ultrasound attenuation was determined as described earlier (Nieminen et al. 2004).

7.3.2 Histological grading of articular cartilage structure

After mechanical testing of the samples, the tissue specimens were immersed in 10% formalin solution for fixation. The tissue was then further processed and embedded in paraffin to enable preparation of histological sections.

In study II, the histological integrity of the samples was determined by using the histological-histochemical grading system (HHGS, also known as Mankin Scoring or Mankin System) introduced by Mankin et al. (1971). In light microscopy, the histological integrity of cartilage was graded using the Safranin-O stained histological sections. Tissue structure, cellularity and Safranin-O stainability of the samples and the integrity of the tidemark were visually evaluated (**Table 7.2**).

The value of this method has been questioned, as its reproducibility has been shown to be low (Ostergaard 1997, 1999). To improve the histological grading, Osteoarthritis Research Society International (OARSI) established a working group to create a novel grading method for OA changes in cartilage. This method was introduced in 2006 (Pritzker et al. 2006). In studies III-IV, the OARSI OA grading was used to evaluate the cartilage integrity (**Table 7.3**).

Table 7.2 Histological-histochemical grading system (Mankin scoring)

	Grade		Grade
I. Structure		**III. Safranin-O staining**	
a. Normal	0	a. Normal	0
b. Surface irregularities	1	b. Slight reduction	1
c. Pannus and surface irregularities	2	c. Moderate reduction	2
d. Clefts to transitional zone	3	d. Severe reduction	3
e. Clefts to radial zone	4	e. No dye noted	4
f. Clefts to calcified zone	5		
g. Complete disorganization	6		
II. Cells			
a. Normal	0		
b. Diffuse hypercellularity	1	**IV. Tidemark integrity**	
c. Cloning	2	a. Intact	0
d. Hypocellularity	3	b. Crossed by blood vessels	1

(Mankin et al. 1971)

Table 7.3 OARSI cartilage histopathology grade assessment – grading methodology

Grade 0: surface intact, cartilage morphology intact
Grade 1: surface intact
Grade 2: surface discontinuity
Grade 3: vertical fissures (clefts)
Grade 4: erosion
Grade 5: denudation
Grade 6. deformation

(Pritzker et al. 2006)

Mankin Score	0	1	3	8
OARSI OA Grade	0	1	2	3
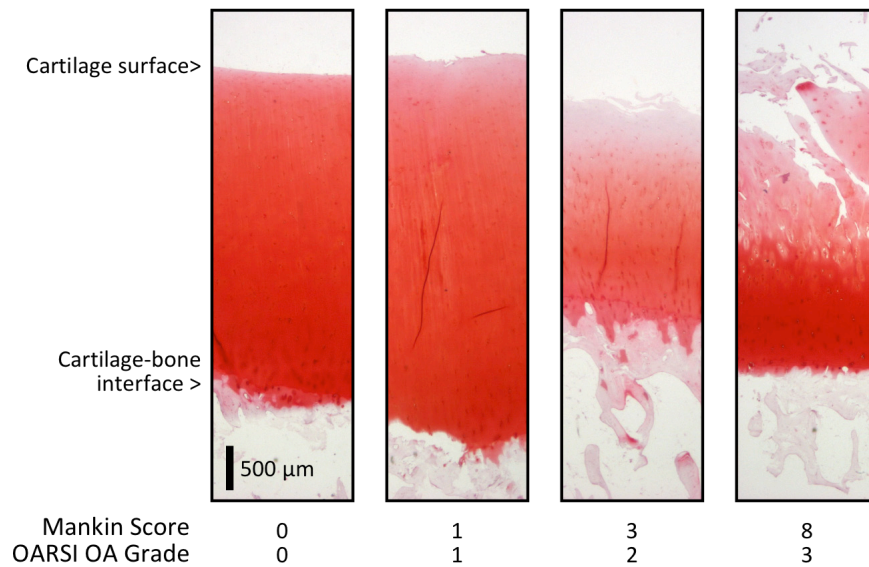
Figure 7.3 Characteristic cartilage sections with different stages of degeneration. Cartilage surface is smooth in healthy cartilage and the cartilage tissue stains intensely with Safranin-O. During the degenerative process, the Safranin-O staining decreases as a sign of PG depletion and the cartilage surface undergoes fissuration and cleft formation.

7.3.3 Optical density (OD) measurements

One typical feature of the OA process is the decrease in the PG content of the cartilage tissue. Optical density measurements can be used to evaluate the PG content and distribution of cartilage. The histological sections were stained with cationic Safranin-O stain, which targets the anionic sulphate and carboxyl groups derived mainly from the PGs of the tissue (Király et al. 1996). The PG content could then be evaluated by the measurement of absorbance of monochromatic light in the sample with a calibrated densitometer system with Leitz Ortholux II microscope (Leitz Wetzlar, Wetzlar, Germany) and a cooled 12-bit CCD camera (Photometrics, Tucson, AZ, USA). This semiquantitative method was used for determination of tissue PG content in studies I and III. Furthermore, the spatial distribution profiles of PGs were determined and included when the mechanical behavior of the samples in studies I and IV were modeled.
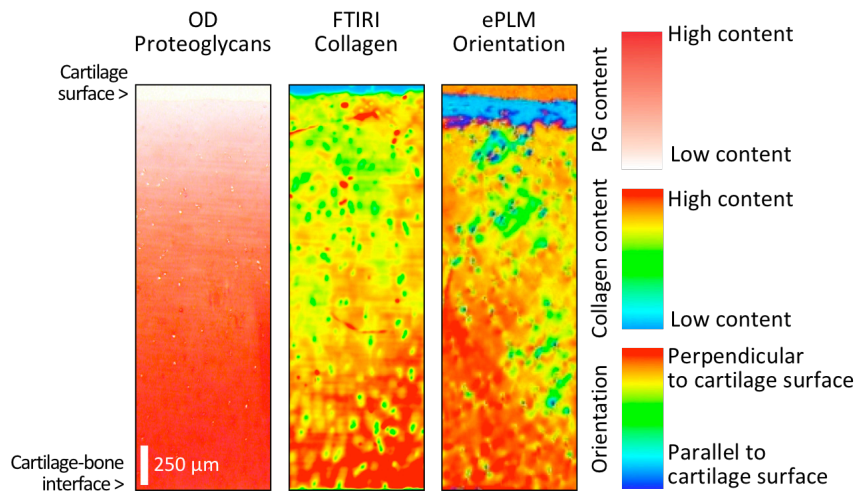
Figure 7.4 Characteristic images from optical density, FTIRI collagen and orientation analyses in normal human patellar cartilage.

7.3.4 Fourier transform infrared imaging (FTIRI)

Infrared spectroscopy has been used to characterize and analyze various chemical components from various samples for long time. The Fourier transform infrared imaging (FTIRI) technique was used to map the infrared absorption spectra from unstained tissue sections with a resolution from 6.25 to 25 µm by using PerkinElmer Spotlight 300 instrument (Perkin Elmer, Waltham, MA, USA). Calculation of specific parameters from the spectrum at each pixel of the sample made it possible to create images which illustrate the spatial distribution of selected macromolecules (Camacho et al. 2001, Potter et al. 2001). In studies I, III and IV, FTIRI was utilized to estimate the collagen content and the spatial distribution of collagen in the samples by calculating the integrated absorption of the amide I peak (wave numbers 1610-1710 1/µm) in each pixel of the tissue sections. Thereafter, the absorption values of each pixel row were averaged horizontally to create a depth-wise collagen content profile of the samples. The averaged value of the profile was considered to represent an estimate of the total collagen content of the sample.

7.3.5 Enhanced polarized light microscopy (ePLM)

Collagen is a birefringent material and can rotate the plane of polarized light. Therefore, polarized light microscopy can be used to specifically analyze the structure and deposition of collagen in articular cartilage. The methods related to enhanced polarized light microscopy (ePLM) have been described thoroughly in a recent article (Rieppo et al. 2008). Briefly, the unstained cartilage sections were analyzed with a computer-controlled semi-automated polarized light microscope system built around scientific grade polarized light microscope (Leitz Ortholux II POL, Leitz-Wetzlar, Wetzlar, Germany). After alignment of the polarizer pair of the system, a series of images was acquired with different positions of the polarizer pairs to collect all signals from the birefringent sample. The acquired images were processed with custom-made software. As the signal intensity in each

pixel follows a sinusoidal function determined by the polarizer alignment angle and the birefringent behavior of the collagen fibrils, the theoretical maximal and minimal signal intensities can be determined through a least square fitting method. The orientation-independent birefringence was determined as the theoretical maximum signal intensity. Anisotropy, or the degree of parallelism is an index to measure the parallel alignment of the collagen fibrils in each pixel of the sample. This was determined by the ratio of minimal to maximal signal intensities (Rieppo et al. 2008). The Stokes parameters were determined to determine orientation of collagen fibrils and the mean orientation of collagen fibrils was calculated by using these parameters in each pixel of the section (Collett 1992). In study I, the birefringence, orientation of the collagen fibrils and anisotropy maps of the samples were analyzed and the obtained depth-wise profiles were processed similarly as the results from the OD and FTIRI measurements.

7.3.6 Biochemical analyses

In study II, biochemical analyses were conducted to determine the proteoglycan and collagen contents of the samples. A detailed description of the measurements has been described earlier (Töyräs et al. 2003). Briefly, the PG content was determined using a spectrophotometric assay of the uronic acid content (Blumenkrantz et al. 1973). The collagen content was quantified by assaying the hydroxyproline content of the samples (Schwartz et al. 1985). In studies II and IV, the interstitial water fraction was measured from the ratio of the wet and dry weights for each sample.

7.4 Numerical modeling of cartilage mechanics

In study I, finite element (FE) analyses were performed to determine the specific role of the PGs and collagen network on the Poisson's ratio. For this purpose, an axisymmetric fibril-reinforced poroelastic model was created as detailed in study I. Quadratic 8-node continuum elements were created to mimic the PG matrix and interstitial fluid, whereas horizontal

spring elements represented the collagen network. The Poisson's ratio of PG matrix was set to 0.42 and the effect of PG matrix and collagen fibril moduli values on the cartilage Poisson's ratio was studied at equilibrium.

7.5 Statistical analyses

Correlation coefficients between continuous variables were calculated as Pearson correlation coefficients. Spearman's Rho was calculated for the correlation analysis with discrete parameters such as OARSI OA-grade and Mankin score (Blalock 1972). Kruskal-Wallis-H-test was used to determine the statistical significance of the site-dependent variation of the parameters in study I as well as the significance of the differences between different degeneration groups in study II. Furthermore, in study II, the Mann-Whitney U-test was utilized to test statistical significance when comparing the healthy samples with the degenerated samples.

In studies III and IV, the linear mixed model was used to determine differences between the sample groups. This test was selected, as it enabled the comparison of samples with possible interdependencies (Brown et al. 2006). Thereby, the potential interrelationships between the samples from the same individual, or from the same location, were taken into account.

In studies II and III, the receiver operating characteristic (ROC)-analysis was conducted to determine optimal cut-off values for the diagnostic variables. The selection was based on finding the point of the ROC-curve where the sum of sensitivity and specificity was maximal.

All statistical analyses were conducted using SPSS statistical software, versions 11.0.2-16.0.2 (SPSS inc., Chicago, IL, USA).

8 Results

8.1 Structure-function relationships in cartilage

In study I, the value of equilibrium modulus was found to be primarily determined by the PG content of cartilage (**Table 8.1**). The Poisson's ratio values were primarily determined by the collagen content and the orientation of the collagen network (**Table 8.1**). This result was also confirmed by the FE model, which indicated that the effect of PG matrix on the Poisson's ratio of cartilage was less significant than the effect of collagen network (**Figure 8.1**). The structure of collagen network in patellar cartilage was often found to differ from the typical three-layered structure, as analyzed by the ePLM measurements. Several samples demonstrated an extra lamina in the middle cartilage layer where the collagen fibrils were oriented in parallel to the surface (**Figure 4** in Study I).
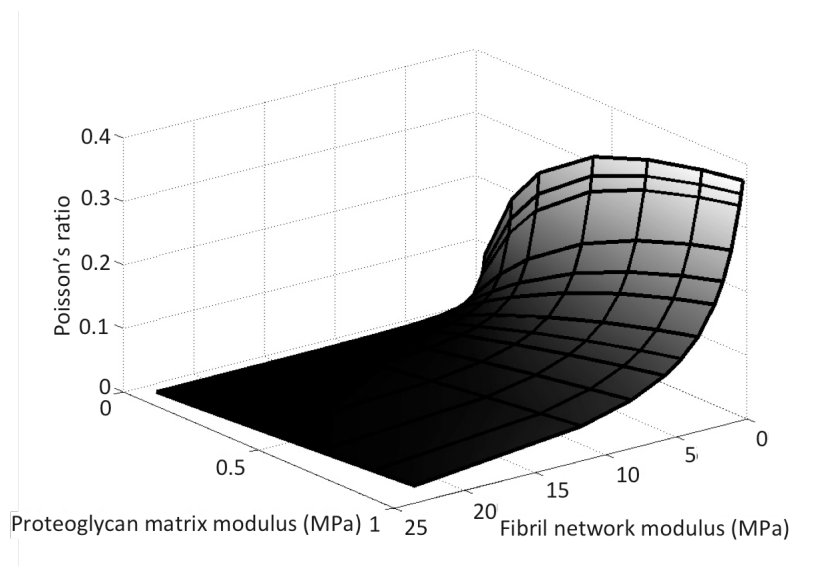
Figure 8.1 According to the FE-model, the effect of collagen fibril network modulus on Poisson's ratio is stronger than that of the PG matrix modulus.

Table 8.1 Linear correlation coefficients between the mechanical and the structural parameters of bovine knee and humeral articular cartilage (n=30).

| | PGs | Collagen | ePLM | | |
			Birefringence	Orientation	Anisotropy
E_{EQ}	0.86 *	0.72 *	-0.53 *	-0.10	0.14
Poisson's ratio	-0.33	-0.59 *	0.15	0.36 *	0.03

* p<0.05

8.2 Topographical variation in properties of cartilage

In study I, the mechanical properties, *i.e.* equilibrium modulus and Poisson's ratio of cartilage displayed a site-dependent variation between different measurement sites (p<0.05) (**Table 1** in Study I). Further, the results from studies III and IV displayed significant site-dependent variation (p<0.05) in mechanical, compositional and acoustic parameters within patellar cartilage surface (**Figure 3** in Study III and **Figure 3** in Study IV). However, in contrast to all other parameters, ultrasound reflection from the cartilage surface exhibited no statistically significant topographical variations in healthy cartilage (p=0.61).

8.3 Detection of cartilage degeneration

The results from MRI, ultrasound indentation and 2D ultrasound measurements revealed a statistically significant variation between the healthy and degenerated samples (p<0.05) (Study II). Values of Mankin score were not significantly different between the samples with normal visual appearance or with early degenerative changes, such as slighty discolored surface (**Figure 4** in Study II). ROC analysis enabled comparison of the specificity and sensitivity between different methods. The relatively low number of samples prevented the comparison of the methods using the AUC (area under the curve) analysis. However, several methods showed promising specificity and sensitivity values. When comparing the diagnostic parameters with each other, the ultrasound

parameters (R_{US}, URI, IRC) showed the highest sensitivity and specificity values, whereas MRI parameters demonstrated lower values from 0.67-0.81 (**Table 8.2**). Furthermore, most of the measured variables showed high correlations with the mechanical and compositional reference parameters (**Table 8.3**). The best predictors of the histological integrity were R, URI and IRC, $|r|$=0.84-0.85. Ultrasound indentation (E_{dyn}) showed the strongest association with the reference mechanical parameters (**Table 8.3**).

Table 8.2 Mean ($\pm$ SD), cut-off, specificity and sensitivity values of cartilage samples categorized by Mankin scoring (0, 1-3, >3). The biophysical methods revealed statistically significant variation between the different levels of degeneration. Furthermore, after determination of an optimal cut-off value for each method (ROC analysis) to detect degenerated samples, good specificity and sensitivity values were revealed for several methods.

	Mankin score			Cut-off	Speci-ficity	Sensi-tivity
	0 (n =11)	**1-3** (n =11)	**3-** (n =10)			
Ultrasound indentation						
E_{dyn} (MPa)*	9.1 ± 6.1	3.7 ± 3.6	1.5 ± 0.5	2.5	0.89	0.71
R_{US}(%)*	3.7 ± 1.2	1.9 ± 1.1	0.6 ± 0.3	2.3	0.89	0.91
MRI						
T_2 (ms)*	43 ± 14	69 ± 59	120 ± 105	44	0.67	0.81
T_{1GD} (ms)*	401 ± 4	371 ± 35	322 ± 64	386	0.67	0.81
Ultrasound						
α (dB/mm)*	2.65 ± 0.58	2.01 ± 0.45	1.76 ± 0.43	2.50	0.78	0.95
SOS (m/s)*	1603 ± 27	1572 ± 15	1548 ± 14	1576	0.78	0.91
IRC (dB)*	-26.7 ± 1.6	-30.9 ± 4.0	-37.6 ± 4.6	-29.0	0.91	0.86
URI (µm) *	7.4 ± 1.2	15.1 ± 8.5	34.3 ± 15.4	10.2	1.00	0.91

*$p<0.01$

Table 8.3 The biophysical parameters revealed strong correlations with the reference parameters. Correlation with Mankin scores was highest for R and for *IRC* and *URI* ($r=0.84$), as measured with the 2D ultrasound imaging device. The best predictor of mechanical properties was E_{dyn}, as measured with the ultrasound indentation instrument. Furthermore, the speed of sound was strongly associated with $E_{dyn,ref}$ and E_{ref}.

	Mankin score	PG content	Collagen content	Water content	E_{ref}	$E_{dyn,ref}$	Average $|r|$
E_{dyn}	-0.73**	0.87**	0.86**	-0.69**	0.60**	0.98**	0.79
R_{US}	-0.85**	0.83**	0.78**	-0.66**	0.58**	0.87**	0.76
α	-0.55**	0.65**	0.64**	-0.65**	0.47**	0.62**	0.60
SOS	-0.76**	0.90**	0.86**	-0.80**	0.79**	0.90**	0.83
T_2	0.61**	-0.36*	-0.33	0.34	-0.33	-0.30	0.37
T_1Gd	-0.54**	0.62*	0.36	-0.56**	0.63**	0.50**	0.54
IRC	-0.84**	0.61**	0.54**	-0.48**	0.58**	0.58**	0.61
URI	0.84**	-0.49**	-0.49**	0.37*	-0.49**	-0.48**	0.53

*$p<0.05$, **$p<0.01$, n=30-32.

The results from study III indicate that both ultrasound indentation and mechanical indentation measurements reflect the true mechanical properties of the tissue (**Figure 8.2** a-b). Furthermore, both these measurements are significantly correlated with the collagen content of the samples (**Figure 8.2** c-d). The ultrasound reflection from the healthy cartilage surface showed no statistically significant (p=0.61) topographical variation. This was the only parameter which exhibited any statistically significant difference (p=0.01) between the healthy samples and samples with signs of early degeneration (**Table 8.4**).

Table 8.4 Values of measured parameters (Mean ± SD) among samples divided into groups with variable cartilage degeneration: Healthy cartilage (OARSI OA Grade 0), Early degeneration (Grades 1.0-1.5), Advanced degeneration (Grades 2 and above). R_{US} was the only parameter that showed any statistically significant difference between the healthy samples and the samples with early degeneration.

	Healthy cartilage n=35	Early degeneration n=17-18	Advanced degeneration n=21-22	All samples n=73-75
	[——————— p<0.001 ———————]			
	[— p=0.01 —] [— p<0.001—]			
R_{US} (%)	2.34 ± 0.74	1.79 ± 0.74	0.80 ± 0.80	1.76±1.00
E_{US} (MPa)	3.51± 1.67	3.88 ± 2.08	2.64 ± 1.98	3.34±1.90
	[——————— p<0.001 ———————]			
	[— p=0.004 —]			
F_{IND} (N)	0.41 ± 0.18	0.40 ± 0.18	0.23 ± 0.16	0.36±0.19
	[——————— p<0.001 ———————]			
	[— p<0.001 —]			
E_{DYN} (MPa)	4.67±2.24	4.76±3.01	1.87±2.32	3.88±2.75
	[——————— p<0.001 ———————]			
	[— p<0.001 —]			
E_{EQ} (MPa)	0.64±0.30	0.70±0.62	0.21±0.26	0.53±0.44
	[——————— p<0.001 ———————]			
	[— p<0.001 —]			
PG content (absorbance)	1.36±0.29	1.19±0.32	0.83±0.37	1.16±0.39
	[——————— p<0.001 ———————]			
Collagen content (absorbance)	[— p<0.001 —]			
	0.25±0.03	0.25±0.05	0.21+0.03	0.24±0.04

8.4 Ultrasound speed and attenuation in healthy and degenerated patellar cartilage

In study IV, values ultrasound speed and attenuation showed a statistically significant spatial variation in patellar cartilage surface in all samples as well as when only healthy samples were analyzed (**Figure 3** in Study IV). The values of ultrasound speed were typically between 1560 m/s and 1640 m/s (**Figure 8.3** a), *i.e.* the average variation was less than 5 %.

Ultrasound speed associated significantly with the compositional and mechanical parameters of the cartilage tissue (**Table 8.5**). Ultrasound speed and reference dynamic modulus displayed a strong positive correlation (r=0.81, n=67, p<0.01). Partial correlation analysis revealed that the water and collagen contents were individual determinants of the ultrasound speed. The water, collagen and PG contents of the tissue were able to explain 55% of the variation in the ultrasound speed between the samples, whereas the compositional properties accounted for 69% and 67 % of the variation in the equilibrium modulus and dynamic modulus of the tissue, respectively.
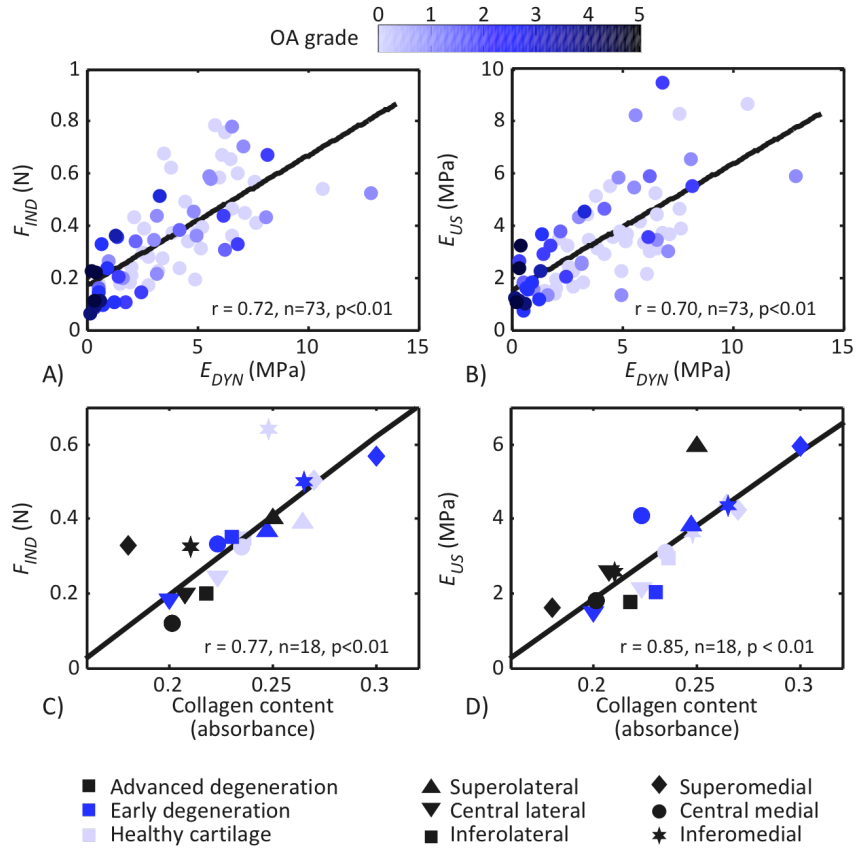
Figure 8.2 Scatter plots of the indentation measurements and the reference mechanical modulus and collagen content. Ultrasound indentation results displayed a linear positive correlation (p<0.01) with both mechanical indentation and reference dynamic modulus. When the samples with different grades of degeneration were averaged at each location, the indentation results showed a significant (p<0.01) positive correlation with the collagen content of the samples.

Table 8.5 Linear correlations and partial correlations between the ultrasound and reference parameters

	OARSI OA-grade	Water content	Collagen content	PG content	E_{EQ}	E_{DYN}
SOS	-0.30*	-0.57**	0.70**	0.44**	0.74**	0.81**
ΔSOS	0.07	0.25*	-0.37**	-0.16	-0.36*	-0.39**
α	0.18	-0.23	0.31*	-0.003	0.08	0.17
Δα	0.41**	0.43**	-0.71**	-0.36**	-0.68**	-0.68
Partial correlations		Water content	Collagen content	PG content	R^2	
SOS		-0.32**	0.52**	0.09	0.55**	
ΔSOS		0.08	-0.29*	0.05	0.14*	
α		-0.12	0.29*	-0.21	0.14*	
Δα		0.10	-0.59**	0.01	0.50**	
E_{EQ}		-0.26*	0.48**	0.56**	0.69**	
E_{DYN}		-0.45**	0.45**	0.38**	0.67**	

n=67-68, *p<0.05, **p<0.01

8.5 Compression induced variation of ultrasound speed

Both ultrasound speed and attenuation typically decreased during compression. The decrease in ultrasound speed during compression was low, being under 2% on average. However, this variation was estimated to cause errors of up to 15 % in the values of mechanical modulus of articular cartilage, as determined by ultrasound indentation (**Figure 8.3** c). Interestingly, the magnitude of error was greatest in those samples with early degenerative changes. In the degenerated tissue, the change in ultrasound speed was minimal (**Figure 8.3** b).
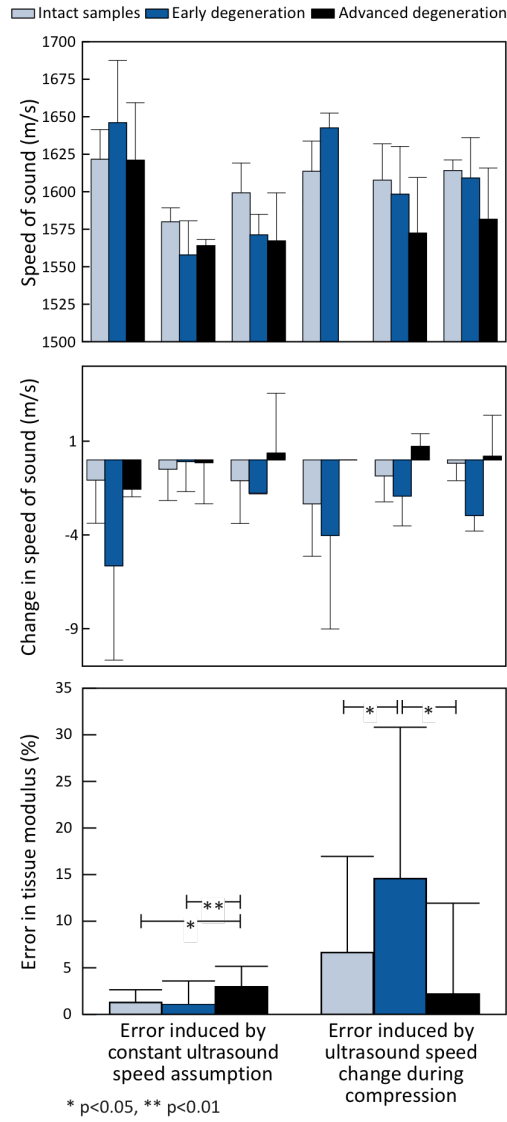
Figure 8.3 Topographical variation of ultrasound speed in human patellar cartilage (A). Change in speed of sound during 2% compression (B). The errors that arise due to the assumption that there is a constant ultrasound speed in cartilage and the ultrasound speed change during compression (C).

9 Discussion

OA is a common disease with major economical and sociological consequences. At present, the treatment is mostly symptomatic, focusing on attempts to decrease the pain of the diseased joints. This palliative treatment is far from satisfactory, but at present there are no therapies available for preventing the progression of the disease.

OA is known to impair the structural, compositional and mechanical properties of cartilage. The earliest changes involve a deterioration of the superficial collagen network and a decline in the superficial PG concentration. These changes have been postulated to take place even before visual changes occur on cartilage surface. Fibrillation of the cartilage surface is the first visible change which can be observed during arthroscopy. This process is followed by a further reduction of the PGs, and an increase in the water content of cartilage and these compositional changes make cartilage softer. As the fibrillation continues, cartilage starts slowly eroding and the end stage of this disease is the denudation of articulating bone end.

Today, the diagnosis of OA is usually based on the combination of clinical examination with conventional radiographs. By using the traditional X-rays, it is possible to evaluate the cartilage changes only indirectly by joint space narrowing, a sign of advanced disease, when a substantial amount of cartilage has worn away (Hayes et al. 2005). Bone-associated changes such as osteophyte formation, subchondral sclerosis and cyst formation are also typical features associated with the advanced disease.

Several therapeutic approaches, mostly pharmaceuticals, are under development for the prevention of OA progression. Furthermore, several cartilage repair techniques for treating local cartilage defects are being developed. Many of the methods are in the pre-clinical phase and being tested in laboratory animals. In these situations, the results of precise histological analysis can be evaluated. However, to prove the efficacy of these methods in clinical trials, it is crucial to have sensitive quantitative

methods for assessing the outcome of the treatment. Radiographs are far too insensitive to allow detection of the early OA changes. If these were to be used, however, the follow-up time would have to be very long and the number of patients would have to be very high. In one clinical study where these kinds of follow-up methods were selected, a mere 13 % of the placebo group displayed any progression of the disease during the 2-year follow-up time (Bingham et al. 2006). Obviously, no statistically significant difference between the placebo and the active treatment groups was found. Therefore, new and more powerful methods might produce substantial financial savings and improve the efficacy of clinical trials. However, at the moment, the only accepted end-point in clinical OA trials is the joint space narrowing (JSN) (Qvist et al. 2008).

Furthermore, the development of novel pharmaceuticals will require that early OA changes can be reliably detected in order to allow targeting of these novel therapies. However, the earliest OA changes are often asymptomatic. For these reasons it is evident that the novel, more sensitive, quantitative methods for diagnosis of early OA changes in cartilage are urgently needed. Research on therapy and diagnosis of OA needs to progress in parallel in order to apply the results of these studies into clinical practice.

Articular cartilage is a highly specialized connective tissue and its functional integrity can be generally determined by its mechanical properties. This necessitates an understanding of the structure-function relationship of the tissue. In study I, we were able to demonstrate that the lateral displacement of the cartilage tissue during compression, *i.e.* Poisson's ratio, is primarily dependent upon the amount and organization of collagen. This clearly indicates that by investigating cartilage structure it is possible to evaluate its functionality.

Several novel methods have been introduced for the determination of cartilage properties and as ways to detect early OA changes. These methods are typically intended to determine mechanical properties of the tissue, grade the degree of fibrillation or to determine the organization of collagen network or the PG content of the cartilage tissue.

Biomechanical properties of cartilage can be determined with several methods. Indentation of the cartilage surface can be conducted *in vivo* and also commercial applications have been developed for this purpose. Indentation testing of cartilage stiffness provides important information about cartilage status, since it gives direct information about cartilage functionality. Softening of cartilage is also known to take place during the degenerative process (Knecht et al. 2006). However, the use of stiffness values to distinguish early degeneration from healthy tissue can be challenging, as healthy cartilage demonstrates significant site-dependent variation. The results in study I highlighted the statistically significant variation in cartilage stiffness present at different joint surfaces. However, significant variation may exist also within one cartilage surface, as exemplified by the patellar cartilage surface in study III. Therefore in order to diagnose cartilage degeneration by measuring cartilage mechanical properties with high specificity, the obtained results should be compared location-wise with a precise map of the stiffness results of healthy cartilage.

Indentation provides useful information about the cartilage, but the thickness of the tissue influences the results. In order to overcome this challenge, a novel ultrasound indentation method has been developed (Laasanen et al. 2002). With this method, it is possible to estimate cartilage thickness and deformation during the compression testing, and an estimate of the true mechanical modulus can be obtained. However, the use of the ultrasound method in estimating tissue thickness and deformation is subject to some errors in the thickness values, as the ultrasound speed has been observed to change during compression (Nieminen et al. 2007). Therefore, in order to minimize errors in stiffness values, methods to correct the ultrasound speed change during compression need to be developed. As these techniques are intended at diagnosing the earliest degenerative changes, it is important that the first signs of the decline in cartilage stiffness can be reliably detected. Despite these challenges, the indentation technique is capable of determining cartilage stiffness, as emphasized by the significant correlation coefficients

with the reference mechanical values. In the present study, the slight discrepancy between the actual measurements and reference values can be partially explained by the differences in measurement geometry, *i.e.* indentation vs. unconfined compression (Korhonen et al. 2002a). In this *in situ* study extensive care was targeted at precise and repeatable localization of the measurements due to site-dependent variation of cartilage mechanical properties. Therefore, this should not cause major variation between the results. However, the indentation setup was different between the techniques. Most likely the most significant reason for variation between the results is the detachment of cartilage plugs from the subchondral bone. This may enable significant swelling of the tissue and alter the mechanical properties of cartilage.

Ultrasound has also been utilized to characterize cartilage structure. According to the present results, ultrasound signal reflection from cartilage surface is a sensitive parameter in distinguishing cartilage deterioration (studies II and III). R_{US} mainly reflects the integrity of the superficial cartilage layer. However, ultrasound reflection at the cartilage surface has been shown to relate closely to other degenerative processes within cartilage. Importantly, in study III, we were able to demonstrate that the R_{US} value exhibits no site-dependent variations in healthy cartilage, and that the results between healthy cartilage and those with early degeneration displayed statistically significant differences. Further, the ultrasound reflection measurements showed the highest sensitivity and specificity values when compared with other diagnostic methods (study II). These findings are of importance as they suggest that ultrasound measurements could be used to detect reliably the earliest degenerative changes in articular cartilage.

The R_{US} measurements face the challenges encountered with any spot-like measurements, whereas 2D ultrasound imaging can be used to map larger areas of cartilage surfaces. Ultrasound imaging can also be used to evaluate changes in the cartilage-subchondral bone interface (Saarakkala et al. 2006). However, the restricted penetration of the high-resolution ultrasound signal limits its potential to assess the intra-articular

structures transcutaneously. Therefore, in order to obtain images with the highest resolution, an intra-articular approach is necessary. The development of the intra-articular ultrasound technique might enable evaluation of intra-articular structures analogously to the way that vascular structures can be evaluated with intra-venous ultrasound catheters (Viren et al. 2009). This would require only a minimally invasive approach and these methods could be used to quantify cartilage status during arthroscopic procedures. Ultrasound imaging of joint structures can be performed non-invasively (Grassi et al. 2005), but evaluation of articular cartilage transcutaneously is challenging due to the limitations of acoustic window, whereby only limited visibility can be achieved of cartilaginous areas of most joints. Ultrasound speed seems to be a sensitive and specific measure of cartilage deterioration, but so far there are no reliable methods for determining ultrasound speed *in vivo*.

Magnetic resonance imaging has long been used as an imaging modality for soft tissues. Typically, cartilage lesions are evaluated by visual assessment by a radiologist. With high-resolution MRI devices, it is possible to quantify cartilage volume, which has been shown to be weakly associated with OA symptoms (Wluka et al. 2004). However, in order to evaluate the early OA changes, volumetric evaluation is not sufficiently sensitive, and therefore quantitative MRI methods have been developed for diagnosing the changes associated with OA (Raynauld et al. 2004). In dGEMRIC, T_1 relaxation of cartilage is determined in the presence of a negatively charged contrast agent (gadopentetate dimeglumine), which is considered to distribute in cartilage inversely to the tissue PG content as both compounds have a negative charge (Bashir et al. 1996). Therefore early loss of the PG molecules in cartilage would be revealed by an increased concentration of gadolinium and this can be indirectly detected through the altered T_1 relaxation times. Determination of T_2 relaxation times has been suggested to permit the assessment of the collagen network of cartilage (Goodwin et al. 1998, Nieminen et al. 2001, Xia et al. 2001). The preliminary studies with these methods showed significant correlations with the cartilage mechanical and compositional properties *in*

vitro. However, recent *in situ* research articles detected only a modest relationship of dGEMRIC and T_2 with the mechanical parameters or composition and organization of the cartilage matrix (Lammentausta et al. 2006, Lammentausta et al. 2007).

The development of MRI methods has been very rapid and a rather limited discussion of the techniques has been conducted before the methods have been adapted to clinical trials as putative reference methods. In early dGEMRIC studies the concentration of the contrast agent was substantially higher than that which can be achieved in the clinical situations via intravenous dosing. In addition, recent studies on contrast agent diffusion times in cartilage indicate that the diffusion of the contrast agents is rather slow in native cartilage (Kallioniemi et al. 2007, Silvast et al. 2009a, Silvast et al. 2009b). Therefore, the penetration of the contrast agent may not have achieved diffusion equilibrium when imaging is conducted 2 hours after the intravenous injection of the contrast agent.

Furthermore, the early studies used enzymatic degradation of collagen or PGs of cartilage (Bashir et al. 1996), an extreme approach, which produced high correlation values. The specific enzymatic degradation of the main components of the tissue differs from the changes occurring during spontaneous OA, where both components alter concurrently. As MRI methods are non-invasive, they are attractive reference methods for clinical studies. However, caution is necessary and one must speculate whether the validation of these methods for clinical use has been inadequate, especially if they are being used as the basis for individual therapeutic decisions. Our results from study II also support this view, since the specificity and sensitivity values did not achieve the levels required for clinical techniques.

As with any diagnostic technique, one should prefer non-invasive methods. However, today the knee arthroscopy procedure is often performed also in young individuals with symptomatic knee joints, and an extensive number of arthroscopic operations are performed annually. It would be optimal if quantitative data on cartilage status could be

obtained during routine arthroscopy. Monitoring these patients carefully and determining the characteristics that can best predict the progression to clinical OA at later age, may represent the best way to improve early diagnostics of OA and help in the prevention of the progression of the disease. The need for arthroscopies is not likely to decrease, and therefore also methods that are based on an arthroscopic approach should be developed in order to collect as much useful information as possible during the same operation.

Most of the methods evaluated in this thesis work may have the potential for clinical measurements as they revealed acceptable specificity and sensitivity in discerning healthy cartilage from samples with signs of early or advanced degeneration. Furthermore, they showed good correlations with the reference parameters. It should be noted that some methods seem to be more strongly associated with specific reference parameters than others. When compared to each other, the individual diagnostic methods have different advantages and challenges (**Table 9.1**). Optimally, the selection of the method for use should be tailored individually, as some parameters seem to reflect biochemical properties, while others are more sensitive at detecting changes in the structure of the cartilage surface. Possibly, the biophysical diagnostic methods can also be divided into different categories similarly as has been suggested for biomarkers (Bauer et al. 2006). While some tests are suitable for early diagnostics of OA, others may be more suitable as prognostic tools or in measuring efficacy of medical interventions. An analogy to clinical MRI can be drawn – the most suited method should be selected based on the clinical phrasing of the question, just as in MRI studies, the most suitable pulse sequences are selected. In particular, in clinical pharmacological research, the selected end-point methods must be chosen so that they measure as accurately as possible the process that the particular drug is intended to alter.

At the moment, the treatment of OA is far from optimal and better methods for diagnosing and treating this disease are needed. The development of this area of research is strictly linked to research into OA

therapeutics. At the point when the methodology is being selected for clinical diagnosis of early cartilage degeneration, it would be beneficial to have accurate, non-invasive and highly sensitive and preferably inexpensive screening methods. The positive results from this screening could be confirmed with further studies at higher specificities.

Potentially these screening methods could be applied after multidimensional OA risk assessment. This risk assessment could use information about known risk factors for OA, such as gender, age, weight, type and time of joint injury, genetic factors and loading conditions. Then the screening tests could be targeted at those individuals at a high risk for OA. These kinds of risk assessment tests do not exist at the moment, but they might be worthy of study. If the sensitive screening method implicates the presence of cartilage degeneration, further studies could be performed to confirm the diagnosis and to help with the decision on how best to treat the patient.

Table 9.1 Advantages and challenges of the novel quantitative methods for sensitive diagnostics of OA

Method	Sensitivity Specificity	Advantages	Challenges
Plain radiographs	- +++	• Non-invasive, easy to perform • Simultaneous bone diagnostics • Cheap • Good availability	• No direct information on cartilage • Poor sensitivity • Radiation exposure
Quantitative MRI	++ ++	• Non-invasive • No radiation exposure • Information on cartilage PGs (T_{1Gd}) and collagen (T_2) • Simultaneous diagnostics of other soft tissues	• Expensive as compared with radiographs • Poor availability
Indentation/ Ultrasound indentation	+++ +++	• Can be performed during routine arthroscopic procedures • Direct information on cartilage function	• Minimally invasive • *In vivo* measurement of certain locations can be challenging in US indentation
Ultrasound speed and attenuation	++ ++	• Changes associate strongly with several determinants of cartilage integrity	• Lack of clinically applicable measurement devices
Ultrasound imaging of cartilage surface	+++ +++	• Changes in cartilage surface seem to reflect alterations in cartilage quality as well as mechanical and compositional properties of cartilage • Initial degeneration alters these parameters	• At the moment no arthroscopic instrumentation exists • Requires at least minimally invasive approach
Streaming potentials	? ?	• Indirect information about cartilage composition	• Minimally invasive
OCT	? ?	• Good spatial resolution • Method could be developed for arthroscopic use	• Information only on superficial cartilage
Biochemical biomarkers	? -	• Availability is good: Samples can be analyzed in a central laboratory • Affordable prizing	• At the moment no methods exist for diagnosis at individual level

The research on OA diagnostics and therapeutics is limited by the lack of knowledge about the etiopathogenesis of the entire OA process: it is not known which changes are signs of the progressive disease and which

changes typically heal by themselves. Furthermore, a major proportion of advanced OA with radiographic changes is asymptomatic (Felson 1987). Therefore, extensive multidisciplinary follow-up studies are needed to elucidate this process. Even though no pharmaceutical solutions are currently available, it has been shown that by targeting specific OA risk factors, such as obesity, beneficial results can be obtained in OA treatment. Therefore, it would be interesting to test whether patients with an early OA diagnosis could be motivated to change their lifestyles and what is the effect on OA progression of this lifestyle modification. Obviously, the importance of early diagnostics of OA will be emphasized after effective medication for OA has been developed.

10 Conclusions

In this study, quantitative diagnostic methods for early OA were used to determine properties of healthy and degenerated articular cartilage. The sensitivity and specificity of these methods was determined. Furthermore, the results of diagnostic tests were compared with histological, mechanical, compositional and acoustic reference parameters. In addition, the effect of cartilage composition on tissue Poisson's ratio was studied. The main conclusions can be summarized as follows:

1. The collagen content and organization were found to be the primary determinants of cartilage dynamic modulus and Poisson's ratio.

2. A comparison of novel methods for the diagnosis of early cartilage degeneration revealed that several methods are capable of sensitively and specifically diagnosing early cartilage degeneration. Some of these methods offer potential for development into clinical use, but the selection of the most suitable method should be made individually.

3. Healthy human patellar cartilage displayed a statistically significant site-dependent variation in the compositional and mechanical properties of the tissue. The OA process was found to affect these properties in a non-uniform pattern.

4. R_{US} was the only parameter, where no site-dependent variation was found in healthy patellae. Furthermore, it was the only diagnostic parameter for which there were statistically significant differences detected between healthy tissue and early OA changes. This parameter was also found to offer high specificity and sensitivity. With respect to the mechanical parameters, no statistically significant differences were found between the healthy samples and those with early degeneration. This was most likely due to the site-dependent variations in the mechanical properties of the patellar cartilage.

5. A change in ultrasound speed during compression was observed. This was found to confer a significant error in the mechano-acoustically determined modulus values. To minimize its impact on cartilage mechanical moduli values, a correction method should be developed. Without correction, the change in ultrasound speed seems to be low in samples with advanced degeneration, and therefore results obtained from these kinds of samples were reliable.

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