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Novel X-ray-based Methods for Diagnostics of Osteoarthritis

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NOVEL X-RAY-BASED METHODS FOR DIAGNOSTICS OF OSTEOARTHRITIS

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Abstract

Osteoarthritis (OA) is the commonest joint disease in the world, and it has a major socioeconomic impact. OA causes progressive degenerative changes in the composition and structure of articular cartilage and subchondral bone. Clinical diagnosis of OA is based on physical examination and qualitative evaluation of changes on plain radiographs. Current clinical imaging methods are subjective or insensitive to early OA changes. Therefore, new methods are needed so as to quantify composition of the cartilage and characteristics of the subchondral bone. The aim of this thesis was to evaluate the potential of clinically applicable X-ray-based methods for the assessment of the cartilage proteoglycan content as well as the structure and density of subchondral bone in a knee joint.

Subchondral bone density and structure (local binary patterns, Laplacian, and fractal-based algorithms) analysis methods for two-dimensional (2-D) plain radiographs were validated against three-dimensional (3-D) bone microarchitecture obtained from micro-computed tomography ex vivo and applied to plain radiographs in vivo. Furthermore, a method developed for the evaluation of articular cartilage proteoglycan content from computed tomography (CT) was validated against a delayed gadolinium-enhanced magnetic resonance imaging of cartilage (dGEMRIC), which is widely used as a proteoglycan sensitive method, in subjects referred for an arthroscopy of the knee joint.

Subchondral bone density and structure evaluated from 2-D radiographs were significantly related to the bone volume fraction and true 3-D microarchitecture of bone, respectively. In addition, bone density- and structure-related parameters from radiographs were significantly different among subjects with different stages of OA. Cartilage proteoglycan content evaluated from CT was significantly related to dGEMRIC method. Furthermore, dGEMRIC was associated with bone structure from a 2-D radiograph.

In conclusion, analysis of bone structure and density is feasible from clinically available 2-D radiographs. A novel CT method sensitive to proteoglycan content should be considered when a 3-D view of cartilage quality is needed.

Keywords: articular cartilage, bone density, bone structure, computed tomography, knee joint, magnetic resonance imaging, osteoarthritis, radiography
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Abbreviations and symbols

2-D  two-dimensional
3-D  three-dimensional
ACL  anterior cruciate ligament
BV/TV bone volume fraction
Conn.Dn connectivity density
CT  computed tomography
dGEMRIC delayed gadolinium enhanced MRI of cartilage
dQCTA delayed quantitative CT arthrography
E_Lap entropy of Laplacian-based image
E_LBP entropy of LBP-based image
FD fractal dimension
FD_Hor fractal dimension of horizontal structures
FD_Ver fractal dimension of vertical structures
FSA fractal signature analysis
FOV field of view
GAG glycosaminoglycan
GV mean greyscale value
HIAngles mean homogeneity index for the orientation of LBPs
ICRS International Cartilage Repair Society
IR-FSE inversion recovery fast spin echo
JSW joint space width
KL  Kellgren-Lawrence
LBP local binary patterns
MRI magnetic resonance imaging
µCT micro-computed tomography
OA  osteoarthritis
ROI region of interest
SD  standard deviation
T1 longitudinal relaxation time
T1_Gd T1 in the presence of gadolinium, dGEMRIC index
T1p longitudinal relaxation time in rotating frame of reference
T2 transverse relaxation time
Tb.N trabecular number
Tb.Sp trabecular separation
Tb.Th trabecular thickness
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>TE</td>
<td>time to echo</td>
</tr>
<tr>
<td>TI</td>
<td>inversion time</td>
</tr>
<tr>
<td>TR</td>
<td>repetition time</td>
</tr>
<tr>
<td>VOI</td>
<td>volume of interest</td>
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</tbody>
</table>
List of original publications

This thesis is based on the following publications, which are referred throughout the text by their Roman numerals:


This thesis also contains previously unpublished data.
Contents

Abstract
Tiivistelmä
Acknowledgements
Abbreviations and symbols
List of original publications
Contents

1 Introduction

2 Articular cartilage and subchondral bone

2.1 Composition and structure of articular cartilage

2.2 Composition and structure of subchondral bone

2.3 Osteoarthritis

2.3.1 Progression of osteoarthritis

2.3.2 Treatment of osteoarthritis

2.3.3 Clinical diagnostic methods for osteoarthritis

3 X-ray-based and magnetic resonance imaging methods

3.1 Basic principles of X-ray imaging

3.2 Plain radiography

3.2.1 Texture analysis of plain radiographs

3.2.2 Texture analysis of osteoarthritic bone changes from plain knee radiographs

3.3 Computed tomography

3.4 Magnetic resonance imaging

4 Aims of the thesis

5 Material and methods

5.1 Study subjects

5.1.1 Human samples ex vivo (I)

5.1.2 Human subjects in vivo (II, III, unpublished data)

5.2 Radiography (I, II, unpublished data)

5.2.1 Selection of regions of interest

5.2.2 Evaluation of bone density from radiographs

5.2.3 Evaluation of bone structure from radiographs

5.3 Computed tomography (III)

5.4 Magnetic resonance imaging (III, unpublished data)

5.5 Reference methods

13
5.5.1 Semi-quantitative grading of radiographs (II, unpublished data)................................. 50
5.5.2 Arthroscopic grading (III) ..................................................................................... 50
5.5.3 Micro-computed tomography (I) ............................................................................ 50
5.6 Statistical analyses .............................................................................................. 51

6 Results 53
6.1 Bone density from plain radiographs (I, II)............................................................. 53
6.2 Bone structure from plain radiographs (I, II).......................................................... 54
6.3 Association of arthroscopy to dGEMRIC and dQCTA (III)...................................... 58
6.4 Correlation between dGEMRIC and dQCTA (III).................................................. 58
6.5 Correlation between dGEMRIC and bone changes on plain radiographs (unpublished data)...................................................................................... 61

7 Discussion 63
7.1 Main findings ........................................................................................................ 63
7.2 Bone density from plain radiographs .................................................................... 64
7.3 Bone structure from plain radiographs .................................................................. 65
7.4 Evaluation of cartilage composition ..................................................................... 68
7.5 Relation between dGEMRIC and bone structure from radiographs......................... 70
7.6 Limitations ........................................................................................................... 71
7.7 Clinical implications and applications in the research ......................................... 72
7.8 Future studies ..................................................................................................... 73

8 Conclusions 75

References 77
Appendices 89
Original articles 91
1 Introduction

Osteoarthritis (OA) is the commonest joint disease, occurring usually in the foot, knee, hip, spine, and hand joints (Buckwalter & Mankin 1998a). OA can be considered as a group of joint diseases which affect all tissues in the joint (Pritzker 2003). The prevalence of OA increases with age and, thus, OA is typically a disorder of middle-aged and older people (Arden & Nevitt 2006, Buckwalter & Mankin 1998b). In Finland, the prevalence of clinically diagnosed knee OA is around 9% in men aged 55–64 years and 11% in men aged 65–74 years while the prevalence is 8% in women aged 55–64 years and 18% in women aged 65–74 years (Arokoski et al. 2007). In addition to reduced quality of life, OA imposes a large economic burden on society, since the direct and indirect costs can be as high as 2.5% of the gross domestic product (March & Bachmeier 1997). OA causes progressive degeneration of the articular cartilage and changes in the subchondral bone density and structure. Typical OA changes in the cartilage include progressive degradation and loss of collagens and proteoglycans and an increase in water content (Buckwalter & Mankin 1998a). Macroscopic changes in the subchondral bone include bone sclerosis (thickening), osteophytes, and bone cysts (Buckwalter & Mankin 1998a).

Clinical diagnosis of OA is based on a physical examination and changes seen on plain radiographs. Typically, the severity of OA is visually evaluated from radiographs using Kellgren-Lawrence (KL) grading scale (Kellgren & Lawrence 1957). However, KL grading is based on a subjective evaluation, it is semi-quantitative, and its inter-rater and intra-rater reliability varies from moderate to substantial (Gunther & Sun 1999, Spector et al. 1992, Spector et al. 1993). Since plain radiography is a cheap, fast, and widely available imaging method, there is a need for development and use of quantitative and user-independent image analysis algorithms that exploit all available information from the radiographs.

Bone tissue can be clearly seen from the plain radiographs, providing a useful solution in the diagnosis of diseases that affect bone density and structure, such as OA. Previously, quantitative evaluation of OA changes from knee radiographs has included measurement of joint space width (JSW) (Buckland-Wright 1999, Reichmann et al. 2011) as well as estimation of bone density (Kinds et al. 2011, Marijnissen et al. 2008, Yamada et al. 2002) and structure (Buckland-Wright 2004, Kraus et al. 2013, Messent et al. 2005c, Podsiadlo et al. 2014, Wołoszynski et al. 2012, Wolski et al. 2010). However, the main limitation of the plain two-dimensional (2-D) radiograph is that it is a projection (summation) through the
actual three-dimensional (3-D) structure. Previous studies with bone samples from human cadavers have shown that textural parameters from 2-D high-resolution radiographs correlate significantly with 3-D trabecular bone parameters (Le Corroller et al. 2013, Ranjanomennahary et al. 2011, Steines et al. 2009). However, these studies used small specimens without soft tissue harvested from a human femur, not the entire bone, and cortical bone was removed from the 3-D analyses. Furthermore, image analysis algorithms in these studies were mainly developed for quantification of the osteoporosis-related changes, not specifically for OA-related changes.

Magnetic resonance imaging (MRI) is typically used to visualise joint morphology in 3-D in clinical practise. For instance, the volume and thickness of articular cartilage as well as changes in the subchondral bone can be evaluated from MR images (Eckstein et al. 2006, Roemer et al. 2011). Furthermore, quantitative methods measuring composition of the cartilage have been developed. One of such methods is delayed gadolinium enhanced MRI of cartilage (dGEMRIC) that has been widely used for evaluation of proteoglycan content of articular cartilage (Dahlberg et al. 2012, Nieminen et al. 2012, Oei et al. 2014).

Computed tomography (CT) provides a detailed 3-D view of a structure of tissues. As the CT imaging is based on the attenuation of X-ray radiation, similarly to 2-D radiographs, bone tissues can be easily distinguished from CT images. On the other hand, soft tissues such as articular cartilage cannot normally be seen properly in the CT images. However, the visibility of cartilage can be enhanced if a contrast agent is injected into the joint. Delayed quantitative CT arthrography (dQCTA), an analogous X-ray technique to dGEMRIC, has been developed for evaluation of the cartilage proteoglycan content, and it also requires injection of a contrast agent (Palmer et al. 2006, Siebelt et al. 2011). Although in vitro studies have demonstrated the potential of dQCTA in assessment of proteoglycan content of cartilage (Bansal et al. 2010, Kallioniemi et al. 2007, Palmer et al. 2006, Silvast et al. 2009, Xie et al. 2010), the method needs to be validated in in vivo studies.

Since the current imaging and image analysis methods for OA have limitations, there is a need for development of more reliable diagnostic methods. Therefore, the purpose of this doctoral thesis was to explore the potential of novel X-ray-based methods to quantify OA related changes in articular cartilage and subchondral bone both ex vivo and in vivo.
2 Articular cartilage and subchondral bone

Articular cartilage is a connective tissue that covers articulating bone ends in synovial joints (Athanasiou et al. 1991). In a knee joint, articular cartilages that covers the patella and condyles of the tibia and femur are typically a few millimetres thick (Ateshian et al. 1991, Faber et al. 2001, Hall & Wyshak 1980). The main functions of articular cartilage are to distribute mechanical loads and, together with synovial fluid, to provide low friction surface between the articulating bones (Sophia Fox et al. 2009).

Beneath the articular cartilage is bone tissue. The main functions of bones are to protect internal organs, enable locomotion, produce blood cells and store minerals (Miller et al. 2007). Bone is a living tissue that constantly renews itself through modelling and remodelling and can adapt its shape and microarchitecture to habitual loadings (Miller et al. 2007, Wolff 1892). In Figure 1, a knee joint and different zones of articular cartilage and two different types of bone are shown.

![Fig. 1. A schematic figure of a knee joint (left) and structure of articular cartilage (right). Different zones of articular cartilage and two different types of subchondral bone are indicated in the figure (right).](image)

2.1 Composition and structure of articular cartilage

Articular cartilage is composed of cells, water, and structural macromolecules. Around 60–80% of the wet weight of the articular cartilage can be water while the remaining 20–40% are mostly structural macromolecules (Athanasiou et al. 1991, Buckwalter & Mankin 1998b, Mankin et al. 2007, Mow et al. 2005). From the structural macromolecules, collagens constitute approximately 60%, proteoglycans
25–35%, and non-collagenous proteins and glycoproteins 15–20% of the dry weight of the tissue (Mankin et al. 2007). The cells (chondrocytes) constitute only about 1% of the articular cartilage volume (Mankin et al. 2007).

In articular cartilage, type II collagen is the most common collagen type accounting 90–95% of collagens (Buckwalter & Mankin 1998b, Mankin et al. 2007). Collagen fibrils are organised into a meshwork (Figure 1) that gives cartilage its tensile strength and form (Mankin et al. 2007, Mow et al. 2005). Furthermore, the meshwork attaches other structural macromolecules and water to the matrix (Mankin et al. 2007).

Cartilage proteoglycans are composed of a protein core and glycosaminoglycan (GAG) side chains. Negatively charged GAGs repel other negatively charged molecules and attract cations into the tissue. Proteoglycans in articular cartilage are mostly large aggregating molecules or aggrecans (about 90% of the total cartilage matrix proteoglycan mass) and smaller proteoglycans. The articular cartilage matrix is mostly filled with aggrecans which form large protein aggregates together with hyaluronan and link proteins. The formation of aggregates helps proteoglycans to attach within the matrix and prevents undesirable displacement of proteoglycans during mechanical loading of the tissue. (Buckwalter & Mankin 1998b, Mankin et al. 2007, Mow et al. 2005).

The only cell type in the articular cartilage is chondrocyte. Chondrocytes do not form cell-to-cell contacts within the extracellular matrix (Buckwalter & Mankin 1998b, Mankin et al. 2007). They sense changes in the cartilage composition and respond by producing and replacing appropriate types and numbers of macromolecules (Mankin et al. 2007). Since articular cartilage is an avascular tissue, chondrocytes receive their nutrition from synovial fluid though diffusion (Buckwalter & Mankin 1998b).

Cartilage tissue fluid consists of interstitial water and solutes such as ions and nutrients (Buckwalter & Mankin 1998b). Tissue fluid has a high concentration of cations to balance the negative charge in the tissue created by the proteoglycans. Hence, the water content of the cartilage depends mainly on the large aggregating proteoglycans and the organisation, stiffness, and strength of the collagen network (Mow et al. 2005). The physical properties of the cartilage depend on the interaction of the tissue fluid and structural macromolecules (Buckwalter & Mankin 1998b).

The structure of the articular cartilage is anisotropic and can be separated into four zones: superficial zone, transitional zone, deep zone, and the zone of calcified cartilage (Figure 1). The boundaries between the zones cannot be exactly defined,
yet each zone has different morphologic features. In the superficial zone, flattened chondrocytes are arranged in parallel to the articular surface. The collagen fibrils are quite thin and are oriented parallel to the articular surface as well. The concentration of water is higher, whereas proteoglycan concentration is lower than other zones of cartilage. In the transitional zone, chondrocytes are spherical and collagen fibrils are oriented more randomly and have a larger diameter. Furthermore, proteoglycan content is higher, but water and collagen content is lower than in the superficial zone. The deep zone contains the largest diameter collagen fibrils that are oriented perpendicular to the articular surface. In this zone, the proteoglycan content is highest and water content lowest compared to the other zones. The collagen fibres of the deep zone pass into the tidemark, which is a thin and irregular boundary between the deep zone and the calcified cartilage. The zone of calcified cartilage separates the deep zone from the subchondral bone. The volume of the cells is lower than in the deep zone, and the cells have low level of metabolic activity. (Buckwalter & Mankin 1998b, Mankin et al. 2007, Mow et al. 2005).

2.2 Composition and structure of subchondral bone

Bone is a composite material that consists of mineral and organic phases, water, and cells (Boskey 2013). The mineral phase comprises approximately 65%, the organic phase approximately 20–25%, and water approximately 10% of the wet weight of the tissue (Buckwalter et al. 1995).

The organic matrix of bone tissue consists primarily of collagen type I (90% of the organic matrix) which gives bone its tensile strength (Miller et al. 2007, Mow et al. 2005). About 5% of the organic matrix is non-collagenous proteins and about 2% is lipids (Boskey 2013, Currey 2002, Miller et al. 2007).

Most of the minerals in bone are hydroxyapatite crystals and calcium phosphate (Mow et al. 2005). The mineral components of bone are closely attached with the collagen fibrils (Miller et al. 2007). Mineral gives bone its compressive strength.

The bone cells include osteoblasts that are responsible for the formation of new bone, osteoclasts that are resorbing the bone tissue, and osteocytes that are osteoblasts which have become embedded with the matrix they have produced (Currey 2002, Miller et al. 2007).

Bones can be categorised by their shape. For instance, the femur and tibia are categorised as a long bone type (Currey 2002). From the long bones, three distinct
zones can be recognised: diaphysis, metaphysis, and epiphysis (Miller et al. 2007). The long bones are hollow in the shaft, i.e. in the diaphysis, and wider at the end of bones, i.e. in the epiphyses. The metaphysis is between the epiphysis and diaphysis. The outer surface of long bones is cortical bone, whereas near at the ends of the long bones, the central part is trabecular bone (Currey 2002).

At the ends of the long bones is a region of subchondral bone. It is located immediately below the zone of calcified cartilage (Figure 1). The cement line, that contains less mineral and collagen than the surrounding bone, separates the calcified cartilage from the subchondral bone. Collagen fibres do not pass the cement line, but blood vessels from the subchondral bone can reach into calcified cartilage through small channels (Madry et al. 2010). The subchondral bone can be further divided into the subchondral bone plate (cortical endplate) consisting of cortical bone and the subchondral trabecular bone (Figure 1) (Burr 2004, Madry et al. 2010).

The cortical bone consists of cylinders called osteons (or haversian system) that typically have a diameter of about 0.2 mm, and each osteon consist of concentric mineral plates called lamellae (Currey 2002). Cortical bone is supplied by the Haversian and Volkmann’s canals which contain a blood vessel or vessels and nerves (Currey 2002, Miller et al. 2007). The thickness of the subchondral bone plate varies within joints, e.g. being in the tibial plateau from twenty micrometres to few millimetres (Milz & Putz 1994).

Subchondral trabecular bone is more porous than cortical bone, but it is typically still denser than normal trabecular bone (Patel et al. 2003). Subchondral trabecular bone is anisotropic, i.e. the trabeculae are not oriented in the same directions and, thus, the mechanical properties of the subchondral trabecular bone vary in the different planes (Burr 2004). Trabecular bone consists of network of trabeculae. Based on the shape, two types of trabeculae can be distinguished. Although the separation between the types is not always clear, the trabeculae can either be rod-like or plate-like. Their maximum thickness is about 0.2 mm (Miller et al. 2007). Trabeculae are mainly oriented according to the daily load. The spaces between the trabeculae are bone marrow, and trabecular bone receives its nutrition from the blood supply in the marrow. The structure of trabecular bone enables it to act as a shock absorber (Miller et al. 2007).

Bone resorption and formation are processes that continue through the lifetime (Miller et al. 2007). Bone mass increases during the childhood and the peak bone mass is achieved around 30 years of age (Miller et al. 2007). After that, the bone
mass starts to slowly reduce. In women, the loss of the bone is accelerated after menopause (Miller et al. 2007).

2.3 **Osteoarthritis**

OA has commonly believed to be purely a disease of mechanical degradation of articular cartilage, but it is nowadays considered as a group of joint diseases which affect all tissues in the joint and have several phenotypes (Bijlsma et al. 2011, Glyn-Jones et al. 2015, Karsdal et al. 2014, Pritzker 2003). Several factors predispose to OA, including but not limited to age, female gender, genetics, nutrition, bone density, obesity, joint injury or surgery, joint deformity, and repetitive joint loading (Arden & Nevitt 2006, Buckwalter & Mankin 1998a). Nowadays, it is widely assumed that harmful biomechanics on a susceptible joint plays a primary role in the development of OA, although OA does not develop on most of the subjects with abnormal joint biomechanics (Glyn-Jones et al. 2015). Aging is the primary risk factor of OA, probably due to a reduction in regenerative capacity and increased number of risk factors present (Glyn-Jones et al. 2015). Female gender increase the risk of OA, probably due to oestrogen (Glyn-Jones et al. 2015). Subjects with high bone mineral density have an increased risk of OA, but low bone mineral density is also associated with the progression of OA (Arden & Nevitt 2006, Nevitt et al. 2010). The possible subtypes of OA that are currently receiving most attention include metabolic, traumatic, inflammation-related, and subchondral bone turnover-driven OA (Karsdal et al. 2014).

2.3.1 *Progression of osteoarthritis*

In the early stages of OA, the water content of the articular cartilage increases, the aggregation of proteoglycans declines, and alterations in the relative number of the collagen fibrils and minor collagens occurs. Minor fibrillation or disruption of the superficial zone of the cartilage, resulting from the disorganisation of collagen meshwork, can be detected. These changes reduce the mechanical stiffness and increase the permeability of the cartilage tissue. Deeper in the cartilage, advancement or duplication of the tidemark may occur. In the mineralised tissues, thickening of subchondral bone plate resulting from the formation of new layers of bone is a typical alteration. Although the amount of bone is increased, the bone may be less dense than normal bone due to incomplete mineralisation. (Buckwalter & Mankin 1998a, Buckwalter et al. 2005, Pritzker 2003).
In the second stage of OA, chondrocytes release mediators that stimulate the cartilage repair process. The repair process, including an increased synthesis of matrix macromolecules and cell proliferation, can last for years. Fibrillation or disruption of the superficial zone of the cartilage is seen, and fissures can even reach into the subchondral bone as the OA progresses. In the subchondral bone, thickening (sclerosis) continues and osteophytes and subchondral bone cysts may be formed. (Buckwalter & Mankin 1998a, Buckwalter et al. 2005).

In the late stages of OA, chondrocytes completely fail to restore the articular cartilage. Articular cartilage is completely lost at this stage, and the subchondral bone is very dense and significantly thickened. Osteophytes and subchondral bone cysts are also common at this stage of the disease. (Buckwalter & Mankin 1998a, Buckwalter et al. 2005, Pritzker 2003). Late stage OA changes causes remarkable pain and loss of joint function.

2.3.2 Treatment of osteoarthritis

Treatment of OA is primarily targeted at alleviating pain and improving joint function (Hunter 2015). According to recent American College of Rheumatology and OA Research Society International guidelines, the treatment of knee OA should first consist of non-pharmacological management including patient education, weight loss, physical therapy, and exercise (Hochberg et al. 2012, McAlindon et al. 2014). After these, medication for pain relief may be used. Sometimes non-surgical treatment consists of paracetamol, oral or topical non-steroidal anti-inflammatory drugs, or intra-articular corticosteroid injections (Hochberg et al. 2012, Karsdal et al. 2014, McAlindon et al. 2014).

When non-surgical treatment options fail, surgery, e.g. periarticular osteotomy that corrects the mechanical axis of the knee or total knee arthroplasty (knee replacement), may be the only treatment option (Glyn-Jones et al. 2015). It should be emphasized that none of the current treatment options can completely cure or slow down the progression of OA, and there are no effective disease-modifying treatments available yet.

2.3.3 Clinical diagnostic methods for osteoarthritis

Diagnosis of OA is based on a patient’s history and symptoms, physical findings, and changes on plain radiographs. Sometimes, MRI can be used as an additional examination if needed. Symptoms and signs of OA include joint pain, restriction in
motion, crepitus with joint motion, joint effusions, and in severe cases, joint deformities and subluxations (Buckwalter & Mankin 1998a, Buckwalter & Martin 2006).

The severity of OA can be evaluated from radiographs using several grading scales, e.g. the KL (Kellgren & Lawrence 1957) and Ahlbäck (Ahlbäck 1968) grading scales. The KL grading is the most typically used, in which the classification criteria are based on a visual evaluation of joint space narrowing, subchondral bone sclerosis, osteophytes, and deformation of bone ends. In Figure 2, representative images for different KL grades are shown. KL grading is based on a subjective evaluation, it is semi-quantitative, and its inter-rater and intra-rater reliability varies from moderate to substantial (Gunther & Sun 1999, Spector et al. 1992, Spector et al. 1993). These can all be considered to be disadvantages of KL grading. Quantitative analysis methods for radiographs are presented in greater detail in section 3.2 of this thesis.

MRI can be used to visualise joint morphology in clinical practice and all tissue types in a joint are visible in MRI (Burstein & Gray 2003). For example, volume, thickness, and curvature of articular cartilage can be quantitatively measured (Eckstein et al. 2006, Roemer et al. 2011). Furthermore, lesions in the articular cartilage as well as changes in subchondral bone, ligaments and menisci can be detected with MRI. Quantitative evaluation of articular cartilage from MRI is discussed in section 3.4 of this thesis.
Fig. 2. Representative images and classification criteria for different Kellgren-Lawrence (KL) grades in the knee. 0 = Normal: No radiographic features of osteoarthritis (OA); 1 = Doubtful OA: Doubtful narrowing of joint space and possible osteophytes; 2 = Minimal OA: Definite osteophytes and narrowing of the joint space; 3 = Moderate OA: Moderate multiple osteophytes, definite narrowing of the joint space, some sclerosis, and possible deformity of bone ends; 4 = Severe OA: Large osteophytes, marked narrowing of the joint space, severe sclerosis, and definite deformity of bone ends.
Arthroscopy is a minor surgical operation where an arthroscope is inserted into the joint through a small incision. The surface of the articular cartilage can be visually evaluated and mechanically palpated during arthroscopy. Arthroscopy can be considered as the gold standard for the assessment of articular cartilage lesions (Spahn et al. 2009). In the arthroscopy, cartilage lesions can be classified, e.g. according to the International Cartilage Repair Society (ICRS) grading system (Table 1) (Brittberg & Winalski 2003). However, qualitative and subjective visual evaluation and manual palpation during the arthroscopy has been claimed to be dependent on the evaluator (Brismar et al. 2002). Furthermore, subchondral bone cannot be investigated during arthroscopy.

**Table 1. International Cartilage Repair Society (ICRS) classification.**

<table>
<thead>
<tr>
<th>ICRS grade</th>
<th>Cartilage appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – Normal</td>
<td>Intact</td>
</tr>
<tr>
<td>1 – Nearly normal</td>
<td>Superficial lesions. Slightly softened indentation stiffness and/or superficial fissures and cracks</td>
</tr>
<tr>
<td>2 – Abnormal</td>
<td>Lesions extending less than 50% of cartilage depth</td>
</tr>
<tr>
<td>3 – Severely abnormal</td>
<td>Lesions extending 50% or more of cartilage depth but not into the subchondral bone</td>
</tr>
<tr>
<td>4 – Severely abnormal</td>
<td>Cartilage lesions extending into the subchondral bone</td>
</tr>
</tbody>
</table>
3 X-ray-based and magnetic resonance imaging methods

3.1 Basic principles of X-ray imaging

X-rays are one type of electromagnetic radiation with shorter wavelengths than visible light being between 0.01 and 10 nm. X-rays penetrate through an object, enabling their use to image the internal structure of human body in medicine. Bones absorb X-rays efficiently, as they contain minerals that are composed of elements with a relatively high atomic number such as calcium (Carroll 2007). Radiography and CT, both using X-rays, are common imaging modalities used in the diagnostics of diseases of bone tissues.

The X-ray beam is attenuated when it travels through the human body and its intensity is reduced. Attenuation of X-rays in the body depends on atomic number, thickness, and density of the tissue as well as energy of the radiation. Attenuation in the matter obeys Equation 1:

\[ I = I_0 e^{-\mu x}, \]

where \( I \) is the intensity of the photons passed the material, \( I_0 \) is the initial intensity of the photons, \( \mu \) is the linear attenuation coefficient that defines the fraction of attenuated photons per unit thickness of the material, and \( x \) is the distance. Sometimes, mass attenuation coefficient is used instead of linear.

The X-ray detector measures the number of photons that have passed through the object. Digital detectors are common nowadays. In the detector, photons are converted to an electrical signal and a digital radiograph is constructed.

3.2 Plain radiography

Plain radiography is widely used in the clinical and research settings of OA. Advantages of plain radiography are that it is cheap, fast to conduct, and widely available. Although subjects are exposed to ionising radiation during radiography, the effective radiation dose is very small, e.g. when the knee is imaged. However, plain radiograph is only a 2-D projection image of a 3-D object.

In the knee joint, X-ray attenuation coefficients of articular cartilage, menisci, and synovial fluid are close to each other and, therefore, these tissues cannot be clearly seen from plain radiographs. However, an evaluation of articular cartilage can still be performed indirectly by measuring JSW from the radiograph, i.e. in
knee the distance between condyles of tibia and femur. Typically, minimum JSW, i.e. the minimum distance between the condyles, is measured (Reichmann et al. 2011). JSW has been measured either manually or using computer-based automatic algorithms (Buckland-Wright 1999, Reichmann et al. 2011). However, early OA cannot be detected using only JSW, as it is insensitive to changes in cartilage composition and cannot detect localised cartilage damage (Glyn-Jones et al. 2015). Furthermore, JSW depends on the positioning of the joint. Despite these limitations, JSW is currently the only structural endpoint measure for the disease-modifying drug research for OA that is accepted by the US Food and Drug Administration and the European Medicines Agency.

Since OA subjects tend to have higher bone density than non-OA subjects, attempts to estimate bone density from plain radiographs have been conducted (Kinds et al. 2011, Marijnissen et al. 2008, Yamada et al. 2002). It is known that image acquisition parameters and post-processing algorithms significantly affect the density estimates, and to overcome this issue, calibration of the greyscale values in an image using an aluminium step wedge have been proposed (Kinds et al. 2011, Marijnissen et al. 2008, Yamada et al. 2002).

In addition to JSW and density estimation, texture analysis is a potential method to extract quantitative and user-independent information of bone structures from plain radiographs. Texture analysis of bone is not as dependent on the imaging conditions as direct evaluation of greyscale values, since the magnitude of the greyscale values is not typically directly evaluated in texture analysis methods.

**3.2.1 Texture analysis of plain radiographs**

Medical image analysis often involves interpretation of tissue appearance. The appearance can be, e.g. smooth, grainy, regular, or homogenous, and these image properties are related to the spatial arrangement of pixel intensities in an image, i.e. image texture (Bankman et al. 2009). Texture analysis attempts to quantify these variations in pixel intensity values. There are several different methods for analysing texture and these methods can be divided into four categories (Bharati et al. 2004, Materka & Strzelecki 1998): statistical, structural, model-based, and transform-based methods. In statistical methods, the distribution and relationships of intensities within a certain region in an image are analysed (Materka & Strzelecki 1998). Grey-level co-occurrence matrix (GLCM)-based methods are one of the most popular methods of statistical texture analysis (Haralick et al. 1973). In structural methods, texture is considered as a set of repeated or regular texture
elements, such as equally spaced lines (Bharati et al. 2004). Mathematical morphology is one example of structural methods (Haralick et al. 1987). In model-based methods, texture is presented as sophisticated mathematical models (Castellano et al. 2004). The fractal model is one typical model-based method (Pentland 1984). In transform-based methods, texture properties are evaluated in a different space (Castellano et al. 2004, Materka & Strzelecki 1998). Transform-based methods include techniques such as Fourier, Gabor, or wavelet transforms (Castellano et al. 2004).

Several different texture analysis methods have been proposed for the evaluation of radiographs. One of the earliest studies involved texture analysis-categorised pulmonary disease from chest radiographs (Sutton & Hall 1972). Nowadays, analyses have been expanded to other applications, e.g. to mammography and bone radiography.

Many potential texture analysis methods for bone have been developed. For instance, Fourier and wavelet analysis (Faber et al. 2004, Vokes et al. 2006), Radon transform (Boehm et al. 2009), methods based on mathematical morphology (Veenland et al. 1997), several different fractal analysis methods (Geraets & van der Stelt 2000, Le Corroller et al. 2013) as well as entropy and GLCM-based measures (Chappard et al. 2010, Pulkkinen et al. 2011, Thevenot et al. 2014b) have been applied to plain radiographs. To remove noise and enhance the appearance of bone trabeculae, the radiographs can be pre-processed, e.g. with median filtering and gradient- or Laplacian-based algorithms (Pulkkinen et al. 2011, Thevenot et al. 2014b). Furthermore, another potential method for bone texture analysis is the local binary patterns (LBP) method that has been widely used in the machine vision field (Ojala et al. 1996). The principle of the LBP method is simple, computationally efficient, and robust to monotonic greyscale variations (Figure 3). It can be considered as an approach that combines statistical and structural methods of texture analysis. The one-dimensional LBP method has recently been applied in the trabecular bone analysis from the calcaneus (Houam et al. 2012).
Fig. 3. LBP is built by thresholding neighbouring pixels by the greyscale value of the centre pixel and multiplying the binary matrix with the weight matrix. In this example \( LBP = 1^*1 + 0^*2 + 0^*4 + 1^*8 + 1^*16 + 1^*32 + 1^*64 + 1^*128 = 241 \).

### 3.2.2 Texture analysis of osteoarthritic bone changes from plain knee radiographs

Fractal analysis is the most popular texture analysis method in OA research. It provides information about the complexity (e.g. details in the image) and roughness (deviation from the mean surface height) of the bone texture (Messent et al. 2005a, Pentland 1984). For example, smooth surface has a low complexity and the deviations from the mean surface height are small, while the surface is rough if the deviations are large. If the image have many details (frequency content is high), the complexity is higher. Progression of OA has been assessed from digital knee radiography using a fractal-based method called fractal signature analysis (FSA) (Kraus et al. 2013) and a signature dissimilarity measure method (Woloszynski et al. 2012). Fractal-based algorithms have also been applied to macro-radiographs, *i.e.* radiographs with better spatial resolution than standard radiographs (Buckland-Wright 2004, Messent et al. 2005b, Messent et al. 2005c, Messent et al. 2006, Messent et al. 2007) and to standard film radiographs from OA knees (Kraus et al. 2009, Messent et al. 2006, Podsadllo et al. 2008a). Although macro-radiographs have better spatial resolution, changes in bone structure can also be detected from standard radiographs (Messent et al. 2006). Selected publications for the evaluation of bone texture related to OA from knee radiographs are given in Table 2.
<table>
<thead>
<tr>
<th>Author</th>
<th>Analysis method</th>
<th>Pixel size</th>
<th>n</th>
<th>Main findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lynch et al. 1991a</td>
<td>FSA1</td>
<td>0.03 mm</td>
<td>20 knees (10 controls, 10 OA)</td>
<td>Overall FD² of horizontal structures in the medial compartment was significantly higher in OA knees.</td>
</tr>
<tr>
<td>Lynch et al. 1991b</td>
<td>FSA</td>
<td>0.03 mm</td>
<td>3 knees (1 control, 2 OA)</td>
<td>Errors related to pixel size, exposure, and subject repositioning were small compared to the changes between FDs of OA and control subjects.</td>
</tr>
<tr>
<td>Buckland-Wright et al. 1996</td>
<td>FSA</td>
<td>0.06 mm</td>
<td>94 knees (28 controls, 66 OA)</td>
<td>FD of horizontal structures was lower in knees with early OA (minor JSN²); FD of vertical structures was highest among subjects with marked JSN. Significant differences were not found in lateral compartment.</td>
</tr>
<tr>
<td>Buckland-Wright et al. 2000</td>
<td>FSA</td>
<td>0.06 mm</td>
<td>19 subjects with ACL¹ rupture in one knee</td>
<td>FD of horizontal structures was lower in knees with ACL rupture compared to contralateral uninjured knees.</td>
</tr>
<tr>
<td>Podsiadlo &amp; Stachowiak 2002</td>
<td>Hurst orientation transform</td>
<td>0.05 mm</td>
<td>1 human proximal tibia</td>
<td>The method can effectively be used to measure roughness and anisotropy of bone.</td>
</tr>
<tr>
<td>Buckland-Wright 2004 (review)</td>
<td>FSA</td>
<td>-</td>
<td>-</td>
<td>Knees with greater JSN had higher trabecular thickness and lower trabecular separation than knees with less severe disease.</td>
</tr>
<tr>
<td>Messent et al. 2005a</td>
<td>FSA</td>
<td>0.025 – 0.05 mm</td>
<td>22 human proximal tibiae without soft tissue (11 controls, 11 OA)</td>
<td>FD of horizontal structures (in medial and lateral subchondral bone) in OA knees was lower, whereas FD of vertical structures (in medial trabecular bone) was higher.</td>
</tr>
<tr>
<td>Messent et al. 2005b</td>
<td>FSA</td>
<td>0.06 mm</td>
<td>137 knees (27 controls, 110 OA)</td>
<td>FDs of vertical and horizontal structures were higher in OA knees in medial and lateral subchondral and trabecular bone. At larger scales, FD of horizontal structures was lower in OA than in control knees in medial subchondral bone.</td>
</tr>
<tr>
<td>Messent et al. 2005c</td>
<td>FSA</td>
<td>0.06 mm</td>
<td>80 OA knees</td>
<td>FDs of horizontal and vertical structures were reduced after 24 months among subjects with slow or detectable JSN.</td>
</tr>
<tr>
<td>Author</td>
<td>Analysis method</td>
<td>Pixel size</td>
<td>n</td>
<td>Main findings</td>
</tr>
<tr>
<td>-------------------------</td>
<td>---------------------------------------------------------</td>
<td>------------</td>
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</tr>
<tr>
<td>Messent et al. 2006</td>
<td>FSA</td>
<td>0.06 mm</td>
<td>34 knees (10 controls, 24 OA)</td>
<td>Changes in bone structure were evident in both macro- and standard radiographs. FD of vertical structures was higher in the OA group.</td>
</tr>
<tr>
<td>Messent et al. 2007</td>
<td>FSA</td>
<td>0.06 mm</td>
<td>117 knees (30 controls, 87 OA)</td>
<td>The decrease in FD of horizontal structures and increase in FD of vertical structures was related to the size of the osteophyte.</td>
</tr>
<tr>
<td>Buckland-Wright et al. 2007</td>
<td>FSA</td>
<td>0.06 mm</td>
<td>1232 knees</td>
<td>FD of vertical and horizontal structures were reduced among subjects with OA receiving placebo and unchanged among subjects with detectable JSN receiving bisphosphonate.</td>
</tr>
<tr>
<td>Podsiadlo et al. 2008a</td>
<td>Augmented Hurst orientation method and FSA</td>
<td>0.05 mm</td>
<td>52 knees (26 controls, 26 OA)</td>
<td>FDs were lower in OA knees. The method is comparable to FSA method.</td>
</tr>
<tr>
<td>Shamir et al. 2009a</td>
<td>WND-CHARM&lt;sup&gt;5&lt;/sup&gt;</td>
<td>0.08 mm</td>
<td>123 knees (84 controls, 115 that initially had KL grade 0 but progressed to KL grade 1, 2, or 3)</td>
<td>The method predicted change from KL0 to KL3 with 72% accuracy and the change from KL0 to KL2 with 62% accuracy. The main predictive signal came from the region adjacent to the tibial spine.</td>
</tr>
<tr>
<td>Shamir et al. 2009b</td>
<td>WND-CHARM</td>
<td>Not reported</td>
<td>350 knees (154 controls, 196 OA)</td>
<td>KL2 and KL3 were differentiated from controls with good accuracy (80 – 92%) when the whole joint area was analysed.</td>
</tr>
<tr>
<td>Kraus et al. 2009</td>
<td>FSA</td>
<td>0.17 mm</td>
<td>248 knees</td>
<td>FDs of horizontal and vertical structures predicted progression of medial JSN (area under receiver operating characteristic curve: 0.75). FD of horizontal structures was lower, whereas FD of vertical structures was higher among progressive OA than non-progressive OA subjects.</td>
</tr>
<tr>
<td>Wong et al. 2009</td>
<td>Algorithm estimating run length and topological parameters</td>
<td>0.08 mm</td>
<td>38 OA knees</td>
<td>Greater JSN was associated with the lower porosity and greater number of free trabecular ends among subjects with advanced knee OA.</td>
</tr>
<tr>
<td>Woloszynski et al. 2010</td>
<td>Signature dissimilarity measure</td>
<td>0.05 mm</td>
<td>137 knees (68 controls, 69 OA)</td>
<td>The method classified knees with 79% accuracy.</td>
</tr>
<tr>
<td>Author</td>
<td>Analysis method</td>
<td>Pixel size</td>
<td>n</td>
<td>Main findings</td>
</tr>
<tr>
<td>------------------------</td>
<td>-----------------------</td>
<td>------------------</td>
<td>-----------------------</td>
<td>-----------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Wolski et al. 2010</td>
<td>Variance orientation</td>
<td>0.05 mm</td>
<td>52 knees (26 controls, 26 OA)</td>
<td>FDs were lower in OA knees.</td>
</tr>
<tr>
<td>Wolski et al. 2011</td>
<td>Variance orientation</td>
<td>0.05 mm</td>
<td>56 knees (28 controls, 28 OA)</td>
<td>FDs were higher (in both medial and lateral trabecular bone area) among subjects with cartilage defects detected with MRI.</td>
</tr>
<tr>
<td>Woloszynski et al. 2012</td>
<td>Signature dissimilarity measure</td>
<td>0.146 mm</td>
<td>203 knees (135 controls, 68 OA)</td>
<td>Progression of medial JSN was predicted in knees with or without pre-existing OA (areas under receiver operating characteristic curve when adjusted with age, sex and BMI: 0.77 and 0.75, respectively).</td>
</tr>
<tr>
<td>Kraus et al. 2013</td>
<td>FSA</td>
<td>0.14 ± 0.03 mm</td>
<td>60 OA knees</td>
<td>FD of vertical structures predicted changes in joint space area, width and cartilage volume after 24 months, and was higher among subjects with progressive OA than without progression. FDs of horizontal structures was not a significant predictor.</td>
</tr>
<tr>
<td>Podsiadlo et al. 2014</td>
<td>Variance orientation</td>
<td>0.1 mm</td>
<td>114 knees (86 controls, 28 with knee replacement)</td>
<td>Subjects who went for knee replacement surgery had lower mean FD and FD of horizontal structures.</td>
</tr>
<tr>
<td>Roemer et al. 2015</td>
<td>FSA</td>
<td>0.05 mm</td>
<td>685 (135 athletes, 550 non-athletes)</td>
<td>FD of horizontal structures was significantly higher and FD of vertical structures lower among athletes than non-athletes. Subjects with previous ACL surgery had lower FDs.</td>
</tr>
</tbody>
</table>

1FSA = fractal signature analysis, 2FD = fractal dimension, 3JSN = joint space narrowing, 4ACL = anterior cruciate ligament, 5WND-CHARM = Weighted neighbour distances using a compound hierarchy of algorithms representing morphology, 6BMI = body mass index.

### 3.3 Computed tomography

In CT, several projection X-ray images are acquired from different angles over a full rotation circle. From the 2-D projection images, a cross-sectional image slice stack is typically reconstructed using iterative or filtered back-projection techniques (Dowsett et al. 2006). Nowadays, it is very common to subsequently calculate a 3-D image from the reconstructed slices. Compared to 2-D radiography,
spatial resolution of clinical CT imaging is lower, but the image contrast is higher (Dowsett et al. 2006).

As in plain radiography, soft tissues in the knee joint, cannot be properly distinguished from native CT images. However, contrast agents can be used to improve the contrast within and between different soft tissues. Iodine-based compounds are typically used in CT imaging, since iodine absorbs X-rays effectively due to its relatively high atomic number. When imaging joints, contrast agent is typically administered intra-articularly directly into the target joint.

CT arthrography has been used clinically to image joint pathologies for many years (Berquist 1997, Vande Berg et al. 2002). In the CT arthrography, the joint is scanned about 5 minutes after the intra-articular injection of iodinated contrast agent and the qualitative evaluation of images for possible pathologies is performed (Vande Berg et al. 2002). Contrast agent has not been diffused into the articular cartilage at the time of imaging, and the surface of the articular cartilage can be well seen in the CT arthrography. Therefore, the thickness of the cartilage can be measured and cartilage lesions can be sensitively detected (Vande Berg et al. 2002).

Quantitative information on the composition of the tissue can be obtained when the delay between contrast agent injection and CT imaging is sufficient and the contrast agent has been diffused into the cartilage. In vivo application of this imaging technique is referred to as dQCTA. Analysis of articular cartilage composition using dQCTA is based on the assumption that negatively charged contrast agent distributes into the cartilage tissue in an inverse relation to the fixed charge density in the cartilage (Bashir et al. 1996, Bashir et al. 1997). The fixed charge density is associated with the GAG content of the cartilage. When cartilage is degraded, its GAG content is reduced and a higher concentration of anionic contrast agent is diffused into the degraded cartilage than into the intact cartilage. However, diffusion and distribution of contrast agents are also affected by other factors including the collagen and water content of cartilage (Evans & Quinn 2005, Palmer et al. 2006, Piscara et al. 2008, Salo et al. 2012, Silvast et al. 2009).

Relation of contrast-enhanced CT and cartilage GAG content has been demonstrated in several in vitro studies (Bansal et al. 2010, Kallioniem et al. 2007, Palmer et al. 2006, Silvast et al. 2009, Xie et al. 2010). Furthermore, dQCTA has been applied ex vivo on knee joints of human cadavers with 10 minute delay between contrast agent injection and imaging (Siebelt et al. 2011, van Tiel et al. 2012). Optimal delay between injection and imaging was suggested to be between 30 and 60 minutes depending on the location in the knee joint (Kokkonen et al. 2012). Although dQCTA has recently been applied in vivo using clinical CT
(Kokkonen et al. 2012) and cone beam CT (Kokkonen et al. 2014), the method has not been thoroughly validated in clinical settings yet.

Similarly to CT, micro-CT (μCT) provides 2-D projection images of an object that can be processed to 3-D images, but the pixel sizes are in a micrometre range. The drawback in μCT is that it is not yet suitable for the scanning of large objects, e.g. organs within a human body. It is typically used for imaging of small objects in laboratory settings. When samples from human bones have been evaluated using μCT, higher bone volume fraction (the ratio of 3-D total bone volume to total volume) and higher trabecular thickness have typically been detected in osteoarthritic subchondral bone than in healthy bone (Bobinac et al. 2013, Ding et al. 2003, Djuric et al. 2013, Fazzalari & Parkinson 1997, Kamibayashi et al. 1995). The structure model index (the relative prevalence of rod-like and plate-like trabeculae) has been reported to be lower in OA bones than in healthy controls indicating that the trabecular architecture is more plate-like than rod-like (Ding et al. 2003, Djuric et al. 2013). Some studies have reported higher connectivity and increased trabecular separation but a lower trabecular number in OA bone compared to controls (Djuric et al. 2013, Kamibayashi et al. 1995). However, there are also studies suggesting that, in OA bone, the number of trabeculae is higher and trabecular separation is lower than in the controls (Bobinac et al. 2013, Buckland-Wright 2004, Djuric et al. 2013, Fazzalari & Parkinson 1997). The discordance between the studies is likely due to the difference in anatomical sites studied and to the different stages of OA in the samples.

### 3.4 Magnetic resonance imaging

In contrast to X-ray based methods, MRI does not involve ionising radiation. Most commonly, MRI utilises the properties of the nucleus of hydrogen, which is a single proton (McRobbie 2007). For example, water and fat in the human body contain hydrogen (McRobbie 2007). In MRI, magnetic moments of protons in the human body are aligned with the direction of the magnetic field in the MR scanner. When a radiofrequency pulse is applied, the protons change their magnetisation alignment relative to the field. When the radiofrequency pulse is switched off, the protons begin to rotate (precess) when returning back their initial orientation. Longitudinal or spin-lattice relaxation time, $T_1$, characterises the time of longitudinal magnetisation to return back to the equilibrium state, whereas transverse or spin-spin relaxation time, $T_2$, characterises the decay of the transverse magnetisation.
Due to the excellent soft tissue contrast of MRI, articular cartilage is well visualised in the MR images (Li & Majumdar 2013). On the contrary, bone tissue is relatively challenging to image with MRI, since it has a much lower proton density compared to the articular cartilage and short $T_2$ relaxation time (Dowsett et al. 2006, Horch et al. 2010). Bone anatomies are visible in MRI due to fatty bone marrow or pathological changes that include the presence of soft tissue (Dowsett et al. 2006).

MRI is considered to be the most important imaging modality of a knee joint in the OA research field (Roemer et al. 2011). In addition to measurement of cartilage thickness and volume, semi-quantitative grading scales have been developed for evaluation of the status of the whole knee joint (Oei et al. 2014, Roemer et al. 2011). However, morphological changes may not be present in the early stage of the OA, and several techniques have been proposed to measure the composition of articular cartilage quantitatively (Li & Majumdar 2013, Nieminen et al. 2012, Oei et al. 2014, Roemer et al. 2011).

Of the currently available in vivo imaging methods, the dGEMRIC method is regarded as one of the best for indirect GAG measurement (Oei et al. 2014). Although the specificity of dGEMRIC to GAG has recently been questioned (Salo et al. 2012, Silvast et al. 2009), it has been reported to detect degenerative changes sensitively in cartilage (Dahlberg et al. 2012). In the dGEMRIC method, a negatively charged contrast agent (gadopentetate dimeglumine) is typically injected intravenously (it can be injected intra-articularly as well), and imaging of the knee joint is usually conducted 90 minutes after injection (Dahlberg et al. 2012). Contrast agent distributes into the cartilage in an inverse relation to the fixed charge density of the cartilage (Bashir et al. 1996, Bashir et al. 1997). The mean $T_1$ relaxation time, also called the dGEMRIC index, is lower in the areas where the concentration of the contrast agent is higher, i.e. in the damaged cartilage area. The dGEMRIC method has been validated in several in vitro studies that have reported association of dGEMRIC to GAG content or degeneration of cartilage (Bashir et al. 1996, Dahlberg et al. 2012, Gray et al. 2008, Li & Majumdar 2013, Nieminen et al. 2012). The method has also been applied successfully in vivo (Dahlberg et al. 2012, Li & Majumdar 2013, Nieminen et al. 2012, Oei et al. 2014). In addition to the question related to the specificity of dGEMRIC to GAG content, contrast agent injection, the delay between the injection and imaging, the dosing bias in obese
subjects and susceptibility to motion artefacts due to lengthy scanning times are the main drawbacks of the dGEMRIC method (Dahlberg et al. 2012).

Another popular method for measuring the composition of cartilage is T2 relaxation time mapping. T2 relaxation time is related to the movement and energy exchange of free water protons inside cartilage (Li & Majumdar 2013). In the cartilage, the integrity and structure of the collagen network and water content affect T2 relaxation time values (Mosher & Dardzinski 2004). T2 relaxation time mapping has been validated in several in vitro and in vivo studies, demonstrating the association of T2 mapping to collagen (Li & Majumdar 2013, Nieminen et al. 2012, Oei et al. 2014).

Other methods for the evaluation of cartilage composition include T1ρ mapping, ultrashort echo time, GAG-specific chemical exchange saturation transfer, and sodium MRI (Li & Majumdar 2013, Nieminen et al. 2012, Oei et al. 2014). However, these methods are out of the scope of this thesis and are, therefore, not discussed here.
4 Aims of the thesis

Current clinical imaging methods are subjective or insensitive to early OA changes. Therefore, new methods are needed so as to quantify composition of the cartilage and characteristics of the subchondral bone. The aim of this thesis was to explore the potential of the novel X-ray-based methods so as to quantify OA related changes in articular cartilage and subchondral bone \textit{in vivo}. The specific aims were:

1. To compare methods evaluating bone density and structure from 2-D plain radiographs with 3-D bone microarchitecture obtained from µCT \textit{ex vivo}.
2. To investigate the potential of bone density- and structure-related methods from plain radiograph so as to discriminate subjects with different stages of OA \textit{in vivo}.
3. To compare the novel dQCTA method with dGEMRIC and their relation to arthroscopic grading of the knee joint \textit{in vivo}.
4. To investigate whether compositional changes in cartilage assessed with dGEMRIC are associated with structural changes in bone assessed from plain radiographs \textit{in vivo}.
5 Material and methods

This thesis consists of three different studies together with unpublished data. Table 3 summarises material/subjects used in this thesis.

Table 3. The summary of material/subjects used in this thesis.

<table>
<thead>
<tr>
<th>Study</th>
<th>Material/subjects</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (ex vivo)</td>
<td>Human cadaver tibias (n = 11)</td>
<td>Radiography, (\mu)CT</td>
</tr>
<tr>
<td>II (in vivo)</td>
<td>Subjects with different stages of knee OA (n = 50) and age-matched controls (n = 53)</td>
<td>Radiography, KL grading</td>
</tr>
<tr>
<td>III (in vivo)</td>
<td>Subjects with persistent knee pain going to an arthroscopy (n = 11)</td>
<td>dGEMRIC(^1), dQCTA(^2), ICRS grading</td>
</tr>
<tr>
<td>Unpublished</td>
<td>Subjects with mild knee OA (n = 80)</td>
<td>Radiography, dGEMRIC</td>
</tr>
</tbody>
</table>

\(^1\text{dGEMRIC = delayed gadolinium-enhanced MRI of cartilage, }^2\text{dQCTA = delayed quantitative CT arthrography.}

5.1 Study subjects

In this thesis, one original study was performed ex vivo and three original studies in vivo. All studies had different subjects or material. Detailed description of the samples used is provided in the following sections.

5.1.1 Human samples ex vivo (I)

In study I, eleven tibial bones from human cadavers (29–77 years of age) with no diagnosed history of joint diseases were studied (Kurkiäervi et al. 2004). The cadaver knees were obtained from Jyväskylä Central Hospital, Jyväskylä, Finland, as approved by the national authority (National Authority for Medicolegal Affairs, Helsinki, Finland, Permission 1781/32/200/01).

5.1.2 Human subjects in vivo (II, III, unpublished data)

In study II, male subjects \(n = 53\), mean age (standard deviation (SD)): 59.4 (5.2) years) with unilateral or bilateral knee OA and healthy age-matched male controls \(n = 50\), age: 59.5 (4.4) years) were investigated. Exclusion criteria included previous hip or knee fracture, surgery of the lower extremities (arthroscopy was allowed), clinical or radiological hip OA, a knee or hip joint infection, congenital
or developmental disease of the lower limbs, paralysis of lower extremities, and rheumatoid arthritis or spondyloarthritis. The detailed exclusion criteria have been published earlier (Liikavainio et al. 2008, Liikavainio et al. 2010). The Ethics Committee of the Kuopio University Hospital approved the study design.

In study III, eleven consecutive patients (8 females and 3 males, 40–68 years of age) referred to an arthroscopic surgery of the knee due to persistent knee pain symptoms were included. Informed consent was obtained from all participants. The study was approved by the Ethical Committee of the Northern Ostrobothnia Hospital District, Oulu, Finland (No. 33/2010).

The unpublished data consisted of 80 postmenopausal (50–65 years of age) women with mild OA (KL1 or KL2) (Multanen et al. 2014). Exclusion criteria included a T-score for femoral neck bone mineral density lower than -2.5 g/cm², a body mass index higher than 35 kg/m², and previous knee instability or severe trauma, inflammatory joint disease, knee intra-articular steroid injections in the preceding 12 months, contraindications to MRI, and any known allergies to contrast agents or renal insufficiency. The detailed inclusion and exclusion criteria have been published earlier (Multanen et al. 2014). The Ethics Committee of the Central Finland Health Care District approved the study design. Informed consent was obtained from all participants.

5.2 Radiography (I, II, unpublished data)

In study I, the bones were imaged using digital radiography (Ysio, Siemens, Germany) using constant imaging parameters (63 kV, 6 mAs, pixel size: 139 x 139 μm², source-detector distance: 151 cm). Subsequently, the effect of soft tissue on the imaging was simulated by immersing the bones into a water bath (radius of the round plastic container: 6 cm) and the imaging was repeated using the aforementioned imaging settings. The water bath increased scattering and reduced the quality of the image. Furthermore, to simulate plain radiography with a very high resolution, a 2-D coronal projection image from the 3-D μCT data was constructed (see 5.5.3 micro-computed tomography).

In study II, anterior-posterior weight-bearing radiographs from both knees were obtained using computed radiography (60 kV, 25 mAs, focus-skin distance: 110 cm) and digitised with a pixel resolution of 200 x 200 μm².

In the unpublished data, posterior-anterior weight-bearing radiographs from both knees were obtained (50 kV, 12.5 mAs, pixel size: 170 x 170 μm², source-detector distance: 120 cm).
5.2.1 Selection of regions of interest

In studies I and II and in unpublished data, four rectangular shaped regions of interest (ROIs) were extracted from the tibia (Figure 4). Two ROIs (size: 6 mm x 14 mm) were placed into the subchondral bone, immediately below the cartilage-bone interface, in the centre of the medial and lateral condyles of the tibia and two ROIs (14 mm x 14 mm) immediately below the dense subchondral bone in the trabecular bone. The trabecular bone ROIs were aligned horizontally with subchondral bone ROIs. Anatomical landmarks for the ROIs were tibial spine, subchondral bone plate, and outer borders of the proximal tibia. The sizes and positions of the ROIs were partly based on previous literature (Lynch et al. 1991b, Podsiadlo et al. 2008b, Woloszynski et al. 2010). A custom-made MATLAB software (The MathWorks, Inc., Natick, MA, USA) was used for the manual placement of the ROIs.

![Fig. 4. Location of regions of interest (ROIs). Two ROIs (6 mm x 14 mm) were placed in subchondral bone, immediately below the cartilage-bone interface, in the centre of the medial and lateral condyles of tibia and two ROIs (14 mm x 14 mm) immediately below the dense subchondral bone in the trabecular bone. The purpose of the white dashed lines is to help place the ROIs in the correct locations.](image)

5.2.2 Evaluation of bone density from radiographs

In studies I and II, bone density was estimated by measuring the mean greyscale value of the ROI (= GV). Furthermore, in study I, an aluminium step wedge was present in the plain radiography to convert GV of bone to the corresponding thickness of the step wedge (= GV_{mmAl}). The corresponding step wedge thickness was calculated using linear interpolation between greyscale values of consecutive
steps in the wedge. In study II, a calibration ball was included in the image. To obtain normalised density estimate (= GV_{cb}), GV was divided by the maximum greyscale value of the calibration ball. Bone density was not measured for the unpublished data, since the images did not contain a calibration object and the imaging conditions were variable, thus, making the estimation of density unreliable.

5.2.3 Evaluation of bone structure from radiographs

In the *ex vivo* study (study I), the radiographs and 2-D projection images of µCT were median filtered (3 x 3 pixels) in order to remove high frequency noise from the images. Bone texture was analysed using Laplacian-based methods (Thevenot *et al.* 2014b), LBP-based methods (Thevenot *et al.* 2014a), and using FSA (Lynch *et al.* 1991a, Lynch *et al.* 1991b).

The Laplacian-based method enhances the appearance of bone trabeculae and quantifies the variation in the greyscale values of the Laplacian-based image (Figure 5). Construction of the Laplacian-based image was based on an earlier study (Thevenot *et al.* 2014b). However, since the ROIs were not oriented completely along the trabeculae, in addition to vertical direction, the Laplacians were also calculated in the horizontal direction, and these results were then summed into one matrix. Subsequently, the unprocessed ROI was multiplied with the square root of the Laplacian matrix to enhance the bone and greyscale values were expanded to full dynamic range to obtain the final Laplacian-based image. To measure the randomness of the greyscale values in the Laplacian-based image, the entropy of the image (E_{Lap}) was calculated using Equation 2:

\[ E = -\sum_i P_i \log_2 P_i, \]

where \( P_i \) contains the normalised count of the greyscale value \( i \) occurring in the image. If \( E_{Lap} = 0 \), all pixel values in the Laplacian-based image are the same, whereas higher values indicate a higher variation in the pixel values of the image.
To measure the randomness of local patterns and variation in the orientation of adjacent local patterns, LBP-based methods were modified from the methods initially developed for \( \mu \)CT data (Thevenot et al. 2014a). The LBP value of a studied pixel is assessed from the greyscale levels of its surrounding, while ignoring the differences in magnitudes (Figure 3). In the current method, the image was initially divided into bone and non-bone regions by determining a local threshold for every pixel in the image using the Otsu method (Otsu 1979) with a window size that was adjusted to the pixel size of the image. The window size was 9 x 9 pixels in study I and in unpublished data, 7 x 7 in study II, and 36 x 36 for the 2-D projection image from \( \mu \)CT. Next, the LBP operator (8-neighborhood on a circle with a radius of 1) was applied in the bone regions and in the non-bone regions next to the bone, i.e. in the bone edge. The pixel was considered to be an edge pixel if at least one of the 8 neighbours of the centre pixel was a bone pixel. The greyscale values of the neighbour pixels were interpolated from their surrounding values. In order to reduce the number of irrelevant patterns, grouping of patterns was carried out by determining the main orientation and the number of valid neighbours (i.e. markers) for each pattern. The main orientation angle was calculated using principal component analysis. The angle (0°, 45°, 90°, and 135°) was calculated only for the patterns which consisted of 2–5 consecutive markers (Figure 6), otherwise the pattern was assigned as non-uniform.

To measure the randomness of the patterns occurring in the image, the entropy of the grouped patterns (\( E_{LBP} \)) was determined using Equation 2. If \( E_{LBP} = 0 \), there
is only single pattern occurring in the image. The homogeneity index for the
orientation of the patterns (HI\textsubscript{Angles}) was derived from the co-occurrence matrix of
the angles. The co-occurrence matrices were constructed similarly to GLCMs
(Haralick \textit{et al.} 1973), \textit{i.e.} by looking the adjacent angle values, and were calculated
in 0°, 45°, 90°, and 135° directions with one pixel distance. The non-uniform and
non-bone areas were excluded from the co-occurrence matrices. Although it is
possible to calculate HI in different directions, only the mean HI (HI\textsubscript{Angles}) of the
four possible directions was used in the analyses in this thesis. If all adjacent
patterns have similar orientation, HI\textsubscript{Angles} is equal to one, while a large variation in
the orientation of local patterns results in a low HI\textsubscript{Angles} value.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure6.png}
\caption{Examples of the main orientation angles of the patterns which consist of three
consecutive markers.}
\end{figure}

In order to estimate fractal dimension (FD), that is related to complexity and
roughness of an image, the FSA method was used (Lynch \textit{et al.} 1991a, Lynch \textit{et al.}
1991b). In brief, the original image was dilated and eroded in the horizontal and
vertical directions with a rod-shaped one-pixel-wide structuring element. The
volume, \(V\), between dilated and eroded images was then calculated. Calculations
were repeated by varying the element length \(r\) from 2 to 4 pixels. Surface area, \(A(r)\),
was obtained from the Equation 3:

\[ A(r) = \frac{(V(r) - V(r - 1))}{2}, \quad (3) \]

After that, a log-log plot was constructed by plotting log of \(A(r)\) against log of \(r\).
Finally, the FD was estimated using a regression line to points between 2 and 4
(between 2 and 32 for the 2-D projection image from µCT). When the structuring
element is pointing in the horizontal direction, the FD of vertical structures (FD\textsubscript{v})
is produced and \textit{vice versa} (Lynch \textit{et al.} 1991a). High FD values are associated
with high complexity of the image, whereas low complexity results in low FD values.

### 5.3 Computed tomography (III)

In study III, a 20 ml dose of ioxaglate–gadopentetate contrast agent mixture (105 mM Hexabrix™ 320, Guerbet, Roissy, France and 2.5 mM Magnevist™, Bayer HealthCare Pharmaceuticals, Berlin, Germany) was injected intra-articularly. After injection, the subject performed active flexion-extension of the knee for 5 minutes so as to enable a smooth distribution of the contrast agent into joint surfaces. Subsequently, the knee joint of the subject was scanned using a clinical 64-slice CT scanner (GE Medical Systems, Discovery™ PET/CT 690, Waukesha, WI, USA) with a tube voltage of 100 kV and a tube current of 160 mA. After the first imaging session, the subject was rescanned at 45 minutes after the contrast agent injection using the same imaging settings. All CT data were resampled to the same isotropic voxel size (312 x 312 x 312 µm³) using linear interpolation (Analyze 10.0 AnalyzeDirect, Inc. Overland Park, KS, USA).

The segmentation of the cartilage was conducted using a region growing method. Dimensions of the 3-D ROI were variable, but they did not exceed the limits that were set at one third of the studied joint surface width. The ROI was located in the centre of the joint surface studied.

Seven cartilage ROIs corresponding to those in arthroscopic evaluation were extracted, including medial and lateral condyles of tibia, medial and lateral condyles of femur, medial and lateral trochlear grooves, and patella. In addition, an ROI from the area of maximal attenuation in synovial fluid was extracted. From the mean X-ray attenuation values of each ROI, cartilage (C) and synovial fluid (SF) parameters at 5 and 45 minutes after the contrast agent injection (C₅, C₄₅, SF₅, and SF₄₅) were calculated. In order to eliminate any variation in equilibrating contrast agent concentration resulting from the dilution by synovial fluid, cartilage parameters at each time point were normalised by that of synovial fluid, i.e. C₅/SF₅ and C₄₅/SF₄₅.

Since the seed point for the region growing could not be defined for all cases due to an absence of cartilage, the eventual sample size in dQCTA analyses was 67.
5.4 Magnetic resonance imaging (III, unpublished data)

In study III, each subject was scanned three times on a 3 Tesla scanner (Siemens Skyra, Siemens Healthcare, Erlangen, Germany) with a dedicated transmit/receive knee coil. Prior to administration of the contrast agent, single-slice T1 mapping was performed at the centre of medial and lateral condyles using an inversion recovery fast spin echo (IR-FSE) sequence (repetition time (TR) = 4060 ms; time to echo (TE) = 8.6 ms; inversion time (TI) = 50, 100, 200, 400, 800, 1600, 3200, and 3900 ms; field of view (FOV) = 120*120 mm²; matrix = 256*256; slice thickness = 3 mm). Subsequently, 0.2 mM/kg (double dose) of gadopentetate (Gd-DTPA₂⁻, Magnevist™) was injected intravenously, followed by active flexion-extension exercises of the knee for 5 minutes and walking for 5 minutes. T1 measurements were repeated at 90 minutes after intravenous administration of Gd-DTPA₂⁻ using the same imaging parameters. Two weeks after the previous imaging session, subjects were given an intra-articular injection of the combination of Gd-DTPA₂⁻ and ioxaglate (see 5.3 Computed Tomography). The post-contrast T1 measurement was repeated the second time at 90 minutes after intra-articular injection using the aforementioned imaging protocol.

In unpublished data, subjects were scanned once on a 1.5 Tesla scanner (Siemens Magnetom Symphony Quantum, Siemens Healthcare, Erlangen, Germany) with a standard transmit/receive knee array coil at 90 minutes after intravenous administration of Gd-DTPA₂⁻ (Magnevist™). Immediately after injection of the contrast agent, subjects performed active flexion-extension exercises of the knee while sitting for 5 min, walking for 5 min, and stair climbing for 5 min. Single-slice T1 mapping was performed at the centre of medial and lateral condyles using an IR-FSE sequence (TR = 1800 ms; TE = 13 ms; TI = 50, 100, 200, 400, 800, and 1600 ms; FOV = 120*120 mm²; matrix = 256*256; slice thickness = 3 mm).

Articular cartilage was segmented manually for the quantitative T1 analysis from T1 relaxation time maps using an in-house MATLAB application. The T1 maps were generated with a pixel-by-pixel three-parameter fit routine. In addition to cartilage, a ROI from synovial fluid was segmented in study III. The segmented cartilage was divided into similar ROIs that were used in dQCTA analyses apart from patella (Figure 7) and the mean T1 relaxation time of each cartilage ROI, i.e. the dGEMRIC index, was calculated separately for dGEMRIC with intravenous and intra-articular injection (T1Gd,IV and T1Gd,IA, respectively). In unpublished data,
\( T_{1Gd,IV} \) was calculated from central and posterior tibia ROIs (divided to bulk, superficial, and deep zones).

Additionally, in study III, the change in relaxation rate was calculated for cartilage and synovial fluid (\( \Delta R_{1,IV}, \Delta R_{1,IA}, \) and \( \Delta R_{1,SF} \)) as follows (Equation 4):

\[
\Delta R_1 = \left( \frac{1}{T_{1Gd}} - \frac{1}{T_{1,0}} \right),
\]

where \( T_{1Gd} \) and \( T_{1,0} \) are relaxation time values with and without Gd-DTPA\(^2\), respectively. Furthermore, \( \Delta R_{1,IA} \) was normalised by \( \Delta R_{1,SF} \) (\( = \Delta R_{1,IA}/\Delta R_{1,SF} \)) analogously to the normalised dQCTA parameters. Since the cartilage surface was not entirely visible in all dGEMRIC slices in study III, the sample sizes for \( T_{1Gd,IV} \) and \( T_{1Gd,IA} \) were 53 and 59, respectively. Sample sizes for \( \Delta R_{1,IV}, \Delta R_{1,IA}, \) and \( \Delta R_{1,IA}/\Delta R_{1,SF} \) were 50, 54, and 54, respectively.

Fig. 7. In study III, regions of interest (ROIs) were extracted from medial and lateral condyles of tibia and femur, and from medial and lateral trochlear grooves. In unpublished data, ROIs from medial and lateral condyles of central (white area in tibia) and posterior tibia were included.
5.5 Reference methods

5.5.1 Semi-quantitative grading of radiographs (II, unpublished data)

In study II and in unpublished data, the knees were classified according to the KL grading scale (Figure 2) (Kellgren & Lawrence 1957). KL grades were not known during the quantitative image analyses. In study II, the lateral side was more affected than the medial side in three knees and these knees were excluded from the analyses (one knee had KL grade 3 and two knees KL grade 4) in order to homogenise the study sample.

5.5.2 Arthroscopic grading (III)

In study III, arthroscopy was conducted for the subjects \( n = 9 \) within 2–12 (mean = 5.4, SD = 2.8) weeks. During the arthroscopy, the predetermined sites at the knee joint (medial and lateral condyles of tibia, medial and lateral condyles of the femur, medial and lateral trochlear grooves, and patella) were classified according to the ICRS grading system (Brittberg & Winalski 2003) by an experienced orthopaedic surgeon (Table 1). ICRS grading was missing from four sites and thus the total number of ICRS graded sites was 59.

5.5.3 Micro-computed tomography (I)

In study I, the bones were cut into halves and both the medial and lateral condyles were imaged with a µCT scanner (SkyScan 1176, Bruker, Belgium, 80 kV, 300 µA, isotropic voxel size of 17.4 µm, 0.04 mm copper + 0.5 mm aluminium filter) separately. Volumes of interest (VOIs) were selected from the same location as the plain radiograph ROIs, and evaluated using SkyScan CTAn software (Bruker, Belgium). Before calculating the conventional 3-D parameters, 3-D median filtering (radius 2) and global thresholding were applied so as to extract bone tissue from background. The calculated 3-D parameters included bone volume fraction (BV/TV, the ratio of 3-D total bone volume to total volume of VOI, in %), average trabecular thickness (Tb.Th, in µm), trabecular separation (Tb.Sp, mean thickness of the non-bone areas, in µm), trabecular number (Tb.N, in 1/mm), and connectivity density (Conn.Dn, the degree to which a structure is multiply connected, 1/mm³) (Bouxsein et al. 2010).
Furthermore, in order to simulate plain radiography, all binarised μCT slices were summed together so as to construct a 2-D coronal projection image from the 3-D μCT data (Figure 8). This high-resolution 2-D projection image was analysed using the same algorithms as were used for the plain radiographs to evaluate bone density and structure.

5.6 Statistical analyses

In study I and for the analysis of the unpublished data, Pearson’s correlation analysis (together with 95% confidence intervals (Altman & Gardner 1988)) was applied to study the relationship between different parameters.

In study II, the differences between different KL groups were evaluated using the linear mixed model to take into account the correlation between the knees of the same subject. In the model, KL group and knee (left or right) were set as fixed variables and the subject was set as a random variable. Restricted maximum likelihood estimation was used in the model. Furthermore, estimated means for the different KL groups were obtained from the fitted model, and Fisher’s least significant difference test was performed in order to ascertain which KL groups differed statistically significantly from each other. In addition, Bonferroni’s post-hoc test was also performed so as to adjust for multiple comparisons between KL groups. However, p-values obtained from the linear mixed models for different parameters were not adjusted for multiple comparisons (Rothman 1990).

In study III, normal distribution of the parameters was tested using the Kolmogorov-Smirnov test, and based on the normality of the parameters either
Pearson ($r$) or Spearman ($\rho_s$) correlation analysis (together with 95% confidence intervals) was applied. The Kruskal-Wallis test was used for group comparisons.

All statistical analyses were conducted with SPSS software versions 19–22 (SPSS Inc., Chicago, USA). The level of statistical significance was set to $p < 0.05$. 
6 Results

6.1 Bone density from plain radiographs (I, II)

In Table 4, mean and SD values for the 3-D µCT parameters evaluated *ex vivo* from human cadaver tibiae are shown (study I).

Table 4. Mean (standard deviation) of 3-D parameters from µCT (*n* = 44).

<table>
<thead>
<tr>
<th>3-D parameters</th>
<th>Mean ± SD (min – max)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BV/TV¹ (%)</td>
<td>21.8 ± 5.9 (11.8 – 33.6)</td>
</tr>
<tr>
<td>Conn.Dn² (1/mm³)</td>
<td>6.32 ± 2.04 (3.14 – 11.51)</td>
</tr>
<tr>
<td>Tb.Th³ (µm)</td>
<td>211 ± 30 (162 – 283)</td>
</tr>
<tr>
<td>Tb.Sp⁴ (µm)</td>
<td>723 ± 113 (526 – 997)</td>
</tr>
<tr>
<td>Tb.N⁵ (1/mm)</td>
<td>1.02 ± 0.18 (0.73 – 1.41)</td>
</tr>
</tbody>
</table>

¹BV/TV = bone volume fraction, ²Conn.Dn = connectivity density, ³Tb.Th = trabecular thickness, ⁴Tb.Sp = trabecular separation, ⁵Tb.N = trabecular number.

In study I, bone density-related parameters (GV_{mmAl} and GV) evaluated from a plain radiograph (Figure 9) and from 2-D projection image of µCT correlated significantly with BV/TV from µCT *ex vivo* (Table 5).

Table 5. Pearson correlation coefficients (95% confidence interval) between bone densities evaluated from both plain radiographs and 2-D µCT projection image and bone volume fraction (BV/TV).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>BV/TV</th>
<th>BV/TV</th>
<th>BV/TV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All (<em>n</em> = 44)</td>
<td>Subchondral bone (<em>n</em> = 22)</td>
<td>Trabecular bone (<em>n</em> = 22)</td>
</tr>
<tr>
<td>GV_{mmAl}²</td>
<td>0.86 (0.75 – 0.92)¹</td>
<td>0.81 (0.58 – 0.92)²</td>
<td>0.61 (0.25 – 0.82)³</td>
</tr>
<tr>
<td>GV³</td>
<td>0.93 (0.87 – 0.96)¹</td>
<td>0.90 (0.76 – 0.96)²</td>
<td>0.86 (0.69 – 0.94)³</td>
</tr>
</tbody>
</table>

¹*p* < 0.01, ²GV_{mmAl} = GV converted to aluminum equivalents, ³GV = mean greyscale value.

In study II, bone density-related parameters (GV and GV_{CB}) evaluated from plain radiograph *in vivo* were significantly higher in KL2–4 groups than in control group in medial tibial subchondral bone and trabecular bone (Table 6).
Table 6. Bone density-related parameters (mean (standard deviation)) in different KL grade groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>KL 0 (n = 104-110)</th>
<th>KL 1 (n = 28)</th>
<th>KL 2 (n = 27)</th>
<th>KL 3 (n = 31)</th>
<th>KL 4 (n = 7)</th>
<th>p-value</th>
<th>Post-hoc&lt;sup&gt;1&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Medial subchondral bone</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GV&lt;sup&gt;2&lt;/sup&gt;</td>
<td>142 (21)</td>
<td>149 (16)</td>
<td>159 (19)</td>
<td>166 (20)</td>
<td>173 (23)</td>
<td>&lt;0.001</td>
<td>0-2, 0-3, 0-4,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1-2, 1-3, 1-4</td>
</tr>
<tr>
<td>GV&lt;sub&gt;cb&lt;/sub&gt;&lt;sup&gt;3&lt;/sup&gt;</td>
<td>0.64 (0.11)</td>
<td>0.65 (0.08)</td>
<td>0.69 (0.11)</td>
<td>0.69 (0.10)</td>
<td>0.78 (0.11)</td>
<td>&lt;0.001</td>
<td>0-2, 0-3, 0-4,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1-3, 1-4, 2-4</td>
</tr>
<tr>
<td><strong>Lateral subchondral bone</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GV</td>
<td>145 (16)</td>
<td>147 (18)</td>
<td>147 (18)</td>
<td>137 (14)</td>
<td>129 (18)</td>
<td>0.006</td>
<td>0-3, 0-4, 1-3,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1-4, 2-3, 2-4</td>
</tr>
<tr>
<td>GV&lt;sub&gt;cb&lt;/sub&gt;</td>
<td>0.66 (0.12)</td>
<td>0.65 (0.15)</td>
<td>0.70 (0.12)</td>
<td>0.71 (0.15)</td>
<td>0.64 (0.10)</td>
<td>0.197</td>
<td></td>
</tr>
<tr>
<td><strong>Medial trabecular bone</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GV</td>
<td>105 (19)</td>
<td>113 (15)</td>
<td>121 (18)</td>
<td>124 (18)</td>
<td>135 (19)</td>
<td>&lt;0.001</td>
<td>0-2, 0-3, 0-4,</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1-3, 1-4</td>
</tr>
<tr>
<td>GV&lt;sub&gt;cb&lt;/sub&gt;</td>
<td>0.52 (0.13)</td>
<td>0.53 (0.11)</td>
<td>0.56 (0.11)</td>
<td>0.57 (0.08)</td>
<td>0.62 (0.08)</td>
<td>0.039</td>
<td>0-3, 0-4</td>
</tr>
<tr>
<td><strong>Lateral trabecular bone</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GV</td>
<td>131 (16)</td>
<td>135 (16)</td>
<td>136 (14)</td>
<td>128 (15)</td>
<td>124 (19)</td>
<td>0.148</td>
<td></td>
</tr>
<tr>
<td>GV&lt;sub&gt;cb&lt;/sub&gt;</td>
<td>0.59 (0.13)</td>
<td>0.61 (0.15)</td>
<td>0.64 (0.13)</td>
<td>0.67 (0.15)</td>
<td>0.63 (0.11)</td>
<td>0.033</td>
<td>0-2, 0-3</td>
</tr>
</tbody>
</table>

<sup>1</sup>Differences between groups using Fisher’s least significance difference post hoc test. Differences between groups using the Bonferroni post hoc test are bolded. <sup>2</sup>GV = mean greyscale value, <sup>3</sup>GV<sub>cb</sub> = GV normalised with calibration ball.

6.2 Bone structure from plain radiographs (I, II)

In study I, significant correlations between bone texture parameters from plain radiograph and 3-D bone architectural parameters from µCT were obtained, although the degrees of correlations varied depending on the parameter and the direction in which the texture measures were calculated (Table 7). The correlations remained significant when subchondral bone ROIs and trabecular bone ROIs were analysed separately (Appendix Tables 11 and 12). The strongest correlations were observed between HIAngles and Tb.Sp and between FDVer and Tb.Sp (Figure 9).

Significant correlations between bone texture parameters assessed from both the original 3-D µCT data and its 2-D projection were also obtained (Table 7 and Appendix Tables 11 and 12).
Table 7. Pearson correlation coefficients (95% confidence interval) between bone texture parameters evaluated from both plain radiographs and 2-D µCT projection image and 3-D µCT parameters. All ROIs pooled together (n = 44).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>BV/TV^1</th>
<th>Conn.Dn^4</th>
<th>Tb.Th^5</th>
<th>Tb.Sp^6</th>
<th>Tb.N^7</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plain radiograph</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ELap</td>
<td>0.61</td>
<td>0.34</td>
<td>0.59</td>
<td>-0.41</td>
<td>0.48</td>
</tr>
<tr>
<td></td>
<td>(0.38 – 0.77)</td>
<td>(0.04 – 0.58)</td>
<td>(0.11 – 0.62)</td>
<td>(-0.74 – -0.32)</td>
<td>(0.34 – 0.75)</td>
</tr>
<tr>
<td>ELBP</td>
<td>0.57</td>
<td>0.61</td>
<td>0.39</td>
<td>-0.57</td>
<td>0.58</td>
</tr>
<tr>
<td></td>
<td>(0.33 – 0.74)</td>
<td>(0.38 – 0.77)</td>
<td>(0.11 – 0.62)</td>
<td>(-0.74 – -0.32)</td>
<td>(0.34 – 0.75)</td>
</tr>
<tr>
<td>HIAngles</td>
<td>-0.66</td>
<td>-0.73</td>
<td>-0.37</td>
<td>0.73</td>
<td>-0.71</td>
</tr>
<tr>
<td></td>
<td>(-0.80 – -0.45)</td>
<td>(-0.84 – -0.55)</td>
<td>(-0.60 – -0.08)</td>
<td>(0.56 – 0.85)</td>
<td>(-0.83 – -0.53)</td>
</tr>
<tr>
<td>FDHor</td>
<td>-0.04</td>
<td>0.27</td>
<td>-0.28</td>
<td>-0.13</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td>(-0.33 – 0.26)</td>
<td>-0.02</td>
<td>0.53</td>
<td>(-0.53 – -0.02)</td>
<td>(-0.42 – 0.17)</td>
</tr>
<tr>
<td>FDVer</td>
<td>0.41</td>
<td>0.69</td>
<td>0.03</td>
<td>-0.70</td>
<td>0.60</td>
</tr>
<tr>
<td></td>
<td>(0.12 – 0.63)</td>
<td>(0.49 – 0.82)</td>
<td>(-0.27 – 0.32)</td>
<td>(-0.83 – -0.52)</td>
<td>(0.36 – 0.76)</td>
</tr>
<tr>
<td><strong>2-D µCT projection image</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ELap</td>
<td>0.71</td>
<td>0.48</td>
<td>0.64</td>
<td>-0.50</td>
<td>0.61</td>
</tr>
<tr>
<td></td>
<td>(0.53 – 0.83)</td>
<td>(0.21 – 0.68)</td>
<td>(0.42 – 0.79)</td>
<td>(-0.70 – -0.24)</td>
<td>(0.38 – 0.77)</td>
</tr>
<tr>
<td>ELBP</td>
<td>0.70</td>
<td>0.72</td>
<td>0.46</td>
<td>-0.71</td>
<td>0.73</td>
</tr>
<tr>
<td></td>
<td>(0.51 – 0.83)</td>
<td>(0.53 – 0.84)</td>
<td>(0.19 – 0.66)</td>
<td>(-0.83 – -0.52)</td>
<td>(0.56 – 0.85)</td>
</tr>
<tr>
<td>HIAngles</td>
<td>-0.70</td>
<td>-0.79</td>
<td>-0.38</td>
<td>0.78</td>
<td>-0.79</td>
</tr>
<tr>
<td></td>
<td>(-0.83 – -0.51)</td>
<td>(-0.88 – -0.64)</td>
<td>(-0.61 – -0.10)</td>
<td>(0.63 – 0.87)</td>
<td>(-0.88 – -0.64)</td>
</tr>
<tr>
<td>FDHor</td>
<td>-0.39</td>
<td>0.16</td>
<td>-0.66</td>
<td>0.01</td>
<td>-0.10</td>
</tr>
<tr>
<td></td>
<td>(-0.62 – -0.11)</td>
<td>-0.14</td>
<td>0.44</td>
<td>(-0.45 – 0.80)</td>
<td>(-0.29 – 0.30)</td>
</tr>
<tr>
<td>FDVer</td>
<td>-0.07</td>
<td>0.59</td>
<td>-0.55</td>
<td>-0.46</td>
<td>0.31</td>
</tr>
<tr>
<td></td>
<td>(-0.36 – 0.23)</td>
<td>(0.36 – 0.75)</td>
<td>(-0.73 – 0.30)</td>
<td>(-0.67 – -0.19)</td>
<td>(0.01 – 0.56)</td>
</tr>
</tbody>
</table>

^1p < 0.05, ^2p < 0.01, ^3BV/TV = bone volume fraction, ^4Conn.Dn = connectivity density, ^5Tb.Th = trabecular thickness, ^6Tb.Sp = trabecular separation, ^7Tb.N = trabecular number, ^8ELap = entropy of Laplacian-based image, ^9ELBP = entropy of grouped local binary patterns, ^10HIAngles = mean homogeneity index for orientation of local patterns, ^11FD = fractal dimension of horizontal structures, ^12FD = fractal dimension of vertical structures.
Fig. 9. Statistically significant correlations between a) bone density from plain radiograph ($GV_{mmAl}$) and bone volume fraction (BV/TV), b) mean homogeneity index for orientation of local patterns ($HI_{Angles}$) and trabecular separation (Tb.Sp), and c) fractal dimension of vertical structures (FD$_{Ver}$) and Tb.Sp.

In study II, significant differences in bone texture parameters from plain radiographs between OA subjects and controls (KL 0) \textit{in vivo} were mainly observed in the medial side ROIs. In medial subchondral bone, $E_{Lap}$ was lower and $FD_{Ver}$ higher among OA subjects (Table 8). In medial trabecular bone, $HI_{Angles}$ was lower and $E_{LBP}$, $FD_{Hor}$, and $FD_{Ver}$ were higher among OA subjects (Table 8).
Table 8. Bone texture parameters (mean (standard deviation)) in different KL grade groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>KL 0 (n = 104-110)</th>
<th>KL 1 (n = 28)</th>
<th>KL 2 (n = 27)</th>
<th>KL 3 (n = 31)</th>
<th>KL 4 (n = 7)</th>
<th>p-value</th>
<th>Post-hoc¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medial subchondral bone</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0-2, 0-3, 0-4, 1-2, 1-3, 1-4, 2-4, 3-4</td>
</tr>
<tr>
<td>ELap²</td>
<td>7.21 (0.11)</td>
<td>7.20 (0.13)</td>
<td>7.10 (0.13)</td>
<td>7.06 (0.09)</td>
<td>6.94 (0.17)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>ELBP³</td>
<td>3.85 (0.04)</td>
<td>3.84 (0.03)</td>
<td>3.86 (0.03)</td>
<td>3.86 (0.03)</td>
<td>3.87 (0.03)</td>
<td>0.182</td>
<td></td>
</tr>
<tr>
<td>HIAngles⁴</td>
<td>0.64 (0.02)</td>
<td>0.65 (0.02)</td>
<td>0.64 (0.01)</td>
<td>0.64 (0.02)</td>
<td>0.64 (0.01)</td>
<td>0.100</td>
<td></td>
</tr>
<tr>
<td>FDHor⁵</td>
<td>2.73 (0.09)</td>
<td>2.72 (0.12)</td>
<td>2.73 (0.11)</td>
<td>2.74 (0.11)</td>
<td>2.75 (0.08)</td>
<td>0.915</td>
<td></td>
</tr>
<tr>
<td>FDVer⁶</td>
<td>3.02 (0.09)</td>
<td>3.03 (0.09)</td>
<td>3.06 (0.11)</td>
<td>3.12 (0.08)</td>
<td>3.13 (0.09)</td>
<td>&lt;0.001</td>
<td>0-3, 0-4, 1-3, 1-4, 2-3</td>
</tr>
<tr>
<td>Lateral subchondral bone</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ELap</td>
<td>7.07 (0.10)</td>
<td>7.07 (0.11)</td>
<td>7.07 (0.13)</td>
<td>7.10 (0.11)</td>
<td>7.05 (0.17)</td>
<td>0.678</td>
<td></td>
</tr>
<tr>
<td>ELBP</td>
<td>3.85 (0.04)</td>
<td>3.84 (0.04)</td>
<td>3.83 (0.05)</td>
<td>3.83 (0.04)</td>
<td>3.82 (0.05)</td>
<td>0.154</td>
<td></td>
</tr>
<tr>
<td>HIAngles</td>
<td>0.64 (0.02)</td>
<td>0.64 (0.02)</td>
<td>0.66 (0.02)</td>
<td>0.65 (0.02)</td>
<td>0.65 (0.02)</td>
<td>0.007</td>
<td>0-2, 1-2</td>
</tr>
<tr>
<td>FDHor</td>
<td>2.86 (0.11)</td>
<td>2.88 (0.13)</td>
<td>2.79 (0.13)</td>
<td>2.80 (0.13)</td>
<td>2.72 (0.09)</td>
<td>0.001</td>
<td>0-2, 0-3, 0-4, 1-2, 1-3, 1-4</td>
</tr>
<tr>
<td>FDVer</td>
<td>3.08 (0.09)</td>
<td>3.09 (0.11)</td>
<td>3.06 (0.12)</td>
<td>3.09 (0.08)</td>
<td>3.07 (0.10)</td>
<td>0.842</td>
<td></td>
</tr>
<tr>
<td>Medial trabecular bone</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ELap</td>
<td>7.07 (0.11)</td>
<td>7.04 (0.11)</td>
<td>7.03 (0.08)</td>
<td>7.04 (0.10)</td>
<td>7.01 (0.12)</td>
<td>0.113</td>
<td></td>
</tr>
<tr>
<td>ELBP</td>
<td>3.72 (0.07)</td>
<td>3.75 (0.07)</td>
<td>3.77 (0.07)</td>
<td>3.77 (0.05)</td>
<td>3.80 (0.03)</td>
<td>&lt;0.001</td>
<td>0-1, 0-2, 0-3, 0-4</td>
</tr>
<tr>
<td>HIAngles</td>
<td>0.70 (0.02)</td>
<td>0.69 (0.02)</td>
<td>0.69 (0.01)</td>
<td>0.68 (0.02)</td>
<td>0.67 (0.01)</td>
<td>&lt;0.001</td>
<td>0-3, 0-4, 1-4, 2-4</td>
</tr>
<tr>
<td>FDHor</td>
<td>2.82 (0.08)</td>
<td>2.84 (0.08)</td>
<td>2.82 (0.10)</td>
<td>2.86 (0.07)</td>
<td>2.92 (0.08)</td>
<td>0.006</td>
<td>0-3, 0-4, 1-4, 2-4</td>
</tr>
<tr>
<td>FDVer</td>
<td>3.01 (0.08)</td>
<td>3.01 (0.08)</td>
<td>3.00 (0.08)</td>
<td>3.06 (0.06)</td>
<td>3.07 (0.08)</td>
<td>0.004</td>
<td>0-3, 0-4, 1-3, 2-3, 2-4</td>
</tr>
<tr>
<td>Lateral trabecular bone</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ELap</td>
<td>6.99 (0.10)</td>
<td>6.96 (0.09)</td>
<td>6.95 (0.10)</td>
<td>6.97 (0.08)</td>
<td>6.84 (0.11)</td>
<td>0.002</td>
<td>0-4, 1-4, 2-4, 3-4</td>
</tr>
<tr>
<td>ELBP</td>
<td>3.81 (0.04)</td>
<td>3.81 (0.04)</td>
<td>3.82 (0.04)</td>
<td>3.81 (0.04)</td>
<td>3.83 (0.02)</td>
<td>0.226</td>
<td></td>
</tr>
<tr>
<td>HIAngles</td>
<td>0.67 (0.01)</td>
<td>0.67 (0.01)</td>
<td>0.66 (0.01)</td>
<td>0.66 (0.01)</td>
<td>0.86 (0.01)</td>
<td>0.199</td>
<td></td>
</tr>
<tr>
<td>FDHor</td>
<td>2.94 (0.07)</td>
<td>2.97 (0.10)</td>
<td>2.95 (0.09)</td>
<td>2.96 (0.07)</td>
<td>2.98 (0.07)</td>
<td>0.278</td>
<td></td>
</tr>
<tr>
<td>FDVer</td>
<td>3.04 (0.09)</td>
<td>3.05 (0.11)</td>
<td>3.04 (0.09)</td>
<td>3.05 (0.07)</td>
<td>3.06 (0.07)</td>
<td>0.880</td>
<td></td>
</tr>
</tbody>
</table>

¹Differences between groups using Fisher’s least significance difference post hoc test. Differences between groups using the Bonferroni post hoc test are bolded. ²ELap = entropy of Laplacian-based image, ³ELBP = entropy of grouped local binary patterns, ⁴HIAngles = mean homogeneity index for orientation of local patterns, ⁵FDHor = fractal dimension of horizontal structures, ⁶FDVer = fractal dimension of vertical structures.
6.3 Association of arthroscopy to dGEMRIC and dQCTA (III)

In the arthroscopy, ICRS classification of the predetermined cartilage surfaces varied from normal (grade 0, \( n = 19 \)) and nearly normal (grade 1, \( n = 19 \)) to abnormal (grade 2, \( n = 19 \)) and severely abnormal (grade 3, \( n = 2 \)).

Although dQCTA showed a trend towards increasing values with severity of cartilage damage seen in the arthroscopy, neither dQCTA nor dGEMRIC parameters were statistically significantly different in different ICRS grade groups (Figure 10).

![Box plots comparing dQCTA and dGEMRIC parameters across ICRS grades](image)

Fig. 10. dQCTA (upper row) and dGEMRIC (lower row) parameters were not statistically significantly different in different ICRS grade groups. ICRS grades 2 and 3 were merged into one group due to low number of lesions with grade 3.

6.4 Correlation between dGEMRIC and dQCTA (III)

In Figure 11, representative MR and CT images, \( T_1 \) relaxation time map overlaid on top of an MR image, and illustrative dQCTA map of \( C_{45}/S_{45} \) overlaid on top of a CT image of a subject with ICRS grade 2 are presented. According to visual evaluation, CT at 5 min after the contrast agent injection had the best diagnostic quality for evaluation of cartilage lesions.
Normalised mean attenuation in cartilage at 45 min after contrast agent injection in dQCTA (C45/SF45) correlated statistically significantly with dGEMRIC with intravenous injection (ΔR1,IV) (Figure 12, Table 9). C45/SF45 correlated significantly with dGEMRIC with intra-articular injection only when ΔR1,IA was normalised by the relaxation rate in the synovial fluid (Table 9).

Fig. 11. Sagittal MR (a, c, and e) and CT (b, d, and f) images from the corresponding location in knee joint with ICRS grade 2 cartilage lesion (arrow). a) Anatomical double echo steady state image (TE/TR = 5/14.1 ms) and c) IR-FSE image (TI/TE/TR = 200/8.6/4060 ms) prior to contrast agent administration. e) T1 relaxation time map of cartilage overlaid on top of a T1-weighted MR image after intravenous contrast agent injection. b) CT at 5 min and d) 45 min after contrast agent injection. f) Illustrative dQCTA map of normalised X-ray attenuation in cartilage at 45 min after contrast agent injection (C45/SF45) overlaid on top of a CT image. Contrast of the images has been adjusted to enhance visibility of the lesion.
60

Fig. 12. Statistically significant correlation between normalised mean attenuation in cartilage at 45 min after administration of the contrast agent (C45/SF45) and $\Delta R_{1,IV}$. Linear fit is for illustrative purposes.

Table 9. Spearman’s rank correlation coefficients (95% confidence intervals) between dGEMRIC and dQCTA parameters ($n = 49 - 56$).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>C5/SF5</th>
<th>C45/SF45</th>
<th>$\Delta R_{1,IV}$</th>
<th>$\Delta R_{1,IA}$</th>
<th>$\Delta R_{1,IA}/\Delta R_{SF}$</th>
<th>T1Gd,IV</th>
<th>T1Gd,IA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$C_r/SF_r$</td>
<td>$C_r/SF_r$</td>
<td>$r$</td>
<td>$p$</td>
<td>$r$</td>
<td>$p$</td>
<td>$r$</td>
</tr>
<tr>
<td>$\Delta R_{1,IV}$</td>
<td>0.42 (0.16 – 0.63)</td>
<td>0.72 (0.56 – 0.83)</td>
<td>0.72</td>
<td>&lt; 0.01</td>
<td>0.72</td>
<td>&lt; 0.01</td>
<td></td>
</tr>
<tr>
<td>$\Delta R_{1,IA}$</td>
<td>0.27 (-0.01 – 0.50)</td>
<td>0.06 (-0.22 – 0.32)</td>
<td>0.06</td>
<td>&gt; 0.05</td>
<td>0.06</td>
<td>&gt; 0.05</td>
<td></td>
</tr>
<tr>
<td>$\Delta R_{1,IA}/\Delta R_{SF}$</td>
<td>0.13 (-0.15 – 0.39)</td>
<td>0.70 (0.53 – 0.82)</td>
<td>0.70</td>
<td>&lt; 0.01</td>
<td>0.70</td>
<td>&lt; 0.01</td>
<td></td>
</tr>
<tr>
<td>T1Gd,IV</td>
<td>-0.48 (-0.66 – -0.23)</td>
<td>-0.68 (-0.80 – -0.50)</td>
<td>-0.68</td>
<td>&lt; 0.01</td>
<td>-0.68</td>
<td>&lt; 0.01</td>
<td></td>
</tr>
<tr>
<td>T1Gd,IA</td>
<td>-0.26 (-0.49 – 0.01)</td>
<td>0.03 (-0.24 – 0.29)</td>
<td>0.03</td>
<td>&gt; 0.05</td>
<td>0.03</td>
<td>&gt; 0.05</td>
<td></td>
</tr>
</tbody>
</table>

$^1p < 0.01$, $^2C_r/SF_r = normalised$ mean attenuation in cartilage at 5 min after administration of the contrast agent, $^3C_r/SF_r = normalised$ mean attenuation in cartilage at 45 min after administration of the contrast agent, $^4\Delta R_{1,IV} = the$ change in relaxation rate in cartilage (intravenous injection), $^5\Delta R_{1,IA} = the$ change in relaxation rate in cartilage (intra-articular injection), $^6\Delta R_{1,IA}/\Delta R_{SF} = \Delta R_{1,IA} normalised$ by the change in relaxation rate in synovial fluid, $^7T_{1,IV} = T_1$ relaxation time (the dGEMRIC index) after intravenously administered contrast agent, $^8T_{1,IA} = T_1$ relaxation time after intra-articularly administered contrast agent.
6.5 Correlation between dGEMRIC and bone changes on plain radiographs (unpublished data)

In the medial compartment, mild associations between \( E_{\text{Lap}} \) in subchondral bone and dGEMRIC index of the central part of the tibial cartilage (Figure 13) as well as between HI_Angles in subchondral bone and dGEMRIC index of deep posterior tibial cartilage were found (Table 10). In the lateral compartment, \( FD_{\text{Hor}} \) in subchondral bone was significantly related to dGEMRIC indices of all tibial cartilage ROIs whereas \( FD_{\text{Hor}} \) was related to dGEMRIC index of the posterior part of the tibial cartilage (Table 10). In Figure 13, statistically significant correlations between \( E_{\text{Lap}} \) in subchondral bone and dGEMRIC index of the central tibial cartilage in the medial compartment and \( FD_{\text{Hor}} \) in subchondral bone and dGEMRIC index of central tibial cartilage in the lateral compartment are shown.

Statistically significant correlations were not found between bone texture parameters from trabecular bone and dGEMRIC index in the medial compartment. Mild associations \( (p < 0.05, n = 80) \) were found between \( E_{\text{Lap}} \) in trabecular bone and of bulk central tibia \( (r = 0.22) \) as well as dGEMRIC index of deep central \( (r = 0.26) \) and deep posterior tibial ROIs \( (r = 0.25) \) in lateral compartment.

Fig. 13. Statistically significant correlations between a) \( E_{\text{Lap}} \) in subchondral bone and dGEMRIC index of bulk central tibial cartilage in the medial compartment and b) \( FD_{\text{Hor}} \) in subchondral bone and dGEMRIC index of bulk central tibial cartilage in the lateral compartment.
Table 10. Pearson correlation coefficients (95% confidence interval) between texture parameters in subchondral bone evaluated from plain radiographs and dGEMRIC indices in tibial cartilage in medial and lateral compartments ($n=80$).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>bcT$^3$</th>
<th>bpT$^4$</th>
<th>scT$^5$</th>
<th>spT$^6$</th>
<th>dcT$^7$</th>
<th>dpT$^8$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Medial compartment</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E$_{lap}^9$</td>
<td>0.29$^2$</td>
<td>0.21</td>
<td>0.24$^1$</td>
<td>0.19</td>
<td>0.23$^1$</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>(0.08 – 0.48)</td>
<td>(-0.01 – 0.41)</td>
<td>(0.02 – 0.44)</td>
<td>(-0.03 – 0.39)</td>
<td>(0.01 – 0.43)</td>
<td>(-0.07 – 0.36)</td>
</tr>
<tr>
<td>E$_{LBP}^{10}$</td>
<td>-0.02</td>
<td>0.03</td>
<td>0.00</td>
<td>-0.01</td>
<td>-0.05</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>(-0.24 – 0.20)</td>
<td>(-0.20 – 0.24)</td>
<td>(-0.22 – 0.22)</td>
<td>(-0.23 – 0.21)</td>
<td>(-0.26 – 0.18)</td>
<td>(-0.20 – 0.23)</td>
</tr>
<tr>
<td>H$_{angles}^{11}$</td>
<td>-0.13</td>
<td>-0.21</td>
<td>-0.11</td>
<td>-0.13</td>
<td>-0.10</td>
<td>-0.23$^1$</td>
</tr>
<tr>
<td></td>
<td>(-0.36 – 0.07)</td>
<td>(-0.42 – 0.01)</td>
<td>(-0.32 – 0.12)</td>
<td>(-0.35 – 0.08)</td>
<td>(-0.34 – 0.09)</td>
<td>(-0.44 – 0.02)</td>
</tr>
<tr>
<td>F$_{Hor}^{12}$</td>
<td>0.00</td>
<td>-0.04</td>
<td>-0.06</td>
<td>-0.11</td>
<td>0.07</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>(-0.22 – 0.22)</td>
<td>(-0.26 – 0.18)</td>
<td>(-0.28 – 0.16)</td>
<td>(-0.32 – 0.11)</td>
<td>(-0.15 – 0.29)</td>
<td>(-0.16 – 0.28)</td>
</tr>
<tr>
<td>F$_{Ver}^{13}$</td>
<td>0.02</td>
<td>0.03</td>
<td>-0.15</td>
<td>-0.03</td>
<td>0.14</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>(-0.21 – 0.23)</td>
<td>(-0.19 – 0.25)</td>
<td>(-0.35 – 0.08)</td>
<td>(-0.25 – 0.19)</td>
<td>(-0.08 – 0.35)</td>
<td>(-0.14 – 0.29)</td>
</tr>
<tr>
<td><strong>Lateral compartment</strong></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E$_{lap}^9$</td>
<td>-0.18</td>
<td>-0.18</td>
<td>-0.18</td>
<td>-0.10</td>
<td>-0.16</td>
<td>-0.19</td>
</tr>
<tr>
<td></td>
<td>(-0.38 – 0.05)</td>
<td>(-0.38 – 0.04)</td>
<td>(-0.39 – 0.04)</td>
<td>(-0.31 – 0.13)</td>
<td>(-0.37 – 0.06)</td>
<td>(-0.40 – 0.03)</td>
</tr>
<tr>
<td>E$_{LBP}$</td>
<td>0.01</td>
<td>-0.08</td>
<td>0.06</td>
<td>0.02</td>
<td>-0.02</td>
<td>-0.11</td>
</tr>
<tr>
<td></td>
<td>(-0.21 – 0.23)</td>
<td>(-0.29 – 0.15)</td>
<td>(-0.16 – 0.28)</td>
<td>(-0.21 – 0.23)</td>
<td>(-0.24 – 0.20)</td>
<td>(-0.32 – 0.11)</td>
</tr>
<tr>
<td>H$_{angles}^{11}$</td>
<td>-0.01</td>
<td>-0.07</td>
<td>0.04</td>
<td>-0.03</td>
<td>-0.04</td>
<td>-0.07</td>
</tr>
<tr>
<td></td>
<td>(-0.23 – 0.21)</td>
<td>(-0.29 – 0.15)</td>
<td>(-0.18 – 0.26)</td>
<td>(-0.25 – 0.19)</td>
<td>(-0.26 – 0.18)</td>
<td>(-0.26 – 0.16)</td>
</tr>
<tr>
<td>F$_{Hor}^{12}$</td>
<td>0.45$^2$</td>
<td>0.43$^2$</td>
<td>0.33$^2$</td>
<td>0.30$^2$</td>
<td>0.49$^2$</td>
<td>0.43$^2$</td>
</tr>
<tr>
<td></td>
<td>(0.26 – 0.61)</td>
<td>(0.23 – 0.59)</td>
<td>(0.12 – 0.52)</td>
<td>(0.08 – 0.48)</td>
<td>(0.30 – 0.64)</td>
<td>(0.23 – 0.59)</td>
</tr>
<tr>
<td>F$_{Ver}^{13}$</td>
<td>0.17</td>
<td>0.28$^1$</td>
<td>0.13</td>
<td>0.23$^1$</td>
<td>0.19</td>
<td>0.26$^1$</td>
</tr>
<tr>
<td></td>
<td>(-0.05 – 0.38)</td>
<td>(0.06 – 0.47)</td>
<td>(-0.09 – 0.34)</td>
<td>(0.02 – 0.43)</td>
<td>(-0.03 – 0.40)</td>
<td>(0.04 – 0.45)</td>
</tr>
</tbody>
</table>

$^1p < 0.05$, $^2p < 0.01$, $^3bcT = bulk central tibia$, $^4bpT = bulk posterior tibia$, $^5scT = superficial central tibia$, $^6spT = superficial posterior tibia$, $^7dcT = deep central tibia$, $^8dpT = deep posterior tibia$, $^9E_{lap} = entropy of Laplacian-based image$, $^{10}E_{LBP} = entropy of grouped local binary patterns$, $^{11}H_{angles} = mean homogeneity index for orientation of local patterns$, $^{12}F_{Hor} = fractal dimension of horizontal structures$, $^{13}F_{Ver} = fractal dimension of vertical structures$. 
7 Discussion

7.1 Main findings

In this doctoral thesis, novel X-ray-based methods were developed and compared to the existing methods of evaluating cartilage, bone, and the stage of OA. Bone density and texture analysis methods for 2-D radiographs were validated against the true 3-D microarchitecture of bone \textit{ex vivo} (Study I). This study demonstrated that bone density evaluated from a plain radiograph was significantly related to bone volume fraction. Texture parameters $\text{FD}_{\text{Ver}}$ and $\text{HI}_{\text{Angles}}$ were related mainly to trabecular separation and connectivity density. Bone density-related and texture parameters from plain radiographs were also compared \textit{in vivo} between age-matched groups of male subjects with different KL grades (Study II). Bone density-related parameters were significantly higher in medial side subchondral bone ROIs, whereas texture parameters showed significant differences between KL0 and KL1–4 ($\text{ELBP}$ in medial trabecular bone ROI) or KL0 and KL3–4 groups ($\text{FD}_{\text{Ver}}$ in medial subchondral bone ROI and all texture parameters in medial trabecular bone ROI). These results indicate that bone density is increased and bone structure becomes more disorganised in OA.

To evaluate the correlation between CT and MRI based techniques, proposed to be sensitive to proteoglycan content of cartilage, a novel dQCTA technique was compared with dGEMRIC (Study III). In this study, dQCTA conducted at 45 minutes after injection of the contrast agent was significantly related to dGEMRIC. The results indicate, that the dQCTA method can be used to evaluate cartilage properties \textit{in vivo}. Finally, the correlation between bone texture parameters and dGEMRIC was determined in order to ascertain whether changes in cartilage composition and subchondral bone structure are simultaneous in a cohort of subjects with mild OA (unpublished data). The dGEMRIC index for the central part of the cartilage in the tibia was significantly related to subchondral bone texture parameters in lateral compartment of joints. Weak associations between cartilage composition and underlying subchondral bone structure was found in the medial compartment of the tibia. The results support the presumption that several tissues are affected in the early OA.
7.2 Bone density from plain radiographs

Bone density of proximal tibia evaluated from 2-D plain radiograph correlated strongly with bone volume fraction, *i.e.* bone volume per total volume, evaluated with µCT *ex vivo*. Furthermore, the mean greyscale value from the 2-D coronal projection image from binarised µCT slices correlated strongly with the bone volume fraction. This finding is in line with a previous study that showed a strong correlation (*r* = 0.90) between bone volume fraction from 3-D µCT data and 2-D density estimate from the 3-D data (Steines *et al.* 2009). These are important findings since osteoarthritic subchondral bone is known to have a higher bone volume fraction than healthy bone (Bobinac *et al.* 2013, Buckland-Wright 2004, Ding *et al.* 2003, Djuric *et al.* 2013, Fazzalari & Parkinson 1997, Kamibayashi *et al.* 1995).

When evaluating the bone density from radiographs *in vivo* among subjects with different stages of knee OA, bone density-related parameters in medial subchondral and trabecular bone ROIs were higher among subjects with OA compared to the controls. Consistently, in a previous study, density estimates from plain radiographs were significantly higher in the medial subchondral bone of the tibia and femur among subjects with OA than among the controls (Marijnissen *et al.* 2008). In that study, bone density was estimated by comparing the intensity values of the bone ROI and of the aluminium step wedge (Marijnissen *et al.* 2008). In study I of this thesis, the greyscale values were also calibrated to the aluminium step wedge by interpolating the step wedge thickness that corresponds to the greyscale value of the bone. Current results show that estimation of bone volumetric density from 2-D radiographs is feasible at least when the greyscale values corresponding to the bone fall into the range of the step wedge greyscale values. However, if the greyscale values of the bone are outside the range of the step wedge’s greyscale values (*i.e.* corresponding aluminium thickness needs to be extrapolated), the method may be less reliable, since the detector response of the X-ray device is usually not perfectly linear.

In study II, instead of the step wedge, a calibration ball was present to calibrate the pixel values in the image. Since the imaging was performed over ten years ago, the step wedge could not be added in the imaging procedure. With the calibration ball, similar conversion of greyscale values to step wedge thickness to that in the *ex vivo* study, was not possible. Therefore, the greyscale values of bone were normalised with the maximum greyscale value of the calibration ball. However, this approach may not be optimal since, as mentioned earlier, the response of the
X-ray device’s detector is not perfectly linear and it is not known exactly how the lower greyscale values would behave in the image. In addition to normalised greyscale values, the plain mean greyscale values were also determined, since the imaging conditions were constant between images. However, direct evaluation of greyscale values may be prone to errors, especially if the imaging conditions (e.g., geometry, imaging parameters, pre-processing) vary. There is also variation in the intensity in the X-ray beam (i.e., heel effect) which may also affect the measured greyscale values. The heel effect may be a problem, particularly if the cathode-anode axis orientation changes or if there is a big difference in the location of the knee on the film between study subjects.

One explanation for the higher values of bone density-related parameters in medial subchondral and trabecular bone ROIs among subjects with OA in study II is that the loading of the medial compartment may be increased due to malalignment of the knee. Similarly, the lower values of bone density-related parameters in the lateral side may be associated to the reduced loading of the lateral compartment. This explanation is supported by the earlier study for this sample set, where the knee varus or valgus alignment was higher among OA subjects (Liikavainio et al. 2008).

Based on the current results regarding the evaluation of bone density from radiographs, we suggest that the step wedge should be included when X-ray imaging is performed for a cohort of subjects. However, if the X-ray device and/or the imaging parameters vary significantly between subjects, the reliability of the density measures remains to be studied.

### 7.3 Bone structure from plain radiographs

In study I of this thesis, significant correlations were obtained between bone texture parameters from plain radiograph and 3-D bone architectural parameters. However, the degrees of correlation varied depending on the parameter and the direction on which the texture measures were calculated. For instance, $\text{FD}_{\text{Ver}}$ and $\text{HIAngles}$ were significantly related to the connectivity density and trabecular separation in 3-D. This finding for the FD is consistent with a previous study (Majumdar et al. 1993). The results are very promising since, as mentioned in section 3.3, in addition to higher bone volume fraction, bone trabeculae are thicker, connectivity is higher, and the number and separation of trabeculae are changed in OA subchondral bone compared to healthy bone (Bobinac et al. 2013, Buckland-Wright 2004, Ding et al. 2003, Djuric et al. 2013, Fazzalari & Parkinson 1997, Kamibayashi et al. 1995).
In study II, $E_{Lap}$ was lower whereas $FD_{Ver}$ was higher in medial subchondral bone among subjects with OA than among the controls. This is consistent with previous studies in which $FD_{Ver}$ have been reported to be higher among subjects with OA compared to controls (Buckland-Wright et al. 1996, Messent et al. 2005b, Messent et al. 2006, Messent et al. 2007) and among subjects with progressive OA compared to subjects with non-progressive OA (Kraus et al. 2009, Kraus et al. 2013). One explanation for these findings is that subchondral trabecular bone in the proximal tibia is not as well organised in OA, i.e. the trabeculae are less oriented to the main loading direction than in healthy bone (Ding et al. 2003). In medial trabecular bone, bone structure was more random (detected with $E_{LBP}$) among people with OA, whereas HI$_{Angles}$ was lower (larger variation in the orientation of adjacent local patterns) and FDs of horizontal and vertical structures were higher among people with advanced OA than among the controls. These findings for the $FD_{Ver}$ in trabecular bone are in line with the previous literature (Messent et al. 2005a, Messent et al. 2005b). Furthermore, and also consistent with the current findings, higher values of $FD_{Hor}$ at small image scales have been reported among OA subjects compared to the controls (Messent et al. 2005b) and higher FDs of both vertical and horizontal structures have been reported among subjects with cartilage defect compared to subjects without defects (Wolski et al. 2011). However, the above-mentioned findings contradict with studies, in which FDs for medial trabecular bone have been reported to be lower among OA subjects compared to controls (Podsiadlo et al. 2008b, Wolski et al. 2010). These studies used the same study sample (subjects with medial meniscectomy and controls) and radiographs, but the analysis methods were different from each other (Podsiadlo et al. 2008b, Wolski et al. 2010). It is possible, that after meniscectomy, changes in bone structure are different compared to some other phenotype of OA that has been developed from a different pathway.

Although more significant changes were found in the medial side ROIs, $FD_{Hor}$ was significantly lower in lateral subchondral bone among subjects with OA than among the controls (Study II). This finding is in line with the previous literature, as loss of bone and bone structure from the lateral side of tibia has been reported in OA (Buckland-Wright 2004, Lindsey et al. 2004, Messent et al. 2005a, Podsiadlo et al. 2008a, Wolski et al. 2010). It has been shown that the knees with medial compartment OA commonly have varus malalignment (Sharma et al. 2001) and thus, the loss of bone and bone structure may be due to associated subluxation and reduction in loading of the lateral compartment (Buckland-Wright 2004, Messent et al. 2005b). It should be noted that in study II, subjects having more OA changes
in the lateral than in the medial compartment were excluded so as to homogenise the study sample.

In the present thesis, several algorithms for evaluating the bone structure were used. However, it should be noted that each algorithm measures different features from the image. Since the HI_Angles parameter is the mean HI value from four different directions, it is less affected by the orientation of the image (in this thesis, results are reported only for the mean HI). It can be hypothesised that FDs or directional homogeneity indices would correlate even more strongly with thickness and separation of trabeculae, if the image is oriented along the trabeculae. The current results support this hypothesis since the degree of correlation varied depending on which direction the FD or homogeneity indices of local patterns were calculated. For example, HI_angle in the horizontal direction and FD_Ver in trabecular bone area were significantly related to the trabecular separation, whereas HI_angle in the vertical direction and FD_Hor were less related (data for the directional HI_angle measures are shown in original publication). This is because the trabeculae were aligned more vertically than horizontally in study I and, therefore, when calculating HI_angle, there was less variation in the orientation of adjacent local patterns in a vertical direction. However, in the previous texture analysis studies of a knee joint, the images have not been oriented along the main direction of trabeculae and, therefore, the images were not oriented in the present studies either.

In study I of this thesis, the degrees of correlation between µCT parameters and entropies (E_Lap and E_LBP) were found to be variable. One explanation for the positive correlation between E_LBP and connectivity density is that, when the bone is highly connected, more different patterns are detected in the texture analysis and eventually the entropy of patterns is higher. Based on the current results, E_Lap is more sensitive to the changes in the structure of subchondral bone than of trabecular bone. The Laplacians were calculated in the vertical and horizontal directions and summed together, which may have reduced the sensitivity of method for bone changes when the trabeculae are well aligned only in one direction, i.e. in the trabecular bone. Originally, the method was designed for the analysis of femoral neck, where the orientation of the trabeculae is usually clear and the ROI can easily be aligned along the trabeculae (Thevenot et al. 2014b).

The detailed comparison between LBP-based, Laplacian-based, FSA and fractal-based algorithms, as well as other texture methods, is a challenging topic. Therefore, further evaluation to establish an optimal procedure for including multiple parameters for bone texture analysis is required in future (Huber et al. 2009). However, the present results suggest that the bone texture analysis may serve
as a complementary method in radiographic OA diagnostics, since good correlations were obtained with 3-D microarchitecture of bone.

### 7.4 Evaluation of cartilage composition

In study III, dQCTA was significantly related to dGEMRIC. The correlation between these two methods was expected since both of these methods use an anionic contrast agent and are proposed for the quantification of the cartilage GAG content (Bansal et al. 2010, Bashir et al. 1996, Bashir et al. 1997, Bashir et al. 1999, Cockman et al. 2006, Siebelt et al. 2011, Xie et al. 2010). However, the correlation depended on the parameter used to quantify the concentration of contrast agent in the cartilage. The strongest correlation between dGEMRIC with intravenous injection and dQCTA were obtained when the dQCTA parameters were normalised with the contrast agent concentration in the synovial fluid. Furthermore, the strongest correlation between normalised dQCTA and dGEMRIC with intra-articular injection was observed when dGEMRIC was normalised by the $\Delta R_1$ value in synovial fluid. Therefore, it is important to consider the contrast agent concentration in the synovial fluid both in dQCTA and dGEMRIC with intra-articular injection, especially among subjects with expected differences in the volume of synovial fluid.

Pharmacokinetically, dGEMRIC conducted at 90 minutes after injection should be closest to the dQCTA at 45 minutes after injection, although there still is considerable difference in time between these two measurement methods. However, the selection of the time points for the imaging was based on the previous literature (Dahlberg et al. 2012, Kokkonen et al. 2012, Tiderius et al. 2003). It is likely that 5 minutes after intra-articular injection, a low amount of the contrast agent was diffused into the cartilage and, therefore, the correlations of dQCTA at that time point with the dGEMRIC parameters were lower. Furthermore, differences in pharmacokinetics between intravenous and intra-articular contrast agent administration, and between contrast agents used (gadopentetate and ioxaglate), may partly explain the variations in correlations. Transportation of the contrast agent into the cartilage between intravenous and intra-articular injections may be different (Bashir et al. 1997), although the diffusion from the subchondral bone was found to be negligible in recent studies (Hawezi et al. 2011, Salo et al. 2012, Silvast et al. 2009). The penetration of gadopentetate and ioxaglate into the cartilage is different due to their molecular masses and charges (gadopentetate: 548 g/mol, -2; ioxaglate: 1269 g/mol, -1) (Bansal et al. 2011, Silvast et al. 2009). However, it
should be noted that equilibrating conditions for dQCTA and dGEMRIC with intra-articular injection were similar, since a strong correlation between ΔR₁ in synovial fluid and mean attenuation in synovial fluid at 45 minutes after ioxaglate injection was observed (data not shown).

In study III, neither dQCTA nor dGEMRIC were significantly different between different ICRS grade groups. This finding is consistent with previous studies in which dGEMRIC did not correlate with the arthroscopic findings (Nojiri et al. 2006, Owman et al. 2008). Owman et al. (2008) calculated the dGEMRIC index for the medial and lateral condyles of the tibia and femur whereas Nojiri et al. (2006) calculated it for the lesion in the patella. However, these results contradict with another study that reported significantly different R₁ and ΔR₁ values between OA and reference compartments (Tiderius et al. 2003). Tiderius et al. (2003) limited their analyses to the load bearing medial and lateral condyles of the femur representing more homogenous cartilage areas. In study III, ROIs from various locations were extracted, and cartilage properties have been shown to differ between different locations (Kurkijärvi et al. 2004, Multanen et al. 2009). The absence of a relation between dGEMRIC and arthroscopic grading might also be because the remaining cartilage is analysed in the dGEMRIC and dQCTA analyses, whereas the absence of cartilage defines the arthroscopic grading. Hence, when there is an arthroscopically defined lesion, the quality of the underlying cartilage is actually evaluated in dGEMRIC and dQCTA analyses. However, it is expected that the remaining cartilage immediately under the lesion should have at least mild degenerative changes. Furthermore, the cartilage area that was analysed in the dGEMRIC may not cover the whole lesion detected in arthroscopy because of the limited slice thickness. It is probable that, dGEMRIC and dQCTA methods may detect the changes earlier than they are manifested in the arthroscopy.

In the present study, dQCTA was in best agreement with dGEMRIC with intravenous injection at 45 minutes after ioxaglate injection. If judged only with visual evaluation by the experienced radiologist, the CT conducted at 5 min after the injection had the best diagnostic quality for evaluation of cartilage lesions. Thus, two separate scans might offer the optimal result for both qualitative and quantitative evaluation of cartilage. However, both logistics and radiation exposure may hinder the application of two separate scans, although low radiation dose procedures are emerging for the imaging of the knee joint. Further studies are still needed to optimise the imaging delay and contrast agent dose for dQCTA. Also, similarly than in dGEMRIC, the specificity of dQCTA to GAG content needs to be clarified.
7.5 Relation between dGEMRIC and bone structure from radiographs

In the present study, when comparing changes in cartilage proteoglycan content, estimated with dGEMRIC, and subchondral bone structure from radiographs among subjects with early OA (KL 1 or 2), stronger correlations were seen in the lateral compartment of the tibia. $FD_{hor}$ and $FD_{vec}$ to some degree, in lateral subchondral bone were related to the dGEMRIC index of the lateral tibial cartilage. The positive correlation between FDs and the dGEMRIC index indicate that, when the cartilage degenerates (more contrast agent is present in the cartilage and eventually the dGEMRIC index is lower), the bone structure also deteriorates (detected as lower values of FDs). Furthermore, a mild but significant relation was found between the structural changes in medial subchondral bone, detected with texture parameters $E_{Lap}$ and $H_{Angles}$, and the dGEMRIC index of medial tibial cartilage. Again, the positive correlation between $E_{Lap}$ and the dGEMRIC index and negative correlation between $H_{Angles}$ and the dGEMRIC index suggest that, when cartilage degenerates, bone structure is also changed in the medial side. $E_{Lap}$ is associated with bone volume fraction and trabecular thickness, whereas $H_{Angles}$ and FDs are more associated with connectivity density and trabecular separation in the subchondral bone area.

Consistent with the current results, previous studies have reported more significant changes in the lateral side than in the medial side when comparing cartilage morphology (Blumenkrantz et al. 2004, Bolbos et al. 2008, Lindsey et al. 2004), composition (Bolbos et al. 2008), or lesions (Wolski et al. 2011) to the bone structure. FD of horizontal structures in the lateral compartment, and FD of vertical structures with small scales in both medial and lateral compartments, were significantly higher among subjects with cartilage defects (in medial, lateral, or both compartments) compared to subjects without defects detected with MRI (Wolski et al. 2011). When cartilage morphology was compared to apparent bone structure parameters calculated from the MRI data, the apparent bone volume fraction, trabecular thickness, and trabecular number correlated positively with the cartilage thickness in the medial side (Blumenkrantz et al. 2004, Bolbos et al. 2008). In the lateral side, cartilage thickness correlated positively with apparent bone volume fraction, trabecular thickness, and trabecular number, and negatively with apparent trabecular separation (Bolbos et al. 2008). Furthermore, MRI parameters related to cartilage composition ($T_1p$ and $T_2$) correlated negatively with apparent bone volume fraction, trabecular thickness, and trabecular number and positively...
with apparent trabecular separation in the lateral side among subjects with mild OA and controls (Bolbos et al. 2008). $T_1\rho$ is related to the proteoglycan content (and may be related to collagen and water content as well) of a cartilage whereas $T_2$ is related to the water and collagen content of cartilage (Li & Majumdar 2013, Nieminen et al. 2012).

In general, the results of the present study support the presumption that several tissues are affected in the early OA. However, the causality of the tissue changes remains to be studied. Although significant correlations between dGEMRIC and subchondral bone structure from radiographs were observed, more detailed studies with carefully selected subjects, MRI parameters, and imaging modalities are warranted in order to fully understand the factors which affect the correlations between the changes in cartilage and subchondral bone.

7.6 Limitations

This thesis has several limitations that should be addressed. The main limitation of this thesis is that different OA phenotypes could not be studied separately, and they were probably mixed in the study samples. However, there is currently no established practise for the selection of subjects with certain OA phenotype and, thus, it was not feasible to control this mixing of phenotypes when selecting the subjects in the studies. Furthermore, in each sub-study, different sets of subjects were used, and therefore the characteristics of the study sample might have been different. For example, in the bone imaging studies of this thesis (studies I, II and unpublished data), the bones might have been different, since presumably there are differences in the bone density and structure between the genders. In study II, male subjects with different stages of OA and age-matched controls were selected, whereas in the unpublished data, postmenopausal women with knee pain and mild OA were selected. These factors should be considered when generalising the current results.

The donors did not have any diagnosed joint disease at the time of their death in study I and, thus, only limited variation in bone density and structure could be expected. However, this does not necessarily mean that they did not have any OA changes in bone or cartilage, since OA changes in the cartilage were seen in the contralateral side (Qu et al. 2007). Consequently, a larger sample set with both non-OA and OA subjects should be studied in future to further clarify the sensitivity of the methods reported here. Furthermore, ex vivo bone samples in study I did not contain soft tissue which reduces quality of the radiograph and, therefore,
generalisation of the methods *in vivo* is partially restricted. However, the effect of soft tissue was simulated by immersing the bones into a water bath during radiography (data shown in the original publication of study I). After the bones were immersed in the water bath, texture parameters were still significantly related to 3-D microarchitecture of bone.

Another limitation of this thesis is related to the grading of OA. KL grading and ICRS grading are subjective and semi-quantitative. In study II, quantitative results were compared to the KL grading, which was conducted from the same image that was used for the bone density and texture analyses. Visual evaluation of bone sclerosis is part of the KL grading, making the quantitative analysis of bone at least slightly dependent on the KL grade. One of the main limitations is that some studies of this thesis had relatively small sample sizes. For example, in study III, site-specific correlations were not calculated due to the limited sample size. To reach statistical significance ($p < 0.05$) for Pearson’s correlation coefficient at level of 0.6 with the power of 80%, at least 16 samples would be required. Finally, intra-articular injection is required in dQCTA. However, it is more challenging to conduct intra-articular injection and this is usually experienced as more uncomfortable than intravenous injection. This may hinder the clinical application of the method.

### 7.7 Clinical implications and applications in the research

Each of the clinical modalities used in this thesis has its own strengths. Although MRI and CT provide 3-D views of a knee joint, radiography has its place in OA imaging since it is widely available, is a low cost method, and is fast to conduct with a minor radiation dose.

MRI is currently the best method by which to clinically evaluate articular cartilage. In addition to morphological measurements of cartilage, MRI methods that are related to the composition of the cartilage are available (*e.g.* dGEMRIC, $T_2$, $T_1\rho$) and new methods will be developed in future. Although bone can be evaluated indirectly with MRI (signal comes from bone marrow), it is likely that sensitive methods to directly evaluate bone tissue will also be available in future. However, the spatial resolution of *in vivo* MRI scanners should also be increased in order to evaluate the structure of bone sensitively.

CT is currently the best available method for evaluating bone tissue, although the resolution in clinical scanners is not yet sufficient for evaluation of bone microstructure. It has been suggested that, for sensitive evaluation of 3-D
microarchitecture of bone, resolutions below 80–100 \( \mu m \) should be used (Isaksson et al. 2011). Bone volume fraction, instead, can be reliably evaluated also with larger pixel sizes. The limitation related to spatial resolution of the scanners is likely to be resolved in the near future. For instance, novel cone beam CT devices can provide isotropic resolution of around 100 \( \mu m \), and high resolution peripheral quantitative CT (HR-pQCT) devices with resolution even below 100 \( \mu m \) have been introduced (Boutroy et al. 2005, Boyd 2008). HR-pQCT has recently been shown to be applicable in the imaging of the knee joint, although imaging times are longer than in conventional CT (Kroker et al. 2015). In addition to evaluation of bone, indirect methods for evaluation of cartilage composition have been developed and applied \textit{in vivo}. Therefore, simultaneous evaluation of cartilage and bone would be a great advantage in OA diagnostics in future.

Based on the availability and ease of the radiography and image analysis methods for plain radiographs, these methods could be applied in the first screening of OA, e.g. in primary health care. After radiography, if the diagnosis is still uncertain or if symptoms are persistent, MRI or CT could be used in specialised health care to acquire a detailed 3-D view of the joint.

Since it is nowadays widely accepted that different phenotypes of OA exist (Bijlsma et al. 2011), it can also be hypothesised that different phenotypes exhibit different changes in subchondral bone during OA. To clarify the role of subchondral bone in the development of OA, subject selection to such studies should be made carefully. For instance, typical alterations in each suggested phenotype should be clarified in order to develop methods that are able to characterise these changes in bone in 2-D and 3-D. However, this kind of subject selection is still very challenging.

### 7.8 Future studies

In future, the texture analysis methods presented for plain radiographs should be thoroughly validated \textit{in vivo} against true 3-D bone structure obtained from CT with sufficient resolution. Furthermore, detailed clarification would be required of how each variable changes at different stages of OA, and in different OA phenotypes.

In CT, algorithms to evaluate bone structure simultaneously with dQCTA method could be developed and applied. Since novel cone-beam CT or HR-pQCT scanners provide a high resolution image stacks, application of such methods to image data with sufficient resolution for the evaluation of bone structure is clinically possible. Furthermore, apart from ioxaglate, other kinds of contrast
agents that might be even more sensitive to compositional changes in cartilage, have been and are being developed (Lusic & Grinstaff 2012, Nieminen et al. 2015).

With regard to MRI, novel methods for evaluation of bone should be developed and validated against 3-D microarchitecture of bone obtained from CT with sufficient resolution in vivo.
8 Conclusions

In this thesis, the potential of the novel X-ray-based methods to quantify OA related changes in articular cartilage and subchondral bone were explored \textit{ex vivo} and \textit{in vivo}. More specifically, 2-D image analysis methods for the evaluation of bone density and structure from plain radiographs were developed and validated \textit{ex vivo}. Subsequently, these were compared with MRI of cartilage, and applied to subjects with different stages of OA \textit{in vivo}. Furthermore, a method developed for the evaluation of cartilage composition from CT (dQCTA) was compared with dGEMRIC, which is an analogous MRI technique to dQCTA.

The main conclusions of the present thesis are summarised as follows:

1. Methods evaluating bone density and structure from 2-D plain radiographs are significantly related to 3-D bone microarchitecture obtained from µCT \textit{ex vivo}, yet the degrees of correlation vary depending on the parameters.
2. Subjects with different stages of OA had significant differences in bone density-related and structure-related parameters.
3. dQCTA method was significantly related to dGEMRIC \textit{in vivo}. However, neither dQCTA nor dGEMRIC were significantly related to the arthroscopic grading of a knee joint.
4. Associations between cartilage composition assessed with dGEMRIC and bone structure assessed from plain radiographs \textit{in vivo} were observed mainly in the lateral side of the tibia.
References


## Appendices

Table 11. Pearson correlation coefficients (95% confidence interval) between bone texture parameters evaluated from both plain radiographs and 2-D µCT projection image and 3-D µCT parameters in subchondral bone ROIs (n = 22).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>BV/TV²</th>
<th>Conn.Dn¹</th>
<th>Tb.Th³</th>
<th>Tb.Sp⁴</th>
<th>Tb.N⁵</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plain radiograph</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E_Lap⁸</td>
<td>0.61²</td>
<td>0.23</td>
<td>0.53¹</td>
<td>-0.44¹</td>
<td>0.47¹</td>
</tr>
<tr>
<td></td>
<td>(0.25 – 0.82)</td>
<td>(-0.21 – 0.59)</td>
<td>(0.14 – 0.78)</td>
<td>(-0.72 – -0.02)</td>
<td>(0.06 – 0.74)</td>
</tr>
<tr>
<td>E_ELBP⁹</td>
<td>0.32¹</td>
<td>0.51¹</td>
<td>0.10</td>
<td>-0.46¹</td>
<td>0.42</td>
</tr>
<tr>
<td></td>
<td>(-0.12 – 0.65)</td>
<td>(0.11 – 0.77)</td>
<td>(-0.34 – 0.50)</td>
<td>(-0.74 – -0.05)</td>
<td>(0.00 – 0.71)</td>
</tr>
<tr>
<td>HIAngles¹⁰</td>
<td>-0.49¹</td>
<td>-0.55²</td>
<td>-0.14</td>
<td>0.67²</td>
<td>-0.59²</td>
</tr>
<tr>
<td></td>
<td>(-0.76 – -0.09)</td>
<td>(-0.79 – -0.17)</td>
<td>(-0.53 – 0.30)</td>
<td>(0.35 – 0.85)</td>
<td>(-0.81 – -0.23)</td>
</tr>
<tr>
<td>FDHor¹¹</td>
<td>-0.04</td>
<td>0.36</td>
<td>-0.37</td>
<td>-0.18</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>(-0.46 – 0.38)</td>
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<td>(-0.68 – 0.06)</td>
<td>(-0.56 – 0.26)</td>
<td>(-0.20 – 0.60)</td>
</tr>
<tr>
<td>FDVer¹²</td>
<td>0.23²</td>
<td>0.56²</td>
<td>-0.14</td>
<td>-0.54²</td>
<td>0.45¹</td>
</tr>
<tr>
<td></td>
<td>(-0.21 – 0.59)</td>
<td>(0.18 – 0.79)</td>
<td>(-0.53 – 0.30)</td>
<td>(-0.78 – -0.15)</td>
<td>(0.03 – 0.73)</td>
</tr>
<tr>
<td><strong>2-D µCT projection image</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E_Lap²</td>
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<td>0.38</td>
<td>0.32</td>
<td>-0.56²</td>
<td>0.56²</td>
</tr>
<tr>
<td></td>
<td>(0.19 – 0.80)</td>
<td>(-0.04 – 0.69)</td>
<td>(-0.12 – 0.65)</td>
<td>(-0.80 – -0.19)</td>
<td>(0.18 – 0.79)</td>
</tr>
<tr>
<td>E_ELBP</td>
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<td>0.74²</td>
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<td>-0.80²</td>
<td>0.82²</td>
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<tr>
<td></td>
<td>(0.35 – 0.85)</td>
<td>(0.46 – 0.89)</td>
<td>(-0.22 – 0.59)</td>
<td>(-0.91 – -0.57)</td>
<td>(0.60 – 0.92)</td>
</tr>
<tr>
<td>HIAngles</td>
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<td>-0.73²</td>
<td>-0.15</td>
<td>0.74²</td>
<td>-0.77²</td>
</tr>
<tr>
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<td>(-0.88 – -0.44)</td>
<td>(-0.54 – 0.29)</td>
<td>(0.46 – 0.88)</td>
<td>(-0.90 – -0.51)</td>
</tr>
<tr>
<td>FDHor²</td>
<td>-0.26</td>
<td>0.33</td>
<td>-0.56²</td>
<td>-0.07</td>
<td>0.08</td>
</tr>
<tr>
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<td>(-0.11 – 0.66)</td>
<td>(-0.79 – -0.18)</td>
<td>(-0.48 – 0.36)</td>
<td>(-0.35 – 0.48)</td>
</tr>
<tr>
<td>FDVer²</td>
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<td>-0.54²</td>
<td>-0.56²</td>
<td>0.48¹</td>
</tr>
<tr>
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<td>(-0.39 – 0.45)</td>
<td>(0.56 – 0.91)</td>
<td>(-0.78 – -0.15)</td>
<td>(-0.79 – -0.18)</td>
<td>(0.08 – 0.75)</td>
</tr>
</tbody>
</table>

¹ρ < 0.05, ²ρ < 0.01, ³BV/TV = bone volume fraction, ⁴Conn.Dn = connectivity density, ⁵Tb.Th = trabecular thickness, ⁶Tb.Sp = trabecular separation, ⁷Tb.N = trabecular number, ⁸E_Lap = entropy of Laplacian-based image, ⁹E_ELBP = entropy of grouped local binary patterns, ᵈHIAngles = mean homogeneity index for orientation of local patterns, ᵉFD = fractal dimension of horizontal structures, ᵊFD = fractal dimension of vertical structures.
### Table 12. Pearson correlation coefficients (95% confidence interval) between bone texture parameters evaluated from both plain radiographs and 2-D µCT projection image and 3-D µCT parameters in trabecular bone ROIs ($n = 22$).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>BV/TV</th>
<th>Conn.Dn</th>
<th>Tb.Th</th>
<th>Tb.Sp</th>
<th>Tb.N</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plain radiograph</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E$_{elp}$</td>
<td>0.16</td>
<td>-0.11</td>
<td>0.31</td>
<td>0.11</td>
<td>-0.01</td>
</tr>
<tr>
<td></td>
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<td>(-0.51 – 0.33)</td>
<td>(-0.13 – 0.64)</td>
<td>(-0.33 – 0.51)</td>
<td>(-0.43 – 0.41)</td>
</tr>
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<td>E$_{elap}$</td>
<td>0.04</td>
<td>0.37</td>
<td>-0.13</td>
<td>-0.17</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>(-0.39 – 0.46)</td>
<td>(-0.06 – 0.69)</td>
<td>(-0.52 – 0.31)</td>
<td>(-0.55 – 0.27)</td>
<td>(-0.29 – 0.54)</td>
</tr>
<tr>
<td>H$_{homog}$</td>
<td>0.01</td>
<td>-0.65$^2$</td>
<td>0.41</td>
<td>0.42</td>
<td>-0.33</td>
</tr>
<tr>
<td></td>
<td>(-0.43 – 0.41)</td>
<td>(-0.84 – -0.31)</td>
<td>(-0.01 – 0.71)</td>
<td>(0.00 – 0.72)</td>
<td>(-0.66 – 0.10)</td>
</tr>
<tr>
<td>FD$_{hoc}$</td>
<td>-0.38</td>
<td>0.06</td>
<td>-0.47$^1$</td>
<td>-0.01</td>
<td>-0.16</td>
</tr>
<tr>
<td></td>
<td>(-0.69 – 0.04)</td>
<td>(-0.37 – 0.47)</td>
<td>(-0.74 – -0.06)</td>
<td>(-0.43 – 0.41)</td>
<td>(-0.54 – 0.28)</td>
</tr>
<tr>
<td>FD$_{voc}$</td>
<td>0.56$^2$</td>
<td>-0.66$^2$</td>
<td>0.42</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(-0.47 – 0.37)</td>
<td>(-0.31 – 0.84)</td>
<td>(-0.84 – -0.30)</td>
<td>(-0.85 – -0.33)</td>
<td>(0.00 – 0.72)</td>
</tr>
<tr>
<td><strong>2-D µCT projection image</strong></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E$_{elp}$</td>
<td>0.23</td>
<td>-0.18</td>
<td>0.47$^1$</td>
<td>0.28</td>
<td>-0.04</td>
</tr>
<tr>
<td></td>
<td>(-0.21 – 0.60)</td>
<td>(-0.56 – 0.26)</td>
<td>(0.07 – 0.75)</td>
<td>(-0.16 – 0.63)</td>
<td>(-0.45 – 0.39)</td>
</tr>
<tr>
<td>E$_{elap}$</td>
<td>0.27</td>
<td>0.48$^1$</td>
<td>0.03</td>
<td>-0.34</td>
<td>0.35</td>
</tr>
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<td></td>
<td>(-0.17 – 0.62)</td>
<td>(0.08 – 0.75)</td>
<td>(-0.39 – 0.45)</td>
<td>(-0.67 – 0.09)</td>
<td>(-0.09 – 0.67)</td>
</tr>
<tr>
<td>H$_{homog}$</td>
<td>-0.34</td>
<td>-0.65$^2$</td>
<td>0.07</td>
<td>0.58$^2$</td>
<td>-0.52$^1$</td>
</tr>
<tr>
<td></td>
<td>(-0.67 – 0.09)</td>
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<td>(-0.37 – 0.47)</td>
<td>(0.21 – 0.80)</td>
<td>(-0.77 – -0.12)</td>
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<tr>
<td>FD$_{hoc}$</td>
<td>-0.28</td>
<td>0.56$^2$</td>
<td>-0.72$^2$</td>
<td>-0.42</td>
<td>0.18</td>
</tr>
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<tr>
<td>FD$_{voc}$</td>
<td>0.17</td>
<td>0.68$^2$</td>
<td>-0.77$^2$</td>
<td>-0.65$^2$</td>
<td>0.39</td>
</tr>
<tr>
<td></td>
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<td>(0.37 – 0.86)</td>
<td>(-0.90 – -0.52)</td>
<td>(-0.84 – -0.32)</td>
<td>(-0.04 – 0.69)</td>
</tr>
</tbody>
</table>

$^1p < 0.05$, $^2p < 0.01$, $^3$BV/TV = bone volume fraction, $^4$Conn.Dn = connectivity density, $^5$Tb.Th = trabecular thickness, $^6$Tb.Sp = trabecular separation, $^7$Tb.N = trabecular number, $^8$E$_{elap}$ = entropy of Laplacian-based image, $^9$E$_{elap}$ = entropy of grouped local binary patterns, $^{10}$H$_{homog}$ = mean homogeneity index for orientation of local patterns, $^{11}$FD$_{hoc}$ = fractal dimension of horizontal structures, $^{12}$FD$_{voc}$ = fractal dimension of vertical structures.
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Jukka Hirvasniemi

NOVEL X-RAY-BASED METHODS FOR DIAGNOSTICS OF OSTEOARTHRITIS