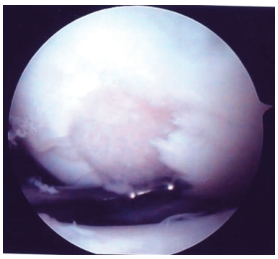
Focal Cartilage Defects in the Knee



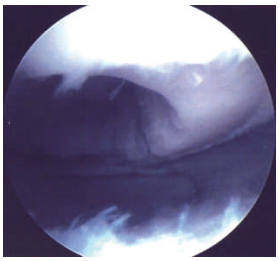


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TABLE OF CONTENTS

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Achieving your goals, I guess, is usually just a matter of not giving up

However, this thesis had never been fulfilled without the help and support of several people to whom I am deeply greatful. I also appreciate the patiency put forward which has finally made the work with this thesis come to an end.

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PAPERS INCLUDED IN THIS THESIS

I. Heir S, Nerhus TK, Røtterud JH, Løken S, Ekeland A, Engebretsen L, Årøen A.
Focal cartilage defects in the knee impair quality of life as much as severe osteoarthritis. A comparison of Knee injury and Osteoarthritis Outcome Score in 4 patient categories scheduled for knee surgery.
Am J Sports Med 2010; 38: 231-237

II. Løken S, Heir S, Holme I, Engebretsen L, Årøen A.
6-year follow up of 84 patients with cartilage defects in the knee: Knee scores improved but recovery was incomplete.
Acta Orthop 2010; accepted for publication.

III. Heir S, Årøen A, Løken S, Sulheim S, Engebretsen L, Reinholt FP.
Intra-articular location predicts cartilage filling and subchondral bone changes in a chondral defect. A randomized, blinded, long term follow-up trial in 82 rabbit knees.
Acta Orthop 2010; accepted for publication.

IV. Heir S, Årøen A, Løken S, Holme I, Engebretsen L, Reinholt FP.
Cartilage repair in the rabbit knee: mosaic plasty resulted in higher degree of tissue filling but affected subchondral bone more than microfracture technique. A blinded, randomized, controlled, long term follow up trial in 88 knees.
Knee Surg Sports Traumatol Arthrosc 2010; submitted.

ABBREVIATIONS

ACI	Autologous Chondrocyte Implantation
ACL	Anterior Cruciate Ligament
BMI	Body Mass Index
CI	Confidence Interval
CPM	Continuous Passive Motion
GAGs	Glucosaminoglycans
H&E	Hematoxylin-Eosin
HA	Hyaluronic Acid
ICRS	International Cartilage Repair Society
IKDC	International Knee Documentation Committee
KOOS	Knee injury and Osteoarthritis Outcome Score
MACI	Matrix-induced Autologous Chondrocyte Implantation
MFC	Medial Femoral Condyle
MPCI	Minimal Perceptive Clinical Improvement
MRI	Magnetic Resonance Imaging
MSC	Mesenchymal Stem Cell
NSAIDs	Non Steroid Anti-Inflammatory Drugs
OA	Osteoarthritis
OCD	Osteochondritis Dissecans
	A-OCD Adult Ostochondritis Dissecans
	J-OCD Juvenile Osteochondritis Dissecans
QoL	Quality of Life
RCT	Randomized Controlled Trial
SD	Standard Deviation
SEM	Standard Error of Means
SF-36	Short Form 36
VAS	Visual Analogue Scale
WOMAC	Western Ontario and McMaster Univeristies Osteoarthritis Index

INTRODUCTION

The knee articular joint surfaces consisting of hyaline cartilage – integrated to the underlying mineralized tissues – are vulnerable to injuries and disease. In contrast to most other tissues, the diarthrodial cartilage-bone unit has limited capacity for healing – potentially leaving lesions and even deep defects in the joint surface unhealed over time (Buckwalter 1998, Hunter W 1743). In a previous study, we observed such full-thickness focal cartilage defects in 11% of all knees undergoing arthroscopic surgery due to knee pain – these high numbers being verified both by MRI studies (Ding et al. 2005) and clinical studies (Aroen et al. 2004, Hjelle et al. 2002, Widuchowski et al. 2007). Focal cartilage defects seem to present with various types and degrees of symptoms (Aarseth L et al. 1999) the reason for the variance – and the factors influencing it – being unknown. In some cases focal cartilage defects even present asymptomatic (Kaplan et al. 2005). The great variance in symptoms and the lack of comparative data on these patients' complaints make it hard to understand the severe complaints some of these patients actually do have. Although patomechanisms related to focal cartilage defects and their long term natural history are not clear, an etiological relationship between these lesions and long term perspective osteoarthritis (OA) has been suggested (Drogset and Grontvedt 2002, Linden 1977) even when the defects are treated with cartilage repair (Knutsen et al. 2007). However, the natural history of focal cartilage defects is not fully understood, neither are the various factors influencing it. Nevertheless, different techniques for cartilage repair have been suggested (Brittberg et al. 1994, Hangody et al. 1998, Lorentzon et al. 1998, Steadman et al. 1997), but no technique has so far been shown to be superior to others – or to the natural history (Bartlett et al. 2005b, Bentley et al. 2003, Gooding et al. 2006, Gudas et al. 2005, Horas et al. 2003, Knutsen et al. 2004, 2007, Magnussen et al. 2008, Messner and Gillquist 1996, Saris et al. 2008, Visna et al. 2004, Zeifang et al. 2010). Moreover, the results of each technique vary widely (Jakobsen et al. 2005). Although the effect of the different techniques to some extent has been verified in experimental animal models (Brittberg et al. 1996, Frisbie et al. 1999, Grande et al. 1989, Hangody 1997), the reasons for the variability in results of one particular technique or between techniques are not thoroughly understood.

When reviewing the "orthopedic" literature there seems to be more focus on new methods and strategies for possibly improving "cartilage repair" than on the understanding of why previous strategies were unpredictable and did not give satisfactory results. Moreover, the major focus seems to be on the layer of non-calcified hyaline articular cartilage; its composition,

organization, biomechanical properties and restoration of its morphology, - with less focus on the mechanical and biochemical interactions with the subchondral mineralized tissues. The concept that subchondral bone and overlying cartilage is one functional unit suggests the importance of pathological changes in and restoration of normal subchondral mineralized tissues as well and the restoration of the interactions between bone and cartilage for a long lasting good result of healing.

When the Oslo Cartilage Group was initiated by Lars Engebretsen and Asbjørn Årøen in 1999, an experimental rabbit model was established to explore histological details in the cartilage-bone unit related to cartilage defects and their treatment. The papers in the current thesis are partly from clinical work and partly from experimental work using the rabbit model. At the time we started the work with this project, the background and the challenges for each study were the following:

Background: Unhealed cartilage defects in the knee cause considerable complaints
Challenge: The degree of symptoms and to which extent they impair patients quality of life are not well documented, and particularly not compared to other knee disorders.
Paper 1: Focal cartilage defects in the knee impair quality of life as much as severe osteoarthritis.

Background: Cartilage defects do not regenerate completely and therefore need cartilage repair.
Challenge: Middle-to-long term outcome following natural history and cartilage repair is not fully known.
Paper 2: 6-year follow up of 84 patients with cartilage defects in the knee: Knee scores improved but recovery was incomplete.

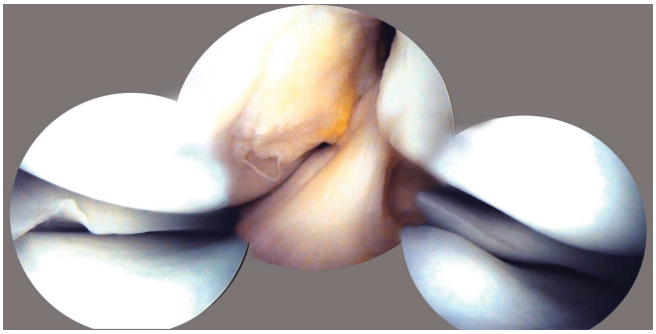
Background: Untreated cartilage defects present with a wide range of symptom intensity and functional impairment.
Challenge: The predictive factors for outcome following natural history of cartilage defects are not fully understood.
Paper 3: Intra-articular location predicts cartilage filling and subchondral bone changes in a chondral defect in the rabbit knee.

Background: The clinical results following cartilage repair vary widely. Moreover, no repair technique has been shown to be superior to others – or to the natural history.
Challenge: The variances in results following certain surgical techniques for cartilage repair have not been thoroughly investigated by comparison in experimental studies.
Paper 4: Cartilage repair in the rabbit knee: Mosaic plasty resulted in higher degree of tissue filling but affected subchondral bone more than microfracture technique.

Adding to previous studies by our group, the general purpose of the papers in this thesis was to clarify some aspects concerning the magnitude of the problem "cartilage defects in the knee", and to enlighten some possible predictive factors and explanations for the success or failure in natural history and repair of cartilage defects.

For the extensive cartilage research ongoing world wide, the ultimate goal is to restore normal function of an injured joint. Obviously, there is a need for better understanding the mechanisms and predictors for successful bone-cartilage regeneration vs. degeneration, including the understanding of why the current techniques for cartilage repair give unpredictable results.

THE NORMAL KNEE JOINT



The knee joint organ consists of articulating surfaces each made up of specialized mineralized tissues covered by a layer of non-mineralized hyaline cartilage. Optimal load distribution within the hyaline cartilage and further into the underlying subchondral tissues is believed to be critical in the absorption of sheare and compressive forces without damaging the structures. Load distribution is determined by the configuration and smoothness of the joint surfaces, the elasticity of the cartilage and the underlying mineralized tissues, and the condition of the menisci. Also contributing to load distribution is the load and motion patterns – guided by bony alignment, ligament conditions and neuromuscular function.

CHARACTERISTICS OF HYALINE ARTICULAR CARTILAGE

The role of hyaline articular cartilage in the knee joint is twofold:

- Shock absorbing; distributing peak load within the chondral tissue and to the underlying mineralized tissues
- Facilitating low friction gliding motion between the joint surfaces; distributing decreased sheare forces to the hyaline cartilage itself and to the underlying mineralized tissues

The composition and architecture of normal hyaline cartilage tissue are well adapted to fulfill these two roles (Suh J-K et al. 1997). The main constituents of hyaline cartilage are the chondrocytes, the matrix and the interstitial water. The interstitial water comprises 60-80% of the cartilage wet weight, and its flow in and out of the solid permeable matrix and the joint cavity provides transport of nutritients to the cells and metabolites away from them (Tyyni and Karlsson 2000).

Chondrocytes

Chondrocytes are the cells responsible for the regulation of synthesis, degradation and remodelling and thereby the turnover of the hyaline cartilage. The cells are originally derived from mesenchymal stem cells, firmly embedded in the matrix they produce, showing low metabolic activity and a low rate of proliferation and turn over. In contrast to most other cells in the body the chondrocytes appear to have little if any direct cell-to-cell contact and they are organized in a tissue lacking direct blood supply and peripheral nerves. The extracellular matrix they produce is responsible for the biomechanical properties of the tissue (Archer and Francis-West 2003, Heinegard 2009). Although the regulation of matrix turnover is poorly understood, mechanical loading is believed to be an important factor (Tyyni and Karlsson 2000).

Glucose is the major energy source in chondrocytes (Archer and Francis-West 2003). Chondrocyte metabolism operates at low oxygen tension within the cartilage matrix, ranging from 10% oxygen tension at the surface to less than 1% in the deep zones. Chondrocytes constitute 2-5 % of the total volume of adult articular cartilage.

Matrix

The extracellular matrix constitutes more than 90% of the tissue volume in non-mineralized articular cartilage and is responsible for the mechanical properties of this most superficial part of the cartilage-bone unit. The matrix is produced by the chondrocytes and composed of macromolecules such as collagen, proteoglycans and non-collagenous proteins and glycoproteins. The collagen network consists mainly of type II collagen fibrils. Other collagen types are type VI, IX, X and XI collagens (Tyyni and Karlsson 2000). The role of the different collagens are not quite clear, but the collagen type IX and XI are believed to interact with collagen type II in building up the collagen meshwork, whereas type VI collagen has been shown to play an important role in the narrow layer of pericellular matrix encapsulating the chondrocytes contributing in their physiology (Alexopoulos et al. 2009).

Several proteoglycan monomers may link to hyaluronic acid (HA) – which is also synthesized and secreted by the chondrocytes – to form large proteoglycan aggregates. The proteoglycan monomers contain a core protein to which several glucosaminoglycans (GAGs) are attached. Chondroitin sulphate and keratin sulphate are the main GAGs. The sulphate groups are negatively charged and thereby tend to repel one another – making the proteglycans swell.

The tendency of distention exerts tensile stress on the collagen meshwork. Furthermore, high consentration of negative charges in the proteoglycans make them hydrophilic – creating an osmotic pressure gradient which promotes fluid flow into the tissue. The degree of hydration in the hyaline cartilage is thereby dependent on this osmotic swelling pressure constituted by the large proteoglycan aggregates opposed by the restrictive tensile forces of the collagen network. In this way, the proteoglycans together with the collagen give the hyaline cartilage its elastisity and resilience (Goldring 2006), and failure in the function of either one may influence on cartilage stiffness and interstitial fluid flow. Compressive loading normally causes extrusion of matrix fluid into the joint cavity as the cartilage is compressed, unloading results in intrusion of synovial fluid into the matrix as the proteoglycans expand the collagen network and thereby reforms the cartilage. This intrusion and extrusion of synovial fluid provides the main route for nutrition of the cartilage, although some nutrition may take place by transportation through the permeable calcified cartilage and tidemark as well.
A large number of other macromolecules, including small proteoglycans and other non-collagenous proteins contribute to the properties of the matrix as well (Heinegard 2009). Once the cartilage is established in the adult, the chondrocytes maintain a low turnover rate of cartilage matrix collagen, GAGs and other cartilage matrix constituents. There are regional differences, and matrix turnover is more rapid in the immediate pericellular zones.

Structure of the non-calcified hyaline articular cartilage

The characteristics of the non-calcified articular cartilage vary from the surface down to the calcified cartilage – being distinguished as different zones (Tyyni and Karlsson 2000). In the most superficial zone, the collagen fibers are aligned parallel to the joint surface. The cells are flattened in the same direction, and the matrix is low in proteoglycan concentration. The organization of the superficial zone allows low friction motion (enhanced by lubrication of the synovial fluid) combined with the ability to withstand the potential damaging effect of sheare forces. In the transitional layer, or middle zone, the collagen fibers are more obliquely oriented and primarily resist compressive forces but also transmit sheare forces to compressive forces further distributing them to the deeper layers. The chondrocytes are more oval in shape and the matrix contains more proteoglycans than in the superficial layer. In the deep zone the collagen fibers are oriented perpendicular to the articular surface mainly in order to resist compressive loads. The chondrocytes are organized in radial columns and the

matrix of this zone has the highest concentration of proteoglycans. The zones are illustrated in figure 1.

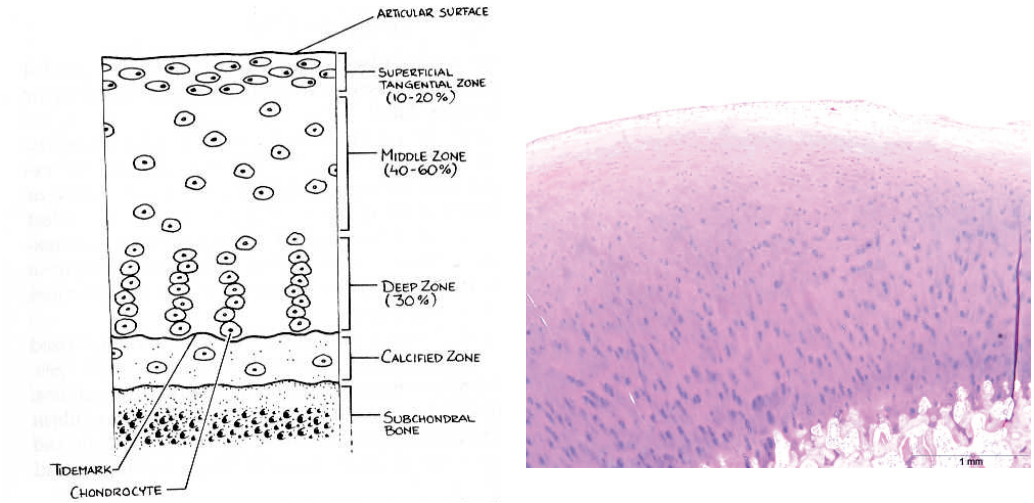


Figure 1. The zones of the articular cartilage. **Left:** Drawing copied from Tyyni A and Karlsson J (Tyyni and Karlsson 2000), **Right:** Histological section of human knee cartilage stained with Hematoxylin-Eosin (from Sverre Løken 2009, personal communication).

CHARACTERISTICS OF SUBCHONDRAL MINERALIZED TISSUES

The roles of the subchondral mineralized tissues are several:

- Firmly attaching the non-calcified chondral layer to the underlying bone
- Mechanically supporting the non-calcified cartilage layer
- Providing shock absorption

The composition and organization of the normal tissues are well adapted to meet the requirements demanded.

Tidemark

The interface between the non-calcified articular hyaline cartilage and the underlying calcified cartilage is referred to as the tidemark (Fawns and Landells 1953) (Figure 1). Although the composition, organization and function of the tidemark is still incompletely understood (Arkill and Winlove 2008), it is described having a trilaminar appearance, being 3-10 μm thick, containing no cells (Lyons et al. 2005). It makes a sharp boundary on Hematoxylin-Eosin (H&E) stained histological sections; it is rich in collagen and contains hyaluronan, but appears to lack the GAGs of regular proteoglycans. The chemical composition diverges, in other words, from the matrices of which it delineates. It is believed that chondrocytes in close apposition to the tidemark produces the components of which the tidemark consists (Lyons et al. 2005). During growth the tidemark is believed to represent a calcification front, at which the non-calcified cartilage is mineralized with hydroxyapatite. The mineralization process is regulated by a number of matrix proteins and the tidemark represents the proteins left after the decalicification process. In the adult joint however, the function of the tidemark is believed to change to rather protect the articular cartilage from progressive mineralization (Lyons et al. 2005, Shibakawa et al. 2005). In osteoarthritis the tidemark commonly becomes replicated, proposed being a sign of the underlying osteoarthritic process with the renewed calcification front advancing into the noncalcified cartilage.

Calcified cartilage

Between the non-calcified articular hyaline cartilage and the underlying bone is the layer of calcified cartilage. Some authors include the calcified cartilage as being one of the zones of the articular cartilage (Tyyni and Karlsson 2000), whereas others define the calcified cartilage as part of the subchondral bone plate (Shibakawa et al. 2005) generally referred to as the mineralized tissues. Some authors emphasize the importance of distinguishing the three different mineralized tissues in the joint from one another – the calcified cartilage, the subchondral bone plate and the subchondral trabecular bone – due to their differences not only morphologically, but also physiologically and mechanically (Burr 2004).
The calcified cartilage is characterized by the extracellular matrix being calcified. The cells are smaller than those of the deep layer of the non-calcified cartilage and contain only a few organelles (Tyyni and Karlsson 2000) indicating low metabolic activity. The tissue is characterized as avascular, but vascular channels are seen within the tissue even in non-

degenerative joints (Shibakawa et al. 2005). At the tidemark there is a process of ongoing endochondral mineralization that may cause the calcified cartilage to thicken. Such thickening contributes to increased stiffness of the subchondral tissues and may be viewed as subchondral sclerosis on radiographs (Burr 2004). The boundary towards the underlying subchondral bone plate is irregular providing a large surface and thereby strong integration, whereas the interface towards the overlying non-calcified cartilage (tidemark) is smooth and may be more vulnerable to delamination. However, in addition to constituting a stiffness gradient towards the subchondral bone, the calcified cartilage is believed to provide adhesive properties.

Subchondral bone plate

Deep to the calcified cartilage is the subchondral bone plate which is compact, i.e. the porosity of the bone is low. It consists of lamellar bone layers and constitutes support for the overlying cartilage. The subchondral bone plate may change its density by remodeling, but may also thicken through direct apposition of bone – in both instances contributing to increased stiffness of the subchondral tissues and subchondral sclerosis as observed on radiographs (Burr 2004).

Cancellous trabecular bone and bone marrow elements

The subchondral trabecular bone differs architecturally from the subchondral bone plate. It is anisotropic i.e. the trabeculae are oriented in different directions. Thus, mechanical properties are different in different planes. Due to the elastic properties by axial compression it is believed that the subchondral trabecular bone contributes substantially to axial compressive load absorption. The bone marrow within the trabecular bone includes multipotent mesenchymal stem cells (MSCs), growth factors and vascular elements taking part in subchondral remodeling and also potentially in osteochondral repair.
MSCs are multipotent cells that may differentiate along several cell lines, thus being the source of various cells, e.g. chondrocytes, osteoblasts, fibroblasts and bone marrow stromal cells (Tuan et al. 2003). Since the MSCs constitute an available cell source, and the MSCs theoretically have the advantage of being able to proliferate without loosing their ability to differentiate into mature chondrocytes (Tuan et al. 2003), bone marrow stimulating

techniques for cartilage repair utilize the properties of MSCs to produce cartilage repair tissue. Besides, MSCs may induce repair of both the mineralized tissues and the non-calcified cartilage in an osteochondral defect (Wakitani and Yamamoto 2002, Yan and Yu 2007).

Subchondral vascular channels

Subchondral vascular channels, also termed “microcracks” or “resorption pits” (Shibakawa et al. 2005), are extensions of vascular tissue from the bone marrow towards the articular cartilage. The vascular channels are present in the subchondral bone plate and in the calcified cartilage of knees without OA, whereas protrusion into the uncalcified cartilage is associated with degenerative OA (Shibakawa et al. 2005). Normally, the vascular channels are considered to take part in the remodeling of subchondral bone, but also possibly in the nutrition of the articular cartilage (Shibakawa et al. 2005).

INFLUENCE OF LOAD ON ARTICULAR CARTILAGE

Although the mechanisms of chondrocyte regulation of matrix synthesis and turnover are poorly understood, mechanical loading is believed to be an important factor in the homeostasis of the hyaline articular cartilage (Tyyni and Karlsson 2000). The hyaline articular cartilage is well adapted to physiological intermittent and gradually increasing compressive loads. As compressive load increases, the pore sizes of the matrix become smaller resulting in a decrease of water flow permeability, which again prevents the interstitial water from being squeezed out of the macromolecular framework too rapidly. On the other hand, if the cartilage is exposed to a sudden impact, the interstitial water does not have time to flow through the matrix framework and thereby contribute to the elastisity of the tissue. Consequently, the load may hit the tissue as in a solid non-elastic phase causing damage to the collagen framework, the cells and eventually the subchondral mineralized tissues (Tyyni and Karlsson 2000). If sheare forces are involved, delamination at a certain level in the cartilage-bone unit may occur.

The effect of load over time is less well understood. There is good evidence that joint cartilage will undergo atrophy (thinning) under reduced loading, such as postoperative immobilization and paraplegia (Vanwanseele et al. 2002). On the other hand, whether the articular cartilage will become thicker after increased load such as weight training or running

is controversial (Kiviranta et al. 1988, Newton et al. 1997). The effect of load and time with regards to the initiation of joint degeneration is not fully understood either.

OSTEOARTHRITIS – END STAGE OF JOINT DISORDERS

Osteoarthritis (OA) is the end stage of degenerative joint disease due to a variety of etiological factors. OA is characterized by a progressive degenerative destruction of the joint organ most often associated with gradually increasing pain, impairment of range of motion, bony deformation, and malalignment and by all this – impaired joint function. The term "primary" or "idiopathic" OA is used when the etiology is unknown – in contrast to secondary or posttraumatic OA following identified knee disorders.
Changes in the cartilage-bone unit morphology are associated with OA (Burr 2004). However, the role of the different changes, the level at which each may be critical and their relative contribution in the initiation and early progression of OA are still not well understood.

RISK FACTORS OF OA

There is good evidence that diseases such as the chronic inflammatory joint diseases and arthritis caused by purulent infections increase the risk of OA. Moreover, in a prospective study carried out in Finland with 22 years follow up Toivanen et al. (2010) found that body mass index (BMI) >30 increases the risk of OA 6.8 times, physical strenuous work in category 6 (1-6) increases the risk 18.3 times, whereas any injury to the knee increases the risk of OA 5.1 times. Buckwalter (2003) claims that sports with high impact and sheare forces increases the risk of OA, however the evidence for that is hard to interpret. The controversies regarding sports and OA include questions whether increased risk of OA is due to extensive supra-physiological loads over time, to initial traumas to the cartilage-bone unit or to the consequence of alterations in biomechanics following trauma. Several experimental models for OA have been proposed; anterior cruciate ligament (ACL) resection in the knee of rabbits or dogs, meniscal resection in the knee of rabbits, and creation of focal cartilage lesions in the knee of dogs (Sniekers et al. 2008). Although the pathomechanisms are unclear, the experimental OA models support the evidence that alterations in knee biomechanics and cartilage load distribution increase the risk of OA.

FOCAL CARTILAGE LESIONS

Pathological changes in the cartilage may be termed "focal" when the pathology is limited to a well defined area of the joint surface and the surrounding cartilage is considered normal or nearly normal. In the literature the terms "injury", "lesion" and "defect" are used without distinction, and therefore do not comprise an etiological explanation. In this thesis "cartilage lesion" is used as a general term covering all changes in the cartilage surface seen macroscopically, whereas the term "cartilage defect" is reserved for an area of the articular surface lacking cartilage substance. The term cartilage injury indicates a possible traumatic etiology in contrast to for instance osteochondritis dissecans (OCD) which is a lesion as long as the fragment is *in situ*, advancing to a cartilage defect whenever the fragment comes loose. Focal cartilage lesions may be present in one or more joint surfaces and compartments, whenever opposing each other termed kissing lesions. Some authors use the term "focal degenerative lesion", however, in the sense of discussing cartilage repair, degenerative changes should probably be distinguished from well defined focal cartilage lesions primarily due to other than local aggravation of general degeneration.
This thesis focuses on persistent focal cartilage defects due to chondral/osteochondral delamination/detachment for reasons such as osteochondritis dissecans or trauma.

OSTEOCHONDRITIS DISSECANS (OCD)

Osteochondritis dissecans (OCD) is primarily a condition affecting the subchondral bone and secondly the articular cartilage. OCD seldom presents in patients below 10 and above 50 years of age (Linden 1976). Males (Bohndorf 1998) and physically active persons (Aichroth 1971) show higher prevalence. The knee is the most often affected joint; in 80% of the cases the OCD is located on the lateral aspect of the medial femoral condyle. Fifty percent of patients having knee OCD show bilateral manifestations. If the lesions do not heal the bony part will gradually detach from the underlying bone and eventually the overlying cartilage will separate from the surrounding cartilage. Finally, the fragment may detach completely and become one or more loose bodies in the joint cavity leaving a focal osteochondral defect in the joint surface – the osseous floor and walls of the defect being sclerotic resembling the subchondral bone plate.

Classifications of OCD

OCD is classified as juvenile (J-OCD) or adult (A-OCD) depending on the time of presentation in relation to the closure of the epiphyseal growth plate. The condition can be graded from radiographs (Milgram 1978), arthroscopically (Guhl 1979), or by MRI findings (Nelson et al. 1990). The arthroscopic and MRI classifications have been shown to be well correlated (O'Connor et al. 2002).

Natural history of OCD

The natural history of J-OCD is different from A-OCD. Linden observed that patients diagnosed with J-OCD seldom developed OA, whereas 80 % of patients with A-OCD developed OA during a 30-year observational period (Linden 1977). Peterson et al reported mean 7.8 years persistent symptoms from defects following OCD detachment in patients at the time of surgical cartilage repair (Peterson et al. 2003), indicating non-healing in long term perspectives. On the other hand, stable OCD in skeletally immature patients will heal in more than 90 % of the cases without surgical intervention (Williams et al. 1998). For patients close to, or passed epiphyseal closure, the prognosis is poor (Williams et al. 1998). Most A-OCDs are probably unhealed J-OCD, but OCD development after closure of the growth plates has been reported (Garrett 1991).

Treatment of OCD (intact fragment)

Skeletally immature patients are treated non-surgically with restricted weight-bearing and modifications of activity in accordance to symptoms and MRI findings. Surgical intervention may be applied if conservative treatment fails or if the patient is close to skeletal maturity or older. Healing of the fragment is promoted, either by drilling (Kocher et al. 2006) or by fixation of the fragment (Williams et al. 1998).

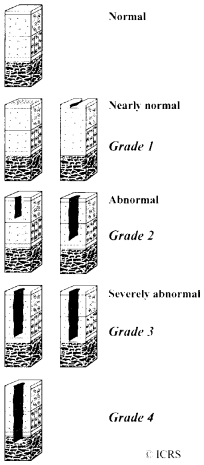
FOCAL CARTILAGE DEFECTS

Focal cartilage defects may be caused by detachment of OCD fragments or by traumatic events and are commonly seen in combination with ACL injuries (Granan et al. 2009,

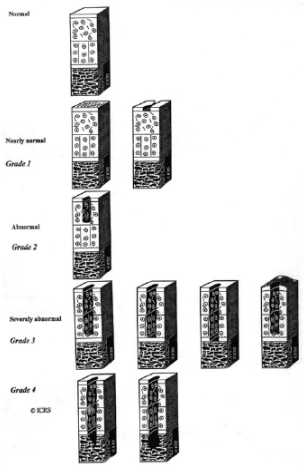
Shelbourne et al. 2003). Cartilage injuries of the patella and lateral femoral condyle may be caused by patellar dislocation (Elias et al. 2002) and frequently include a subchondral bone fragment. In many cases the cause of the focal cartilage defect is unknown and the lesion is discovered at arthroscopy or by MRI. Localized chondral or osteochondral defects with sharp edges and normal surrounding cartilage may be regarded as traumatic defects or sequela following detachment of an OCD fragment.

Classification of focal cartilage defects

Over the years, several classification systems have been presented to describe the extent of cartilage damage observed by inspection of the articular surfaces. The Outerbridge classification (Outerbridge 1961) was originally developed to classify chondromalacia of the patella. With increasing knowledge of cartilage and cartilage injuries, obvious limitations with this and other systems were recognized (Noyes and Stabler 1989). With the classification system published by Noyes and Stabler in 1989, the extent of involvement regarding the depth of the lesion was emphasized. However, the grading was still somewhat qualitative. Through recent years most researchers involved in focal cartilage defects and their repair have replaced other classification systems by the International Cartilage Repair Society (ICRS) classification system first introduced in 1998 (Brittberg and Peterson 1998) and later revised in 2003 (Brittberg and Winalski 2003) (fig 2a and b).



2a



2b

Figure 2a: ICRS classification system of depth of cartilage injuries introduced in 1998 (Brittberg and Peterson 1998) used in study II included in this thesis. Grade 1: nearly normal (superficial fissuring). Grade 2: abnormal (deep fissures/defect, but not down to bone). Grade 3: severely abnormal (fissures/defect down to bone). Grade 4: severely abnormal (fissures/defect extending into the subchondral bone).

Figure 2b: The revised version comprises three main differences from the former: Defects protruding down to – but not into the calcified layer (grade 3b) are distinguished from those involving the calcified layer (grade 3c). Secondly, grade 2 lesions are limited to involvement of less than 50% of the cartilage thickness. Third, blisters are defined as a separate subgroup (grade 3d) (Brittberg and Winalski 2003)

Prevalence of focal cartilage defects

In a study registering cartilage pathology in 993 consecutive knee arthroscopies by our group, focal cartilage defects were found in 20% of the knees, whereas a defect ICRS grade 3-4, exceeding 2 cm^2 in size, was found in 6 % of the knees (Aroen et al. 2004). The findings were in agreement with those of Hjelle et al. (2002) disclosing an ICRS grade 3-4 defect of more than 1 cm^2 in 7 % of the patients below 50 years of age. In a study of 25.124 knee arthroscopies similar findings were reported showing localized ICRS grade 3-4 defects in 9 % of patients below 50 years of age (Widuchowski et al. 2007). In a US database of 31.516 arthroscopies (Curl et al. 1997) Outerbridge grade 4 lesions, i.e. lesions extending through the non-calcified cartilage, where reported in 20 % of the patients and in 5 % of patients below 40 years of age. The prevalence of focal cartilage defects has also been investigated using MRI. In a cross sectional MRI study the prevalence of cartilage defects was 31 % in individuals below and 54 % in those above the age of 45 (Ding et al. 2005). A high prevalence in asymptomatic high level basketball players has also been reported with 31 MRI detected defects in 19 of 40 players (Kaplan et al. 2005). However, the MRI studies also report small defects that may not be clinically relevant. Furthermore, the sensitivity of the MRI may play a role; in a study using 1.0 Tesla MRI cartilage defects were found in 3 out of 54 asymptomatic subjects (age 19-39) (LaPrade et al. 1994), whereas in a recent study using 3.0 Tesla MRI 9 out of 20 asymptomatic subjects (age 25-45) presented cartilage defects (Stahl et al. 2009). Focal cartilage defects may frequently be found in combination with other knee injuries. Granan and coworkers reported focal cartilage defects in 26% of ACL reconstructed patients (Granan et al. 2009). Data from the Norwegian National Cruciate Ligament Registry further demonstrate increasing prevalence of cartilage and meniscal injuries with increasing time from injury to reconstruction (Granan et al. 2009).

Clinical presentation of focal cartilage defects

Focal cartilage defects in the knee may cause pain and limitations in activities of daily living, working ability, sport and recreational activities and impair the patient's quality of life. In addition to pain, the patients may experience swelling and mechanical symptoms such as snapping, locking and even instability (Aarseth L et al. 1999). Frequently a cartilage defect in the

knee is diagnosed in combination with ACL or meniscal injuries (Granan et al. 2009, Shelbourne et al. 2003), and the symptoms may be hard to distinguish from these accompanying disorders. The symptoms may present in connection with an acute injury, but the symptoms may as well start vaguely, increasing with time finally making the patient seek medical help. Patients undergoing cartilage surgery present with lower preoperative Lysholm score compared to patients undergoing ACL reconstruction (Aarseth L et al. 1999). On the other hand, in some cases focal cartilage defects are asymptomatic (Kaplan et al. 2005). The great variance in the types and degree of symptoms – and the lack of comparative data on these patients' complaints, makes it hard to accept the severe complaints some of these patients actually do have. Besides, the reasons for the variance in clinical presentation – and the factors influencing it – are poorly understood.

Natural history of focal cartilage defects

The natural history of focal cartilage defects is largely unknown. Thus, it is not known to what extent a focal cartilage injury leads to OA – or what factors that may predict such a progression. A favorable outcome was reported in a 14-year follow up study on 28 young patients with isolated Outerbridge grade III or IV chondral defects due to trauma or OCD (Messner and Gillquist 1996). Ten of the patients received no treatment, three patients underwent Pridie drilling and the rest either shaving or removal of loose bodies. Twenty-two of the patients showed excellent or good results according to Lysholm score. Except for the study of Linden on OCD (Linden 1977), this is the only study with long term follow up on isolated focal cartilage defects. On the other hand, patients scheduled for surgical cartilage repair are reported to have persistent symptoms for a prolonged period of time (Knutsen et al. 2004, Solheim et al. 2010). From studies on ACL-reconstructed patients with concomitant cartilage lesions some information is available: Shelbourne et al (2003) found that ACL reconstructed patients with a focal cartilage injury exhibited equal functional results as ACL reconstructed patients without such injury after 8.7 years. These findings are supported by data from the Norwegian Cruciate Ligament Registry showing no difference in preoperative Knee injury and Osteoarthritis Outcome Score (KOOS) in ACL patients with or without cartilage injury (Hjermundrud et al. 2010). On the other hand, Drogset et al reported that patients with a cartilage injury detected at the time of ACL reconstruction were more likely to develop OA during an 8-year follow up than patients without

concomitant cartilage injury (Drogset and Grontvedt 2002). According to animal studies, the natural history of focal cartilage defects may be influenced by the depth and the size of the defect, and the age of the individual (Tyyni and Karlsson 2000).

Pain from focal cartilage defects

The reason(s) for pain associated with cartilage pathology is still poorly understood. The non-calcified articular cartilage contains no peripheral nerves or neural elements (Tyyni and Karlsson 2000). The nociceptors of the subchondral bone and soft tissues of the knee joint, however, consist of free nerve endings which are sensitive to both mechanical and chemical stimuli. As for OA there is a poor relationship between the patients' symptoms and the clinical and macroscopic findings. Thus, the pain experienced is believed to be related to the subchondral bone or synovial membrane rather than the lesion within the cartilage tissue (Tyyni and Karlsson 2000).

Spontaneous healing of connective tissue injuries in general

The basic response to injury at the tissue level in general consists of three phases:

1. Inflammatory phase (0-7 days)
2. Proliferative phase (5-21 days)
3. Maturation and remodeling phase (14 days - months)

The inflammatory phase is initially a vascular, cellular and chemical mediated cascade reaction providing a wide supplement of different mediators needed for initiating a repair process; inflammatory cells, interleukins, cytokines, growth factors and MSCs. The initial target is the clot formed by the fibrin, blood cells and platelets deposited at the injury site. Clinically the acute inflammatory phase is characterized by dolor, calor, rubor, tumor and functio lesae.
The inflammatory phase is gradually progressing into the proliferative phase, characterized by clearance of necrotic tissue at the injury site and the evolvement of granulation tissue. This process includes migration of cells, phagocytosis and the proliferation of fibroblasts, synthesis and secretion of matrix together with neovascularization. Proliferation and migration is enhanced by the stimuli of growth factors released from blood platelets and macrophages.

The maturation and remodeling phase is overlapping the proliferation phase. There is a gradual reduction in proteoglycan and water content in the tissue. The density of vessels decreases and there is a normalization of the orientation of the collagen tissue over time.
The granulation tissue initially present will gradually convert to a more host-specific connective tissue – over time differentiating and maturing to tissue quite similar to the surrounding host tissue – being closely integrated.

Spontaneous healing of focal cartilage defects

The term "cartilage defects" includes both pure chondral and osteochondral defects. In regards to healing, some distinguishing between the two types of defects ought to be made (Hunziker 1999). Pure chondral defects may histologically extend down to the tidemark, the interface between the calcified cartilage and the non-calcified articular cartilage. This interface seems vulnerable to delamination; the non-calcified articular cartilage being pealed off the underlying calcified tissue, causing an ICRS grade 3b lesion (Brittberg and Winalski 2003). Since the non-calcified articular cartilage contains no peripheral nerves or vessels, no harm is done to neural or vascular tissue, hence no vascular mediated healing response is initiated. Although the vascular mediated response is believed to be the main contributor in the acute inflammatory process seen in spontaneous connective tissue healing in general, chemotaxis by local cells is always operative in tissue injury and may promote a local inflammatory response initiating some healing. Thus, the chondrocytes are believed to be able to restore minor damage to the matrix components, however, the extent of restorational capability is not clear (Buckwalter 1998) but generally believed to be small. On the other hand, some defects persist with no symptoms, indicating that partial healing may be sufficient and/or nearby tissue is compensating for functional loss due to the defect (Kaplan et al. 2005). Whether partially healed asymptomatic defects have less risk of developing OA than symptomatic ones are not known.
The spontaneous healing of acute osteochondral injuries, on the other hand, is well described by Shapiro and coworkers (Shapiro et al. 1993). Acute osteochondral defects penetrating the mineralized tissues may present a different healing pattern than pure chondral defects due to access to blood and other bone marrow elements. Some authors even claim that experimentally induced lesions penetrating the subchondral mineralized tissues heal, whereas pure chondral

lesions do not (Hunziker 1999). However, the spontaneous repair tissue of osteochondral defects has been shown to degenerate with time (Shapiro et al. 1993), even in young individuals with immature cartilage (Wei et al. 1997).

The tissue types obtained through spontaneous healing are mainly fibrous tissue and fibrocartilage. The fibrocartilage differs from hyaline cartilage in several aspects: The dominating collagen is type 1 in contrast to collagen type 2 in hyaline cartilage (Mandelbaum et al. 1998). The collagen orientation is random in contrast to hyaline cartilage which has specific orientation (Kaab et al. 1998) and thickness (Hedlund et al. 1993) of the different layers. The shape of the cells diverge from normal chondrocytes being similar to fibroblasts.

TREATMENT OF FOCAL CARTILAGE DEFECTS

The purpose of treating patients with persistent cartilage defects may be two-fold:

I. Diminishing symptoms and thereby restitute good knee function.

II. Minimizing the risk of long term complications such as development of OA.

The best treatment for both these purposes would probably be a method resulting in full regeneration of the injured cartilage-bone unit. However, as of today, no such treatment exists. Moreover, no treatment has so far proved to reduce the risk of OA. Therefore, the different treatment modalities currently offered are mainly aimed towards reduction of symptoms – the long term effects still being uncertain. Unfortunately, even the short- and midterm effect regarding symptom relief is unpredictable (Jakobsen et al. 2005).

However, since there is strong evidence of certain risk factors for development of OA in general (Toivanen et al. 2010), the first step should probably be to reduce these risk factors; i.e. avoiding or reducing overweight, avoiding longstanding heavy load during work and preventing additional knee injuries. According to Buckwalter these patients should also avoid activities which include high impact and torsional loads (Buckwalter 2003).

NON-SURGICAL TREATMENT OF FOCAL CARTILAGE DEFECTS

Physical exercise

The effect of strength exercises and other training modalities have not to our knowledge been investigated in patients with focal cartilage defects. However, there is some knowledge regarding OA patients. In a recent Cochrane report the authors conclude that there is at least a short term benefit from exercising in terms of reduced knee pain and improved physical function for patients with knee OA. The magnitude of the treatment effect is small, but comparable to the effect of non-steroid anti-inflammatory drugs (Fransen and McConnell 2008). In an ongoing randomized controlled trial (RCT) concerning surgical techniques of cartilage repair, patients undergo 3 months physical strength training before surgery. For the time being, the majority of patients have improved their subjective knee function to such an extent that they want to postpone surgery (Årøen 2010, personal communication).

Systemic medication

The major symptom of patients with a cartilage defect in their knee seeking medical help is pain. Pain is often treated with analgesic or non steroid anti-inflammatory drugs (NSAIDs). Glucosaminoglycans and chondroitin sulphate have been introduced as possible modulators of OA. Since one of the aims of treating cartilage defects is to avoid the initiation and progression of OA, these drugs are prescribed also to patients with focal cartilage defects. Regarding OA, a metaanalysis have concluded that there was no effect from chondroitin sulphate alone on pain and function (Reichenbach et al. 2007), whereas a Cochrane report concludes that there is a possible effect of glucosamine sulphate, but only for one particular brand, and no effect of glucosamine hydrochloride (Towheed et al. 2005). The effect of these drugs on the course of focal cartilage defects is, however, still unknown.

Intra-articular injections

Intraarticular injections with corticosteroids have traditionally been used to treat the synovitis that often accompanies the OA. Hyaluronic acid (HA) products have also been introduced for intra-

articular injections in the treatment of OA, so-called viscosupplementation. In a metaanalysis the authors concluded that such viscosupplementation had a moderate to large effect compared to placebo with maximum effect 5-13 weeks after the injection. The effect was comparable to NSAIDs and intraarticular effect of corticosteroids (Bellamy et al. 2006). Whether viscosupplementation has any symptomatic effect on focal cartilage defects in patients is unknown. Rabbit experiments have shown that hyalorunan injections may improve the repair of osteochondral defects (Miyakoshi et al. 2005) and repair after microfracture (Strauss et al. 2009). Moreover, hyaluronan viscosupplementation following osteochondral autografting showed beneficial affects on graft cartilage in an ovine model (Tytherleigh-Strong et al. 2005).

SURGICAL TREATMENT OF FOCAL CARTILAGE DEFECTS

The spectrum of surgical alternatives for treating articular cartilage defects range from simple lavage and debridement to replacement of the knee joint surfaces. Choice of treatment depends on multiple factors: the patient's symptoms and complaints, the number of defects, the location, size and depth of the defects, the degree of accompanying OA and the age of the patient. The etiology of the defect and the desired level of activity also need to be taken into consideration when selecting a specific therapy.

The surgical treatment options can be divided into three main categories:

I. Symptomatic treatment
II. Cartilage repair
    a. Bone marrow stimulating techniques
    b. Transplantation of osteochondral grafts
    c. Induction of chondrogenesis
        i. Soft tissue grafting
        ii. Cell mediated techniques
III. Joint surface replacement

Symptomatic treatment (Lavage and debridement)

One of the most basic and traditional methods of treating articular cartilage injuries is lavage (Jackson 1974). The effect is suggested to be due to removal of articular debris and inflammatory mediators known to be generated by the synovial lining of damaged joints (Jackson and Dieterichs 2003). The reduction of pain is, however, short-term and the underlying pathology is not addressed.

Debridement is an arthroscopic surgical technique used to remove cartilaginous loose flaps/fragments, osteophytes and loose bodies that may cause mechanical symptoms. Synovium may be trimmed or removed if it is hypertrophic and interferes with joint motion. Symptomatic relief from debridement has been reported (Jackson and Dieterichs 2003). However, the effect of both lavage and debridement has been questioned, the doubt being supported by the results of two randomized controlled trials in which arthroscopic debridement was compared to sham operation in the treatment of OA. There was no difference between the groups (Kirkley et al. 2008, Moseley et al. 2002). Thus, at least in OA the effect of debridement seems primarily to be a placebo effect. On the other hand, a focal cartilage lesion is not a general joint disease, and based on the current knowledge, arthroscopic debridement with removal of loose chondral flaps may diminish mechanical symptoms and be justified as a first-line therapy before more extensive procedures are performed. In addition, the procedure may provide valuable diagnostic information.

II Cartilage repair

II a. Bone marrow stimulating techniques

The bone marrow stimulating techniques are based on the principle of establishing access to bone marrow elements in an attemt to improve cartilage repair. Abrasion arthroplasty, spongialization, drilling and microfracture technique have in common that they cause hemorrhage and fibrin clot formation in a contained focal cartilage defect. Bone marrow elements such as mesenchymal stem cells (MSCs), leukocytes and growth factors are believed to contribute to remodeling the

fibrin clot into fibrocartilaginous repair tissue. Ossification of the areas deepest in the defect may then occur, while the rest of the primary repair tissue gradually is transformed into fibrocartilage (Breinan et al. 2001, Frisbie et al. 1999, Shapiro et al. 1993).

A major concern with the bone marrow stimulating techniques is the mechanical and biochemical properties of the fibrocartilage repair tissue and the durability of the tissue to withstand degeneration. Mow et al. (1991) refers to fibrocartilage as being an inherently weak tissue which is unorganized in respect to direction of load and poorly integrated with adjacent tissue. On the other hand, in an average 11 years follow up study patients with traumatic full-thickness chondral defects treated with microfracture technique reported significant improvement of both Lysholm and Tegner scores (Steadman et al. 2003).

Another concern with methods involving injury to the subchondral mineralized tissues is what effect the procedure will have on the properties of those tissues. If the subchondral bone is traumatized, the mineralized tissues may remodel and alter stiffness (Radin and Rose 1986). Although the implications of such changes in relation to bone marrow stimulating techniques for cartilage repair have not been thoroughly investigated, changes in the subchondral mineralized tissues have been associated with the initiation and progression of OA (Burr 2004). However, there are no objective criteria as to what changes indicate early OA (Sniekers et al. 2008). Furthermore, both human and experimental work show variable alterations in bone parameters such as bone volume fraction and trabecular thickness in association with OA. In some studies these parameters are increased whereas in others they are decreased. Some authors even suggest a two phase occurrence of these bone parameters; an initial decrease followed by an increase (Sniekers et al. 2008).

II a.1. Drilling

In 1959 Pridie introduced drilling as a method for resurfacing what he called osteoarthritic knee joints (Pridie KH 1959). Multiple holes were drilled through the subchondral bone plate into the trabecular bone to initiate the formation of a repair tissue. Symptomatic improvement by pain relief was reported by a number of investigators following this procedure (Dzioba 1988, Insall 1967). For the treatment of chondral defects in the knee the procedure has lost interest during recent years due to the introduction of other techniques considered more promising. However, no randomized studies have included drilling as a treatment option.

II a.2. Abrasion Arthroplasty

Abrasion arthroplasty involves debriding both the edges and the surface of the articular cartilage defect. By stabilizing the edges, mechanical symtoms may be prevented and containment of the defects is established. The surface of the subchondral mineralized tissues is exposed by using a 1-2 mm motorized burr. The idea is to keep most of the subchondral bone plate intact, but advancing deep enough to induce bleeding. Abrasion arthroplasty thereby creates a defect corresponding to ICRS grade 3c (Brittberg and Winalski 2003). Clinically, Johnson (1986) reported a success rate of 77% in 95 patients after a two year follow-up. Compared to arthroscopic debridement alone, however, inferior results were reported (Bert and Maschka 1989). In a randomized study the abrasion technique was compared to matrix-induced autologous chondrocyte implantation (MACI) yielding significantly different Lysholm scores of 74 and 86 respectively (Visna et al. 2004).

II a.3. Spongialization

Another technique of getting access to subchondral bone marrow elements was introduced by Ficat et al. (1979); a technique called spongialization. This technique includes removal of the entire subchondral bone plate from the underlying cancellous bone, corresponding to ICRS grade 4 (Brittberg and Winalski 2003). Ficat reported 79 % success rate with two years follow-up, however similar results have not been reproduced by others.

II a.4. Microfracture technique

The microfracture technique (Mfx) was introduced by Steadman in 1997 and is similar to drilling in the manner of producing channels through the subchondral bone plate establishing access to the bone marrow elements (Steadman et al. 1997). However, instead of drilling, the perforations are made by arthroscopically tapping an awl through the subchondral bone plate using a mallot. Multiple channels 3-4 mm apart are created within the defect. The importance of creating a contained defect with stable and right-angled edges prior to the perforations is emphasized (Steadman et al. 2001, 2002). By reducing the water induced intraarticular pressure, blood and fat pearls are supposed to present from the channels induced. After the procedure, the patient follows

a rehabilitation program including continuous passive motion (CPM) and restricted weight bearing to stimulate differentiation of the repair tissue into cartilage. Steadman claims that the advantage of Mfx compared to drilling is that the subchondral bone plate is largely preserved and the awls do not produce heat necrosis. So far, no clinical randomized studies comparing the two techniques have been published. In a meta-analysis the Mfx has been reported with a 95% confidence interval for Lysholm score between 78 and 97 (Jakobsen et al. 2005). In randomized controlled clinical trials the Mfx has similar results compared to autologous chondrocyte implantation (ACI) (Knutsen et al. 2004, 2007, Saris et al. 2008), and inferior results compared to mosaic plasty (Gudas et al. 2005, 2009).

Today, Mfx is often used as a primary treatment option, and if not successful, more extensive cartilage repair methods are performed at a later stage. There has been a concern with the Mfx whether it can influence the results of future secondary procedures. In a recent study patients primarily subjected to bone marrow stimulating procedures showed equal improvement following secondary ACI as patient who primarily had undergone debridement only (Zaslav et al. 2009). On the other hand, in another recent study previous bone marrow stimulating procedures were associated with less favorable outcome following ACI (Minas et al. 2009).

II b. Transplantation of osteochondral grafts

Transplantation of osteochondral grafts may be achieved by harvesting grafts either from cadaver knees or from areas in the patient's knee being prone to less load – either substituting the cartilage defect by one solitary graft, or by inserting multiple grafts of one or different sizes by press fit fixation in predrilled holes. The main proposed advantage of osteochondral grafting is the immediate restoration of the defect with articular hyaline cartilage.

II b.1. Osteochondral Allografts

The graft from the cadaver knee with cartilage attached is trimmed and press fitted into a prepared hole – or attached with screws. To ensure the congruence the size and shape of the allograft needs to be close to a perfect match. In addition the knee joint has to be stable and properly aligned. To obtain that, realignment procedures are frequently used to remove stress from the grafted area (Gross et al. 1975). There are two types of allografts in use - fresh or frozen. By using fresh allografts, defined as harvested less than 12 hours after death (Gross et al. 1975),

up to 25 years graft survival has been reported (Gross et al. 2008). Concerns regarding fresh allografts include the risk of immunological reactions and disease transmission. However, a 5-year follow up study reported no instances of tissue rejection (Langer et al. 1978). Frozen allografts have shown decreased cell viability with time and inferior results compared to fresh allografts (Branam and Johnson 2007).

II b.2. Osteochondral Autografts

The transplantation of multiple autologous osteochondral grafts to a chondral defect within the same knee was first reported by Matsusue et al. (1993). The mosaic plasty technique (Mos) was later introduced by Hangody and coworkers implying the use of circular osteochondral grafts of different sizes being placed in the defect in a mosaic pattern (Hangody et al. 1997). The defect is debrided, measured and the sizes of the osteochondral grafts planned. The grafts are harvested from the periphery of the proximal anterior medial and/or lateral femoral condyles and/or the area just above the intercondylar notch. The drill holes in the recipient defect are size-matched allowing press fit fixation of the grafts. It is a one step procedure that may either be performed by a mini invasive arthrotomy or arthroscopically in the cases of small defects on the femoral condyle. In some cases e.g. in treating patellofemoral defects, larger arthrotomies with luxation of the patella may be needed.

Several advantages were proposed by using this technique compared to other techniques for cartilage repair: As for other osteochondral transplantation techniques, the articular surface could be restored immediately in a one step procedure. By using multiple small (φ 2.8-8.0 mm) grafts harvested from less weight-bearing portions of the joint, previously described problems with osteochondral grafting related to restoration of the curvature of the joint surface, fitting and fixation of the transplant and creation of large donor site defects were supposed to be avoided. Other advantages compared to allografts are the elimination of concerns regarding rejection and transmission of diseases as well as grafts, although limited in amount, always being available. Some authors have reported good clinical results following Mos, both in case series (Hangody and Fules 2003, Hangody et al. 2010, Solheim et al. 2010) and in some RCTs (Gudas et al. 2005, Horas et al. 2003), whereas another RCT questioned if the method could be justified (Bentley et al. 2003). By repetitive measures, Solheim and coworkers found a deterioration of clinical outcome with time (Solheim et al. 2010). As an explanation for this the authors propose that there may still be unsolved problems related to the healing of both the grafts and the donor sites. The

concerns regarding donor site morbidity and failure of graft ingrowth are shared by other authors as well (Huntley et al. 2005, LaPrade and Botker 2004). Some authors report a negative correlation between clinical outcome and age, and the technique is not recommended for patients above 50 years of age based on a higher incidence of osteoporosis, osteoarthritis and reduced healing potential (Hangody and Fules 2003). Another limiting factor is the size of the donor area available. Although good results have been published in defects ranging in size up to 8.5 cm^2 (Hangody et al. 1998), the technique has more recently been recommended to be reserved for defects between 1 and 4 cm^2 (Hangody and Fules 2003). In a series of 831 patients with a long term follow up, good-to-excellent outcome was reported in 92% of femoral condyle defects, 87% of tibial defects and 79% of defects in the patellofemoral joint indicating that the intraarticular location may be a predictive factor for the outcome (Hangody and Fules 2003). By second-look arthroscopies in 23 patients with unsatisfying clinical results following Mos, Solheim et al found healing of all donor sites in all knees, however, loss of the cartilage cap was seen in 6 patients and new cartilage defects in another 6 patients. Furthermore, one case report and several experimental studies have shown persistent clefts between transplanted and recipient cartilage and change in subchondral stiffness (Kock et al. 2004, Lane et al. 2004). From experimental studies accuracy of graft delivery has been identified as a major determinate of success with this technique (Hangody and Fules 2003). Perpendicularity of the grafts to the recipient joint surface, adequate press fit fixation to insure sufficient stability, and the grafts not being left prone to the surrounding surface are some of the surgical tricks that are emphasized (Hangody 1997, Hurtig et al. 2001, Pearce et al. 2001). Faulty graft delivery has been associated with graft instability, graft subsidence, subchondral cyst formation and overgrowth of the grafts with fibrous tissue (Hangody 1997, Hurtig et al. 1998, 2001, von Rechenberg et al. 2003). Besides, the use of circular grafts initially leaves some denuded interstitial space between the grafts and the adjacent cartilage. These gaps are supposed to heal by cartilage flow and extrinsic repair (Hurtig et al. 2001). Previous studies suggest that this kind of repair is unreliable (Hurtig et al. 1998, 2001). Grafts left prone to the surrounding surface increase the risk of poor interstitial filling (Pearce et al. 2001). Lack of filling of the interstitial spaces with the clefts obvious apparent could represent a substantial reduction in total tissue filling. Grafts left prone to the surrounding surface also increases the risk for graft instability which again increases the risk of subchondral cyst formation (Pearce et al. 2001, von Rechenberg et al. 2003).

II c. Induction of chondrogenesis

II c.1. Soft tissue grafting

Treatment options with chondrogenic potential have been investigated both in animal models as well as in humans, two of them being perichondral and periosteal grafts.

II c.1.i Perichondral grafts

Up to the early nineties perichondral grafts were investigated as an interesting and promising source of cells with chondrogenic potential for cartilage repair (Homminga et al. 1990). Perichondrium has an outer layer of dense connective tissue and an inner layer of perichondral cells which may differentiate into chondrocytes. Following the use of rib perichondrium with the chondrogenic layer facing the joint cavity and securing the grafts in the defects with fibrin glue, complete filling in 27 of 30 knee defects was observed by arthroscopic examination 10 months following surgery (Homminga et al. 1990). However, another study performed by the same research group with identical treatment but a mean of 52 months follow up, reported satisfactory results in 38 % of the patients only (Bouwmeester et al. 1997). The latter results terminated the use of perichondral grafts (Tyyni and Karlsson 2000).

II c.1.ii Periosteal grafts

Similar to the perichondrium, the periosteum consists of an outer layer of connective tissue and an inner cellular layer (cambium layer). The cells are mesenchymal progenitor cells with osteogenic as well as chondrogenic potential (Tyyni and Karlsson 2000). The chondrogenic potential has been demonstrated in several experimental animal studies both *in vitro* and *in vivo*, mainly in rabbits (O'Driscoll and Fitzsimmons 2001). The chondrocyte phenotype is promoted by low oxygen tension, low access to nutritients and by cell aggregation (Nakahara et al. 1990). Furthermore, the ability to produce cartilage seems to be age dependent: An *in vitro* study demonstrated a linear decline in the capability to synthesize hyaline cartilage from 2 months of age up to 12 months in rabbits that were considered skeletally mature at 6 months of age (O'Driscoll et al. 2001). The formation of hyaline cartilage following periosteal transplantation is stimulated by continuous passive joint motion (CPM) (Salter et al. 1980). The technique of

periosteal transplantation is similar to that of perichondrium: The graft may be placed with the cambium layer facing the joint cavity as recommended by O'Driscoll and Fitzsimmons (2001) or with the cambium layer facing the bone as recommended by Lorentzon et al. (1998). The periosteal flap is usually secured to the base of the defect by a combination of sutures and fibrin glue. The technique is different from that of autologous chondrocyte implantation (ACI) in which the periosteum flap is attached with sutures to the rim of the defect as a cover for the cells injected underneath (Brittberg et al. 1994). In clinical series the results following periosteal grafting have been good to excellent in 70-80% of cases with the most promising results on the patella, particularly when followed by CPM (Alfredson and Lorentzon 1999). On the other hand, a Danish study of 18 patients treated with periosteal grafts for OCD defects showed inferior results and concluded that the method was not justified (Madsen et al. 2000). The technique has not been compared to other methods in any RCTs. In recent years, cell mediated techniques have evoked increasing interest whereas research on periosteal grafts seems to have subsided.

II c.2. Cell mediated techniques

II c.2.i Autologous chondrocyte implantation (ACI)

First generation ACI

The technique of ACI was introduced by Brittberg and coworkers in 1994 (Brittberg et al. 1994). Although the study was single series with short term follow up, the results were considered promising. The publicatiton in The New England Journal of Medicine seemed to put forward a renewed interest and optimism regarding surgical restoration of cartilage following focal defects. The technique was based on preclinical work with short term follow up in the rabbit patella by Grande et al. (1989) followed by another short term follow up study also in the rabbit patella published in 1996 (Brittberg et al. 1996). The technique is performed in 2 steps: The first step is an arthroscopic procedure in which biopsies of healthy cartilage are harvested from the knee. The cartilage biopsies are treated with enzymes releasing the chondrocytes which are then expanded 40-200 times by proliferation *in vitro*. Originally the cells were expanded in bovine serum, however, in recent years autologous serum has been used more frequently. Two-three weeks following the harvesting procedure an arthrotomy is performed. The focal cartilage defect is

debrided, a periosteum graft harvested from the tibia is sutured to the rim of the defect and sealed with fibrin glue, and the suspension of cultured chondrocytes is injected underneath the cover. Until July 2004, 61 studies on ACI had been published (Jakobsen et al. 2005). Most of these studies are single series with short term follow up (Brittberg et al. 1994, Fu et al. 2005). A limited number of long term follow up studies have been published as well (Peterson et al. 2000, 2003). In general, the study designs are poor (Jakobsen et al. 2005) and only a few RCTs are available (Bentley et al. 2003, Horas et al. 2003, Knutsen et al. 2004, 2007, Saris et al. 2008). Furthermore, no clinical studies have so far included a control group without treatment for comparison. Although clinical results from single series studies have been promising, with 80-90% excellent to good results, the results from the RCTs available vary. As of today, ACI has not shown to be superior to any of the other techniques in use (Jakobsen et al. 2005, Magnussen et al. 2008). Moreover, with the extensive use of the method, several challenges became apparent such as leakage of the cell suspension from the defect, loosening of the periosteal flaps and periosteal hypertrophy causing mechanical complaints (Niemeyer et al. 2008). These challenges gradually led to the development of new generations of ACI.

Second and third generation ACI

In 2nd generation ACI the periosteum used as cover of the defect was exchanged with non-human biomaterial. Third generation ACI implies the use of scaffolds as carriers for the cells, which means that the cells are expanded or seeded in the biomaterial *in vitro* prior to the implantation. The 2nd generation ACI was meant to address the adverse effects related to the periosteum, whereas 3rd generation ACI additionally proposes containment of the cells during the implantation procedure and initial healing process and thereby reducing the leakage of cells into the joint. In addition, the 3-D growth pattern of cells is considered advantageous allowing the cells to redifferentiate and resume synthesis of collagen type II (Shahdadfar et al. 2008). Besides, a scaffold with 3-D distribution of cells already prior to implantation represents a biological "implant" which may be delivered arthroscopically as a gel or paste (Kim et al. 2009).
So far the results following 2nd and 3rd generation ACI have not proved to be better than those following 1st generation ACI (Iwasa et al. 2009). However, a more simple technique avoiding the suturing of periost – which in addition may be performed arthroscopically, is probably an advantage compared to 1st generation ACI (Kim et al. 2009).

II c.3.ii Mesenchymal stem cell (MSC) implantation

Theoretically, the use of MSCs may have advantages compared to the use of chondrocytes in cartilage repair: Human MSCs may be isolated from donor sites causing little or no morbidity, such as from bone marrow, adipose or synovial tissue (Yoshimura et al. 2007), thereby avoiding morbidity to the knee joint (Whittaker et al. 2005). Secondly, dedifferentiation of the cells during expansion is avoided. The optimism regarding MSCs in cartilage repair is supported by promising preliminary results shown in the regeneration of injured tissue in organs such as the heart, central nervous system, liver, kidney, and others (Brooke et al. 2007). Despite extensive basic laboratory and experimental animal research, however, the proposed advantages of MSCs in cartilage repair are still controversial. Furthermore, the so far few clinical studies on MSCs used for cartilage repair have not proved the MSCs to provide better clinical results than other treatments.

III. Joint surface replacement

Treatment of pathological conditions in the articular surfaces by joint surface replacement is always an option, either as unicompartmental, bicompartmental or total knee replacement. Knee replacement is well documented as reliable treatment concerning pain relief. However, despite good results in the elderly, the results of knee replacement in the younger knee-active population are less favourable and not to be recommended (Furnes et al. 2007).
Smaller implants only partially covering the condylar surface have been evaluated in experimental studies with less favourable results (Custers et al. 2010).

REHABILITATION FOLLOWING CARTILAGE REPAIR

The clinical evidence of a positive effect of designed rehabilitation protocols following cartilage repair is limited. In a study of Hangody et al. (1997) 18 defects in the medial femoral condyle (MFC) (weight bearing) and 18 defects in the patellofemoral joint (non-weight bearing) of dogs were treated with mosaic plasty. More than one third of the grafts in the weight bearing area

subsided. Consequently, initial weight bearing restriction was recommended. Some authors propose rigid restricted rehabilitation in their experimental work (Frisbie et al. 1999) whereas others propose immobilisation using for instance external fixation a few days before free mobilisation (Dorotka et al. 2005). Most rabbit studies allow the animals to move freely in cages (Kuo et al. 2006). In a non-human primate model Gill et al. (2005) showed a significant improvement in both quality and extent of cartilage repair following Mfx from 6 to 12 weeks follow up. They concluded that the initial immature tissue needs protection beyond 6 weeks. Moreover, both experimental studies (Salter et al. 1980) and clinical studies (Alfredson and Lorentzon 1999) have shown advantages of the use of CPM following cartilage repair. Steadman et al (Blevins et al. 1998) advocate the use of CPM 8 hours a day for 8 weeks following Mfx. Such a demanding program has by many surgeons been replaced by low load stationary bicycling as soon as range of motion allows it. In a retrospective study comparing the strict original rehabilitation protocol to a program without CPM – and with weight bearing as tolerated, no clinical difference was detected (Marder et al. 2005). Some authors claim that the postoperative rehabilitation program needs to be individualized (Reinold et al. 2006). As of today, most centers seem to recommend partially weight bearing during the initial 6-8 weeks. The range of motion is usually not restricted except in cases of patellofemoral lesions where flexion is usually restricted for the first 4 to 6 weeks to unload the patellofemoral joint.

GOALS OF THE PRESENT THESIS

Adding to previous work by our group, the general purpose of the studies in this thesis was to clarify some aspects concerning the magnitude of the problem “cartilage defects in the knee”, and to analyze some possible predictive factors and explanations for the success or failure in natural history and repair of cartilage defects.

The specific goals were:

1. To document the degree of complaints patients with focal cartilage defects do have and particularly how the complaints affect quality of life as compared to other knee disorders.

2. To investigate the effect of time on focal cartilage defects in the knee with respect to expectations concerning future knee function. Furthermore, to evaluate the effect of additional injuries and/or cartilage repair on the middle term outcome.

3. To investigate the relationship between the intraarticular location and the natural history regarding tissue filling of an experimental chondral defect and changes in the subchondral mineralized tissues.

4. To investigate the differences in outcome between two surgical techniques for cartilage repair regarding tissue filling of an experimental chondral defect and changes in the subchondral mineralized tissues.

Hypotheses:

According to the goals above, the following hypotheses were tested:

1. Complaints due to localized cartilage defects in the knee reduce quality of life measured by KOOS to a different extent than those due to ACL deficiency and osteoarthritis when comparing patients within the working population scheduled for surgery (paper I).
2. Complaints related to knee function in patients with a cartilage defect in the knee remains stable over a 6-year observation period (paper II).
3. The intra-articular location is a predictive factor for the outcome of natural history tissue filling and changes in subchondral mineralized tissue of a chondral defect (paper III).
4. Microfracture technique and mosaic plasty as treatments for a chondral defect lead to different outcome regarding filling of repair tissue and changes in the subchondral mineralized tissues (paper IV).

SUMMARY OF THE PAPERS

PAPER I

Focal Cartilage defects in the knee impair quality of life as much as severe osteoarthritis. A comparison of KOOS in four patient categories scheduled for knee surgery

Background: Patients with focal cartilage defects in the knee may suffer from both pain and functional impairment. Treatment options are often insufficient. It is not known, however, to what extent their complaints affect quality of life, compared to other knee disorders. Knee injury and Osteoarthritis Outcome Score (KOOS) is a validated global knee score suitable for comparison of patients with knee complaints attributable to different causes.

Material and methods: Previously registered KOOS baseline data on patients enrolled in different knee treatment studies were included in the present study; the patients were 18 to 67 years of age (working population) at data registration. The different patient categories were: (1) patients with knee osteoarthritis enrolled for knee arthroplasty, (2) patients with knee osteoarthritis enrolled for osteotomies around the knee, (3) patients with focal cartilage lesions enrolled for cartilage repair, and (4) patients with anterior cruciate ligament deficient knees enrolled for anterior cruciate ligament reconstruction. The KOOS subscale quality of life was the main parameter for comparison of complaints.

Results: At preoperative baseline, patients with focal cartilage defects in the knee scored 27.5 on the KOOS subscale quality of life, not significantly different from the 28.8 and 27.2 in the patients with osteoarthritis enrolled for knee osteotomies and arthroplasties, respectively. For all the subscales of KOOS, the cartilage patients scored significantly lower than the patients with anterior cruciate ligament deficiency.

Conclusion: Patients with focal cartilage lesions have major problems with pain and functional impairment. Their complaints are worse than those of patients with anterior cruciate ligament

deficient knees, and quality of life is affected to the same extent as in patients scheduled for knee replacement.

PAPER II

6-year follow up of 84 patients with cartilage defects in the knee: Knee scores improved but recovery was incomplete

Background: The natural history of focal cartilage injury is largely unknown. This study investigated 6-year outcomes in patients with arthroscopically verified, focal, full thickness cartilage injuries in the knee.

Material and methods: In a previous report (baseline study) of 993 knee arthroscopies, 98 patients were less than 50 years old at baseline, and showed a grade 3-4 focal cartilage injury, assessed with the International Cartilage Repair Society (ICRS) scale. In the present study, 84 of the 98 patients completed follow ups at median 6.1 years (range 5.3-7.8) after baseline assessments. At baseline the patients underwent different types of cartilage repair (n= 34) or had no treatment or only debridement (n=64) for their cartilage injury. The follow up included evaluations with the ICRS Knee Evaluation form, the Lysholm score, and other knee evaluation tests. Sixty-eight patients underwent weight bearing radiographic assessments.

Results: Improvements compared to baseline were noted in the average ICRS functional score, Visual Analog Scale pain score, and the patients' rating of the function in the affected knee compared to the contra-lateral knee. However, the average ICRS activity level had decreased from baseline. The average Lysholm score was 76 (SD 21). 19 patients exhibited Kellgren-Lawrence grades 2-3 in the affected knee, and 6 patients exhibited grades 2-3 in the contra-lateral knee and there was a statistically significant difference between affected and contra-lateral knees.

Conclusion: Patients with arthroscopically diagnosed ICRS grade 3-4 cartilage injuries in the knee may improve their knee function over the subsequent 5-8 years, with or without cartilage

repair. However, knee function remained substantially affected. Further studies are needed to determine whether cartilage surgery can yield better functional outcomes than non-surgical or less invasive surgical treatments.

PAPER III

Intra-articular location predicts cartilage filling and subchondral bone changes in a chondral defect. A randomized, blinded, long term follow up trial in 82 rabbit knees

Background: The natural history and predictive factors for outcome of cartilage restoration in chondral defects are poorly understood. We investigated the natural history of cartilage filling and subchondral bone changes, comparing defects at two locations in the rabbit knee.

Material and methods: In New Zealand rabbits aged 22 weeks, a 4 mm pure chondral defect (ICRS grade 3b) was created in patella in one knee and in the medial femoral condyle in the other. A stereomicroscope was used to optimize the preparation of the defects. The animals were sacrificed 12, 24 and 36 weeks after surgery. Defect filling and the density of subchondral mineralized tissue was estimated using Analysis Pro® software on micrographed histological sections.

Results: The mean filling of the patellar defects was more than twice that of the medial femoral condylar defects at both 24 and 36 weeks follow up. There was a statistically significant increase in filling from 24 to 36 weeks after surgery at both locations.

The density of subchondral mineralized tissue beneath the defects subsided with time in the patellas, in contrast to the density in the medial femoral condyles which remained unchanged.

Conclusion: The intraarticular location is a predictive factor for spontaneous filling and subchondral bone changes of chondral defects corresponding to ICRS grade 3b. Disregarding location, the spontaneous filling increased with long term follow up.

PAPER IV

Cartilage repair in the rabbit knee: Mosaic plasty resulted in higher degree of tissue filling but affected subchondral bone more than microfracture technique. A blinded, randomized, controlled, long term follow up trial in 88 knees

Background: Discrepancies and variances in outcome following different surgical techniques for cartilage repair are poorly understood. Successful repair rely on proper tissue filling without initiating degenerative processes in the cartilage-bone unit. Consequently, the objective of the current study was to compare two available techniques for cartilage repair; i.e. microfracture technique (Mfx) and mosaic plasty (Mos), regarding tissue filling and subchondral bone changes in an experimental model.

Material and methods: A 4 mm pure chondral defect (ICRS grade 3b) was created in the medial femoral condyle of both knees in New Zealand rabbits, aged 22 weeks. A stereomicroscope was used to optimize the preparation of the defects. In one knee (randomized) the defect was treated with Mfx, whereas in the other with Mos. The animals were sacrificed 12, 24 and 36 weeks post surgery. The degree of defect filling, new bone formation above the level of the tidemark and the density of subchondral mineralized tissue were estimated by histomorphometry.

Results: Mosaic plasty resulted in a significantly 34% higher degree of tissue filling than microfracture at 36 weeks, SD of mean difference being 34%. Mos resulted in significantly more new bone formation and change in subchondral mineralized tissue density compared to Mfx. The differences between the two techniques were apparent mainly at the long term follow up.

Conclusion: Tissue filling is a limiting factor regarding Mfx when compared to Mos, whereas Mos resulted in more bone changes than Mfx – the implications of the latter remain to be settled. This study underlines the difficulty in predicting outcome in the single case with any of these two techniques, particularly in a long-term perspective.

GENERAL DISCUSSION

MATERIAL

Clinical studies (paper I and II)

Patient data selection:

In paper I previously registered KOOS baseline data from patients enrolled in different knee treatment studies were included and compared. By "baseline data" we meant data registered at the time the patients were included in the different studies, before any specific treatment was initiated. The data being compared was recruited from 4 different knee patient categories. The patients were all scheduled for surgical interventions due to their clinical complaints. Thus, the different patient groups probably do not reflect the whole range of complaints of all patients with the respective diagnoses. For example, many patients with focal cartilage defects do well and are not scheduled for surgery. The same phenomenon is valid for the other diagnoses as well, but the percentage of patients within each diagnose enrolled for surgery may differ. We therefore emphasized in the paper that the results concerned patients already enrolled for surgery only. A weakness of the study, however, is that the threshold of offering patients surgical treatment may vary from one type of disability to another – mainly dependent on the expected "cost-benefit analysis" outcome for that particular treatment. Patient populations with knee complaints such as ligament injuries and osteoarthritis are offered surgical procedures that have a high success rate in relieving symptoms, i.e. ligament reconstruction for ACL injuries (Pinczewski et al. 2007) and either osteotomies in the middle age patients (Birmingham et al. 2009, Ekeland et al. 2009a, Ekeland et al. 2009b) or joint replacement in the elderly suffering from osteoarthritis (Nilsdotter et al. 2009). The results of surgical treatment for patients with localized cartilage defects in the knee have been subject to controversies (Messner and Gillquist 1996) and have not been equally successful (Bentley et al. 2003, Gudas et al. 2005, Jakobsen et al. 2005, Knutsen et al. 2007, Loken et al. 2009, Minas et al. 2009, Mithofer et al. 2005, Rosenberger et al. 2008). That in turn may have lead to narrower indications for being enrolled in the cartilage group, the consequence being that the data from this group represented an overestimate of complaints compared to data from the other groups. A major advantage, however, was that all data were recruited from

recently performed or ongoing clinical studies at the same hospitals, which secured that the system of data collection was similar.

The patients in paper II were a subgroup of patients included in a previous baseline study by our group (Aroen et al. 2004). In the baseline study articular cartilage lesions were registered in 993 consecutive knee arthroscopies. A localized cartilage defect was noted in 20% of the knees. Of these, patients younger than 50 years of age with a focal ICRS grade 3-4 defect at the time of the baseline study were included in the current study (n=98, 13.8% of patients < 50 years) for re-examination 6 years after initial arthroscopy. These patients represented a heterogeneous group; some patients presented cartilage defects only whereas some suffered from accompanying injuries as well. Some patients underwent cartilage repair and/or treatment for accompanying injuries at baseline and some did not. Ideally the natural history of cartilage defects should be studied in a group of patients with both symptomatic and non-symptomatic isolated lesions in a long term follow up. Such a study would be very difficult to execute. Many patients with severe complaints would probably not accept non-surgical treatment for several years in a time where new methods and surgical techniques are continuously being introduced, and the information of such being easily available for the patients.
However, in RCTs comparing cartilage treatment modalities, a group of patients with non-surgical treatment should be included, since the natural history of cartilage injuries is still not well understood, and no surgical treatment so far has proved to be superior to it.

Experimental studies (paper III and IV)

The rabbit knee model was still the most widely used experimental animal model for cartilage restoration according to a PubMed search at the time these studies were conducted (Årøen 2005). Although some scientists claim that a large animal model is to be preferred (Frisbie et al. 2003), the rabbit knee is a useful experimental model for cartilage restoration and repair, and currently no other animal model has been proved to be superior. The rabbit model has also been used previously by our group in experimental cartilage surgery (Aroen et al. 2005, 2006, Loken et al. 2008). There are data indicating that adolescent rabbits are associated with better results following cartilage repair than adult rabbits (O'Driscoll et al. 2001). New Zealand rabbits which

were used in the current studies are believed to reach skeletal maturity between 4 and 6 months. Some authors claim that this correlates with weight above 3.2 kg (Messner et al. 1993b). In our study the rabbits were 22 weeks old at the time of surgery weighing average 4.2 kg.
The animals showed to be vulnerable to complications. Of the altogether 85 experimental animals, two animals died during surgery whereas eleven of the animals sustained sudden unexpected death during follow up. In addition, seven animals had to be sacrificed during follow up due to impaired general health conditions. Although unexpected loss of 20 out of 85 animals, the knees of most of these animals were unaffected and therefore maintained in the studies. However, complications related to the knee were observed in 10 % of the knees: Ten knees (5.9%) were excluded due to patellar dislocation and seven knees (4.1%) because of infection. Although complications and loss of animals related to the rabbit model have previously been described by other authors as well (Brittberg et al. 1996), our numbers are high and constitute a major weakness of these two studies. A properly conducted pilot study may have identified the problems so that proper measures could have been taken prior to the full scale experiment.

METHODS

Clinical studies (paper I and II)

Functional Outcome Scores

Study I and II have in common that we investigated patients' outcome applying socalled "functional" knee scores. Several such functional outcome scores are in use. They are meant to serve as instruments to register complaints of an individual related to function. They may be used to compare groups or individuals, and to detect changes over time in a group or in an individual. However, most scores do not exhibit results of physical performance based on tests. Thus, there is a discussion whether or not these scores should be named "functional" scores (Roos 2009, personal communication).
Another aspect of using any kind of outcome measures is the relationship between differences observed and their clinical relevance. Although differences may be statistically significant, the extent of difference may be too small to have any clinical implications. Moreover, regarding functional scores; the difference observed may not be detectable by the patients. Thus, the

minimal perceptible clinical improvement (MPCI) represents the difference in functional score associated with the smallest change in the score detectable by the patient. As claimed by some authors; understanding the minimal perceptible differences may permit a better assessment of the clinical relevance of therapeutic interventions (Ehrich et al. 2000). Unfortunately, most functional scores in use do not have the level of MPCI stated.

Knee injury and Osteoarthritis Outcome Score – KOOS (paper I and II)

The KOOS is well described in the literature (Nilsdotter et al. 2009, Paradowski et al. 2006, Roos et al. 1998b): It is a 42-item self-administered, self-explanatory knee-specific questionnaire with five separate outcome subscales, assessing Pain (9 items), other Symptoms (7 items), Activities of daily living (ADL) (17 items), Sport and recreational function (Sport/Rec) (5 items), and knee-related Quality of life (QoL) (4 items). For each item five standardized answer options are given in Likert boxes of which one is to be ticked. The answers are rated with scores from 0 to 4. Total subscale scores are calculated and missing data handled according to the users guide (http://www.koos.nu). The subscale scores are presented separately, ranging from 0 to 100, where 100 is the best possible result to be obtained. According to the KOOS user guide, missing data for each subscore is handled separately, thus the number of patients may vary between subscores as they did in the current study I. The KOOS has been validated for patients undergoing ACL reconstruction (Roos et al. 1998b), meniscectomy (Roos et al. 1998a), total knee replacement (Roos and Toksvig-Larsen 2003) and recently, also for the treatment of focal cartilage lesions (Bekkers et al. 2009). The items of the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) LK 3.0 (Bellamy et al. 1988) are included in the first three KOOS subscales. KOOS has been considered reliable and responsive for assessment of knee complaints in a comparative review of knee-specific outcome measures (Garratt et al. 2004). Patient-reported outcomes assessing knee problems do, however, vary with age and gender in the general population (Demirdjian et al. 1998, Jinks et al. 2002). Therefore, KOOS reference data from a "normal" Scandinavian population was established (Paradowski et al. 2006), making it possible to correct the subscales for age and gender.

The minimal perceptible clinical improvement (MPCI) of the KOOS has not been formally assessed. However, since the full and original version of the WOMAC score is incorporated in KOOS as the subscores "pain", "other symptoms" and "activities-of-daily-living", the MPCI of

10 obtained for the WOMAC (Ehrich et al. 2000) has been applied to KOOS as well (Roos and Toksvig-Larsen 2003). Furthermore, KOOS data from ACL surgery were compared with the clinical knowledge of rehabilitation phases following ACL reconstruction (Roos and Toksvig-Larsen 2003). From this comparison it seemed that a change in score of 8 points or more represented a clinically relevant change following ACL reconstruction. The authors therefore suggested that 8–10 points may represent the MPCI of the KOOS (Roos and Toksvig-Larsen 2003).

Lysholm score (paper I and II) and Tegner score (paper II)

The Lysholm score published in 1982 was meant to be used for ACL patients (Lysholm and Gillquist 1982). A modified version published together with Tegner activity score has, however, been widely used for knee conditions in general (Tegner and Lysholm 1985). The Lysholm score is a set of subscores (each with 5-25 points as maximum score) and the maximum total score is 100. In a recent study of patients considering their knee function as normal, the average Lysholm score was 94 (range 43-100) (Briggs et al. 2009). The score has been validated for cartilage conditions (Smith et al. 2009). Smith et al. (2009) showed a high level of agreement between the physiotherapist- and patient-completed Lysholm scores. But the authors suggested a modification of the score changing the relative weight of the different subscores and removing the subscore "swelling".

The Tegner score is an activity scale ranging from 0 to 10 in which the patients state the highest level of the most demanding activity they are able to perform. Zero represents the patient being on sick leave whereas 10 represents a top league soccer player. The score was intended to be used as a supplement to the Lysholm score and gives complementary information. It was developed as an improvement to the less precise term "return to sport". Tegner score has not been validated or compared to other activity scores. However, since the Tegner score is considered useful as a supplement to Lysholm score, and no other activity score has proved to be superior, it is still in use.

ICRS functional score (paper II)

The ICRS form was published by the ICRS in 1998 (International Cartilage Repair Society 1998) and represented a "package" of evaluating systems including a new standard of how to

arthroscopically evaluate and map cartilage lesions of the knee (fig 2a and fig 3) and how to evaluate the results after cartilage repair. The clinical score contains patient information including sex, age, weight, height, mechanism of injury, previous surgery etc. It also contains a grading of functional level and activity level. When planning our previous baseline arthroscopy study (Aroen et al. 2004) we regarded this as an upcoming and up to date tool for the study. Although the questionnaire may be useful in a surgeon's practice, it has not been widely used in research, and, moreover, it has not been validated. When performing our follow up study (paper II) we obviously had to repeat the use of the ICRS score in addition to the more commonly used scoring systems.

IKDC score (paper II)

The IKDC score was published by the International Knee Documentation Committee in 1993 (Hefti et al. 1993), whereas a modified version was published in 2001 (Irrgang et al. 2001). The score has been validated for ACL patients (Irrgang et al. 2001, Risberg et al. 1999) and for knee patients in general (Higgins et al. 2007).

Cincinnati Knee Rating System (paper II)

The Cincinnati Knee Rating System published in 1983 was meant to be used for ACL patients as well (Noyes et al. 1983b, Noyes et al. 1983a). Just like the Lysholm score it consists of several subscores with a maximum score of 100. The Cincinnati Knee Rating System is validated for ACL-patients (Barber-Westin et al. 1999) and was proven superior to Lysholm score and IKDC in detecting changes over time (Risberg et al. 1999).

Modified 10-point scales of the Cincinnati Knee Rating System (paper II)

This score was part of an evaluation package provided by a cell laboratory (Genzyme, Boston, Massachusetts, USA). The score has never been validated, however, it has been included in similar studies (Browne et al. 2005, Micheli et al. 2001). The score reports on the same variables (pain, locking, knee collapse and swelling) as the Cincinnati knee rating system and other well established scoring systems.

SF-36 (paper II)

Short Form-36 (SF-36) is a general health score (Ware Jr. and Sherbourne 1992). It is widely used in clinical papers – also within the field of orthopedic research. SF-36 contains subscores reflecting both the physical and mental state of the patient. By comparing it to Lysholm score evaluating cartilage patients the authors recommended an additional use of a knee specific score (Bartlett et al. 2005a). Reference scores for men and women of different age groups in different populations are available, including the Norwegian population (Loge and Kaasa 1998).

General comments on the knee scores

The purpose of which the different knee scores are developed may vary, and thus the variables emphasized within each score may vary. In a study from 2007 patients with different knee disorders rated the questions in the questionnaires of several knee scores in regard to how important the questions were to them (Tanner et al. 2007). Of the outcome measures concerning knee disorders in general, IKDC and KOOS were rated top two ensuring the patients' perspective being considered. Based on this, a similar comparison of IKDC and KOOS was performed in a group of patients who had undergone cartilage surgery (Hambly and Griva 2008). The authors concluded that IKDC was superior to KOOS, however, an incorrect use of the KOOS in that study was later pointed out (Roos et al. 2009).

The patients in both study I and II had already been registered at previous baseline inclusions. As for study I, the KOOS was considered suitable also for the comparison of complaints between the different knee patient groups, the natural choice of a main outcome variable being the subscale Quality of Life (QoL). As for study II the main baseline variable selected was the non-validated ICRS functional level (Aroen et al. 2004). At the time the baseline study was initiated (1999), the ICRS scores were considerated the most appropriate, thus, KOOS and Lysholm score were not included as baseline scores. To broaden the scope of study II, however, we added some validated knee scores and SF-36 at follow up. The importance of using validated disease-specific outcome measures in evaluating knee patients has been emphasized by other authors as well (Mohtadi 1998).

Registration of arthroscopic findings (paper II)

In the previous baseline arthroscopy study (Aroen et al. 2004) the arthroscopic findings were registered using the cartilage evaluating package published by ICRS in 1998 (Brittberg and Peterson 1998). The data concerning depth (Figure 2a), size and localization (Figure 3) of the defects were used in study II. The accuracy of estimating size of cartilage defects at arthroscopy has been demonstrated to be poor in an *ex vivo* model (Oakley et al. 2002). However, accuracy improved when using specially designed measurement tools (Oakley et al. 2003). A study on videos of cadaveric knee arthroscopies showed reasonable accuracy using the Outerbridge grading system (Cameron et al. 2003). The interobserver variability in locating the cartilage lesion within the knee joint was satisfactory in a mapping system similar to the ICRS mapping (Hunt et al. 2001). In an on-going study the size of the lesions measured at arthroscopy were similar to the size measured at later knee arthrotomies (Årøen 2010, personal communication). In the current study a standard arthroscopic probe was used in estimating the size of the defects, but we did not investigate the accuracy of the surgeons' measurements.

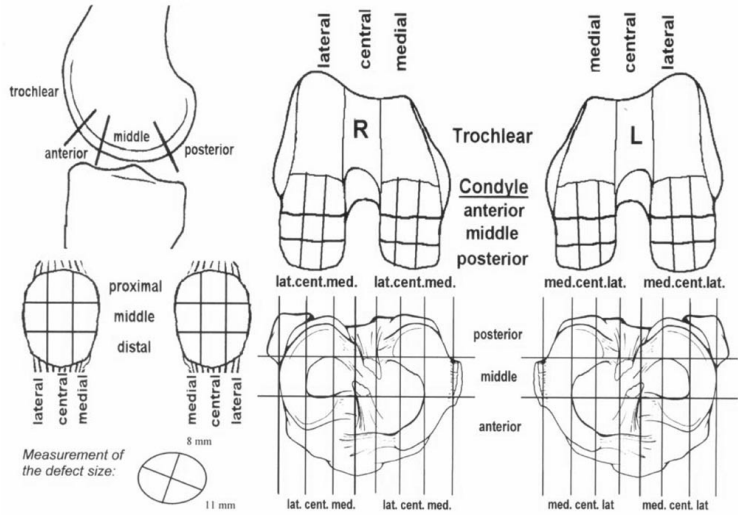


Figure 3. ICRS mapping system for cartilage lesions (Brittberg and Peterson 1998)

Radiological grading (paper II)

In the previous baseline arthroscopy study (Aroen et al. 2004) classification of radiological findings was based on the presence or non-presence of degenerative changes on plain radiographs (weight-bearing or non weight-bearing) or MRI, as evaluated by the orthopedic surgeon. In paper II 68 of 84 patients were examined by weight-bearing radiographs, the findings classified according to Kellgren and Lawrence (1957) (Table 1). The advantage of this system is that it is widely used in previous investigations, enabling comparison between studies. The disadvantage is that the grading is non-quantitative. An alternative would be the Albäck classification which is solely based on mm joint space narrowing (Ahlback 1968).

Table 1. Kellgren-Lawrence Grading Scale (Kellgren and Lawrence 1957).

Grade 1	Doubtful narrowing of joint space and possible osteophytic lipping
Grade 2	Definite osteophytes, definite narrowing of joint space
Grade 3	Moderate multiple osteophytes, definite narrowing of joints space, some sclerosis and possible deformity of bone contour
Grade 4	Large osteophytes, marked narrowing of joint space, severe sclerosis and definite deformity of bone contour

Experimental studies (paper III and IV)

In study III a 4 mm pure chondral defect (ICRS grade 3b) was created in patella in one knee and in the medial femoral condyle (MFC) in the other, whereas in study IV the defects were created in the MFC of both knees. The main parameter to be evaluated was tissue filling of the defect – the natural history at different locations in study III, and the outcome following two different surgical techniques in study IV. Although all parameters that could be controlled were standardized, some variance in healing response was to be expected. Therefore, to minimize a possible effect (and thereby bias) of the individual, bilateral surgery was performed allowing pair wise comparison.

In both studies we investigated pure chondral defects, i.e. defects including the depth of all the non-calcified articular cartilage down to, but not penetrating the tidemark and the underlying calcified tissues. The importance of defining the depth of experimentally produced defects in

relation to the different layers of the joint organ – and evaluating the results in relation to that – has been emphasized (Breinan et al. 1997, Brittberg et al. 1996, Burr 2004, Hunziker 1999). Even the bias of not removing all the tissue of a layer as intended, or causing damage to the layer beneath, has been a topic in discussing the results of cartilage restoration (Breinan et al. 1997, Frisbie et al. 1999). During clinical cartilage repair procedures, some authors recommend "the zone of calcified cartilage" to be removed "without damaging the subchondral bone" (Steadman et al. 2001). Consequently, a number of experimental studies on cartilage repair in animal models have adapted this recommendation producing "full thickness" baseline defects (ICRS grade 3c). In a study comparing the effects of retaining vs removing the calcified cartilage preceding Mfx in horses, Frisbie et al. (2006) reported 16% higher amount of tissue filling in defects where the calcified cartilage assumingly was removed, thereby verifying the recommendation of removal. However, the 16% difference in filling may not be of any clinical relevance; no significant difference was noted in clinical examination of pain, radiographic or immunohistochemical evaluations. Moreover, the authors claimed having developed an accurate arthroscopic technique for removal vs retention of the calcified cartilage. The validation data verifying that the proper depth was reached histologically are, however, still unpublished. As emphasized by Hoemann et al. (2005), it is difficult to consistently remove the entire calcified cartilage without deeply interrupt the subchondral bone plate. Even when aiming for complete removal of the calcified cartilage without damaging the subchondral bone in their sheep model, histological analyses revealed 48% (SD14) - 60% (SD28) residual calcified cartilage (Hoemann et al. 2005). This is not surprising taking the histoanatomy into account; in contrast to the interface between the non-calcified and calcified cartilage (histologically viewed as the tidemark) which is smooth, the interface between the calcified cartilage and the subchondral bone plate is highly irregular with deep indentations of the two tissues into each other, making removal of all calcified cartilage "without damaging the subchondral bone plate" extremely difficult, if not impossible. The consequenses of iatrogenic damage to the calcified cartilage are not well understood. In their horse model, Frisbie et al. (2006) reported that complete removal of the calcified cartilage, and thereby presumingly causing excessive damage to the calcified cartilage, significantly increased new bone formation defined as bone observed above the level of the adjacent tidemark. The ramification of the finding is unknown, but the same excessive bone formation has been observed in other studies on Mfx in full thickness defects (ICRS grade 3c) as well (Frisbie et al. 1999).

The effect of cartilage repair on the subchondral mineralized tissues underlying a well defined pure chondral defect (ICRS grade 3b) is not well known either, and studies including quantitative outcome measures in this respect are limited. In their sheep model involving the MFC Dorotka et al. (2005) measured the percentage of length of the calcified cartilage of normal appearance and found this to be 33% in microfracture defects compared to 79% in untreated defects. Other authors have reported changes in the thickness of the subchonral bone plate following different surgical procedures (Aroen et al. 2006). Whether such changes in the "thickness of the bone plate" observed is due to direct apposition of bone towards the trabecular tissue or to endchonral ossification of the calcified cartilage, with new bone formation above the level of adjacent tidemark as a consequence, is not clear.

Since the calcified cartilage is an integrated part of the subchondral mineralized tissues, and alterations in the calcified cartilage have been associated with the development of OA (Burr 2004), we agree that defects involving vigorous iatrogenic damage to this layer (ICRS grade 3c) should be distinguished from pure chondral lesions (ICRS grade 3b) in evaluating the natural history and the effect of cartilage repair on the subchondral mineralized tissues.

To reduce the risk of damage to the calcified cartilage in experimental studies, we have stressed the importance of using a stereomicroscope in preparing the defect. This is in line with authors not using a stereomicroscope claiming that the procedure demands great care and is hard to perform without leaving residual cartilage or damage to the calcified cartilage (Breinan et al. 1997, 2000).

The animals in the current two studies were sacrificed 12, 24 and 36 weeks after surgery which allowed us to do longitudinally observations as well as the pair wise comparison at each time point. At the time of harvesting, the specimens were immersed in phosphate-buffered paraformaldehyde for one week and decalcified in formic acid until the bone was soft enough for sectioning. In contrast to the methology used in other studies performed by our group on the same rabbit model (Aroen et al. 2005, 2006, Loken et al. 2008), the cubes containing the defect were embedded in an epoxy resin. The reason for doing it this way was the expectations of non-homogenous distribution of tissue filling in the defects due to the techniques of cartilage repair used in study IV. Embedding the specimens in epoxy resin gave us the opportunity to obtain sections from several well-defined levels as section thickness is much more homogenous with an

epoxy resin than with paraffin-embedded tissue. Thus, the cubes were sectioned from one longitudinal surface, the orientation of the defect at random as recommended for quantitative analysis (Gundersen et al. 1988). From the point where the rim of the defect was reached, sections were captured at 5 different levels, each level 700 µm further into the defect. In study IV comparing Mfx and Mos, sections from the three most central of the 5 levels were used for evaluation. However, in study III correlation analyses revealed corresponding results using the one very most central lesion compared to the three, thus only one was analysed. The technique ensured that when using three sections they would all be within a maximum distance of 1050 µm from the very centre of the defect.

Unfortunately, the procedure of preparing the specimens was vulnerable to technical failures. Eighteen knees obtained from the experimental animals, and three out of twenty knees meant to serve as control knees without surgery – a total of twenty-one knees (11.1%) – had to be excluded from the studies, the reason being that sections from these specimens were not available/the quality was too poor for evaluation by light microscopy. As a consequence of that the group of knees meant to serve as controls without surgery had to be excluded from statistical comparison. Thus, the high number of failures in histological preparation was a major weakness of the two experimental studies. There are three previous papers by our group on the same rabbit model with no such numbers of animal and/or technical complications (Aroen et al. 2005, 2006, Loken et al. 2008).

Evaluation of cartilage repair tissue by light microscopy (paper III and IV)

In experimental studies the animals are sacrificed and the entire joint is available for histological analysis. In contrast to a biopsy from a small area used in studies of humans, this enables the investigator to evaluate the degree of filling of the defect and quantitation of tissue components. In the current two rabbit studies we analyzed tissue filling, new bone formation and changes in subchondral mineralized tissues by quantitative measures. From the literature available, few if any studies have used this approach in evaluating cartilage defects and repair. The majority of studies apply semiquantitative histological scores for their outcome measures (Mankin et al. 1971, O'Driscoll et al. 1986, Pineda et al. 1992, Pritzker et al. 2006, Wakitani et al. 1994, Mainil-Varlet et al. 2003). The scores have in common that they include different variables presenting a total

sum of points as the result. The relevance of such a sum score may be questioned as the importance of each parameter in relation to the others is unknown. Besides, the reproducibility of semiquantitative histological scoring systems has been poor and thus the validity is questionable (Hyllested et al. 2002, Mainil-Varlet et al. 2010). In the current studies we quantitatively measured the height of tissue filling above the level of tidemark, both total filling and new bone formation, relating it as degree of filling relative to the shoulder height of the defect. Furthermore we quantitatively evaluated the density of the subchondral mineralized tissues by point counting (Gundersen et al. 1988) (fig 4). In a previous study by our group (Loken et al. 2008) we performed inter- and intraobserver tests of the method and the method turned out to be reproducible with good agreement between the two observers and between the two time-points.

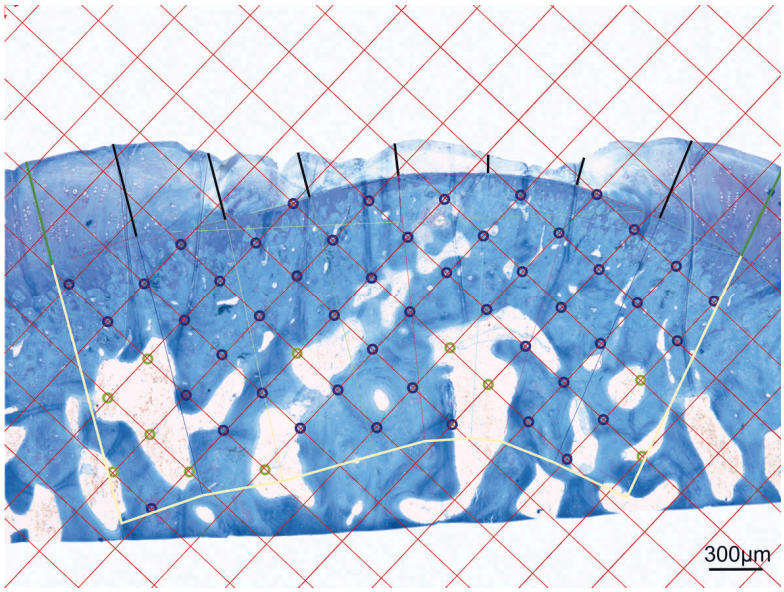


Figure 4. Histological section of a defect in the patella stained with tolouidin blue and micrographed at 40x magnification. The section is modified high-lightening some of the graphics superimposed by the software Analysis Pro®. The two green lines outline the height of the shoulders measured. The seven black lines indicate the height of the tissue filling the defect. The yellow lines together with the tidemark frame the area of interest for subchondral mineralized tissue evaluation. A red grid with 300µm between test lines is superimposed to the micrograph. The intersections of the grid are marked with blue circles whenever overlying mineralized tissue, and green circles whenever not.

Statistical methods

All statistical analyses in this thesis were performed with the Statistical Package for Social Sciences (SPSS statistical package, Chicago, Illinois, USA version 14 or 15).

Paper I

In paper I the main outcome variable was KOOS subscore Quality of Life (QoL). The data obtained from KOOS are ordinal rather than continuous which would indicate the use of non-parametric tests. However, the sample size in each group was considered large enough to apply the central limit theorem with normally distributed sample means, enabling us to use oneway ANOVA analysis of variance for comparison between groups supplied by post hock Bonferroni tests due to multiple comparisons (Paradowski et al. 2006). QoL, with the smallest number of items to be answered, was controlled by non-parametric tests which were in agreement with the ANOVA test. To investigate the impact on QoL of the other four subscales, a linear regression analysis was performed - the results given as R square and corresponding p-values. Lysholm score was compared between two of the groups only, namely the cartilage patients and the osteotomy patients. Thus, an independent sample t-test could be used for that purpose.

Paper II

In paper II the main outcome variable was change in ICRS functional level on a 1-4 scale. We regarded an average difference in functional level of 0.5 units (17%) on the 1-4 functional level scale as a difference of clinical interest. Sample size calculation showed that 47 patients would be sufficient to detect a 0.5 unit change in functional level from baseline with a SD of 1.2 units at 80 % power and 5 % significance level. Consequently, we did have a sufficient number of patients to investigate changes with time for the whole group, but not sufficient number of patients to compare subgroups. Wilcoxon test was applied for changes from baseline for the variables ICRS functional level, ICRS activity level and ICRS rating of knee in comparison with contralateral knee. All these variables are categorical variables with four levels (1-4), the patients served as their own control, thus a non-parametric paired test could be used. A paired t-test was used for the visual analogue scale (VAS) of pain variable since this was regarded being close to a continuous variable. To detect possible predictors for the outcome, both univariate and multivariate linear regression analysis were performed for the dependent outcome variables – investigating the independent parameters: size and localization of the injury, and cartilage repair

and/or additional ligament/meniscal surgery. In addition, confounding factors were investigated such as: age at start of symtoms, age at surgery, sex and body mass index (BMI) at baseline. In such analysis, the influence of different independent parameters on the outcome of the dependent variables can be calculated. Side to side difference of the Kellgren-Lawrence radiological grading was evaluated using a Wilcoxon test, since this was a paired categorical variable as well.

Paper III and IV

The main parameter in these two studies was tissue filling of the defects. One of the strengths of doing experimental animal studies is the opportunity to standardize more or less all parameters that can be controlled. Still some variance due to other factors than those intentionally manipulated has to be expected. To minimize possible effects of the individual, bilateral surgery was performed allowing pair wise comparison of the main variables to be investigated. To our knowledge there is no consensus regarding the minimum change in tissue filling considered being clinically relevant regarding cartilage defects. Based on literature available and previous experience (Aroen et al. 2006) a filling difference of 25% was considered a proper level to discard the H_0-hypothesis of no difference. Preexperimental analysis using a power of 0.80 and a significance level of 0.05 and a standard deviation for the differences of less than 24 % indicated a need of 9 animals in each group. To evaluate the difference in tissue filling from one follow up time point to another regarding each location/ method separately (the effect of time), the need of animals was estimated to be 12 for each of the three follow up time points due to an unpaired experimental situation.

Power and sample size estimations were not performed for the secondary endpoints. This was a weakness of the studies. However, the reason we did not do it was the lack of knowledge regarding new bone formation and changes in subchondral bone density as well as what their implications are related to cartilage regeneration vs degeneration.

Tissue filling, new bone formation and subchondral mineralized tissue density were all considered being continuous parameters allowing us to use oneway ANOVA analysis of variance and paired and unpaired t-tests for detecting possible interactions and possible effects of the different variables.

RESULTS

Paper I and II

Study I showed that KOOS-QoL in patients with focal cartilage defects in the knee enrolled for cartilage repair is impaired as much as in older patients with osteoarthritis signed up for knee replacement. Moreover, the cartilage patients had similar low scores for all five subscales of KOOS, the scores being equal to those of the osteoarthritic patients enrolled for osteotomies around the knee. The similarity between these two patient categories was confirmed by the Lysholm score as well. Patients in the cartilage category were as a mean 28 years younger than the patients in the arthroplasty category, and as a mean 15 years younger than the patients in the osteotomy category. In a paper by Paradowski et al. (2006) all the subscales of KOOS were found to be age dependent in a "normal" Scandinavian population, the values of QoL declining significantly with age. On this background, the patients with cartilage defects do relatively speaking even worse than the patients in the arthroplasty and osteotomy categories.
In study I the baseline KOOS-QoL for the cartilage patients was mean 27.5 and the baseline Lysholm score 49.5. As for study II, KOOS and Lysholm were not included as baseline scores at the time of surgery (1999). However, at the follow up the patients showed mean KOOS-QoL 61.0 and mean Lysholm score 76, i.e. considerable higher values than the baseline scores registered in study I. The difference is in agreement with the main finding in study II; according to the main parameter ICRS knee functional level and the other parameters of the ICRS knee function assessment system, which were registered both prior to surgery and at the 6-year follow up, the patients improved their knee scores significantly regardless of the type of treatment given. Furthermore, linear regression analyses demonstrated that there was no effect of cartilage repair compared to non-repair, and there was no effect of concomitant treatment such as ACL reconstruction or meniscal surgery. Unfortunately, a major limitation with study II is the small number of patients that underwent cartilage repair (n= 30), making direct comparison between cartilage repair versus non-repair subgroups less powerful. In paper II we argue that most of the cartilage repair performed in the study is microfracture and therefore may not be representative for cartilage repair overall. However, no other technique of cartilage repair has shown to be superior to microfracture (Jakobsen et al. 2005).

The main finding of study II is that 84 patients with cartilage defects grade 3-4 improved their knee scores with time regardless of treatment given 6.2 years prior to the follow up. Fifty-four patients were registered with no cartilage repair, but 10 of these were treated with cartilage debridement. Still, according to the data obtained, 44 patients had their defects left "untouched". However, only 13 patients underwent diagnostic arthroscopy only (with possibly additional procedures later on) meaning that at least 41 of the 54 knees with non-repair of the cartilage defects may have improved their knee scores due to other procedures. The reason they ended up with unsatisfactorily results, however, may be due to residual cartilage defects. On the other hand, cartilage defects have been reported to be asymptomatic (Kaplan et al. 2005), and also reported not to influence on the outcome after ACL surgery (Shelbourne et al., 2003, Widuchowski et al. 2009).

The above issues make the data in paper II hard to interpret and thereby conclude. Moreover, in paper I the patients had already had their symptoms for a mean period of almost 6 years still with considerable lower KOOS and Lysholm scores than those registered at 6-year follow up in study II. Thus, as we underline in paper II, the reason for the improvement seen among the patients over time in study II is not at all clear. There are several possible contributing factors to the improved scores; firstly, the baseline scoring was performed by the patient the same day as the arthroscopic surgery, while at the second scoring the patient was coming for a follow up visit. These two different settings may influence how the patient rates the knee function. Secondly, the improved score in our study may also partly be a result of a placebo effect, which has been demonstrated to be important also in orthopedic surgery (Kirkley et al. 2008, Moseley et al. 2002). Finally, the improvement in functional score and less pain stated on the VAS for pain may be due to reduction in activity level; with less physical activity the patients may experience less pain.

The uncertainties concerning the nature of cartilage defects and the complaints they may cause, is also outlined by the finding in study I that 13 % of the ACL patient population showed ICRS grade 3-4 cartilage injuries with apparently no influence of these lesions on the QoL of the patients. This is also in agreement with the findings of Hjermundrud et al. (2010). Moreover, as mentioned above, focal cartilage defects have been shown not to influence the outcome following ACL reconstructions (Shelbourne et al. 2003, Widuchowski et al. 2009). These findings suggest that cartilage defects may play a minor role in ACL deficient and reconstructed knees. On the

other hand, the multivariate analyses in study II showed that ACL reconstruction had no effect on the time course of grade 3-4 cartilage defects, the 6-year Lysholm score of 76 in fact being in the same range as that found in patients with ACL-injury awaiting surgery (Drogset et al. 2005). Most papers on results after cartilage surgery report improvement in functional scores both in case series and in randomized controlled trials (Jakobsen et al. 2005). The patients in study II have a functional level comparable to patients following cartilage surgery in randomized controlled trials with comparable data (Horas et al. 2003, Knutsen et al. 2007, Saris et al. 2008). However, patients in RCTs must fulfill strict inclusion criteria; thus, those results may not be generally applicable to all patients with cartilage injuries (Engen et al. 2009). Nevertheless, conclusions from RCTs are often generalized to all patients with cartilage injuries.
Although the population in study II was young (mean age 32 years at baseline), the radiological examination showed significant osteoarthritic changes in the affected knee 6.2 years after baseline surgery, even in the groups without additional ligament or meniscal injuries. The finding suggests a relationship between focal cartilage injuries and early development of osteoarthritis. Our results are in accordance with the findings in the study by Knutsen et al. (2007) who reported osteoarthritis in more than 30% of the patients 5 years after ACI or microfracture. An association between OCD diagnosed in patients after closure of the epiphyseal plates (average age 29.4 years) and osteoarthritis later in life (average age 61.9 years) has also been shown (Linden 1977). In the regression analysis we noted that a high BMI was associated with a higher Kellgren-Lawrence grade as shown in several other studies (Spector et al. 1994, Toivanen et al. 2010).

In summary from study I and II, patients with cartilage defects grade 3-4 may have severe complaints, even to the same extent as patients signed up for knee replacement. The mechanisms of complaints and their variance are, however, to a large extent unknown. The time course of untreated as well as treated defects is also poorly understood, and, moreover, the relationship to osteoarthritis; the underlying mechanisms and predictive factors are still being largely unknown.

Paper III

A multivariate analysis in study II showed that the location of the grade 3-4 cartilage defects within the knee did not have any effect on the time course regarding functional knee scores. This may not be surprising due to the many locations possible and the small number of patients included in the study. However, other clinical studies have shown discrepancy of results after cartilage repair possibly related to the intraarticular location (Brittberg et al. 1994, Hangody and Fules 2003, Krishnan et al. 2006). Brittberg et al. (1994) and Hangody et al. (2003) both showed inferior results in the patellofemoral compartment following ACI and mosaic plasty respectively, and Krishnan et al. (2006) reported the lateral femur condyle to be the most favourable location for ACI surgery. These findings were the background for study III, an attempt to clarify some predictive factors in the regenerative/degenerative process of the bone-cartilage unit following a cartilage defect ICRS grade 3.

Study III showed higher degree of filling in the patella defects compared to the medial femur condyle defects at 24 and 36 weeks follow up. At 36 weeks the mean difference in filling was 28 % with a SD of 27 % ($p=0.001$). Such large variances in spontaneous filling of ICRS grade 3b defects have previously been reported by other authors as well (Breinan et al. 1997). Tissue filling is to some extent assumed to be crucial in cartilage restoration of a chondral defect. However, the critical amount of tissue filling necessary to significantly discriminate one clinical outcome from the other as far as joint function, pain, disability and reduced risk of osteoarthritis go, is not well understood (Frisbie et al. 2006). At 36 weeks follow up the mean difference of filling in study III was 28 %, thus, our H_{01}-hypothesis could be discarded.

The finding indicates that the intraarticular location influences the natural history of filling of a pure chondral defect ICRS grade 3b in a long term follow up. To our knowledge this issue has not previously been thoroughly investigated. On the other hand, the potential effect of the intraarticular location on the outcome following surgical repair of chondral defects ICRS grade 3 has been discussed in previous experimental papers (Breinan et al. 1997, 2000). Due to small number of animals the authors (Breinan et al. 2000) were not able to show a significant difference in filling of 2 different defects in the trochlea of dogs following ACI, however the percentage of

reparative fibrocartilage was significant different, thereby supporting our observation that the intraarticular location may play a role.

Study III also demonstrated a change in tissue filling of both the patellar and MFC defects with time. The changes in tissue filling was similar at the two locations, i.e. the amount of filling increased significantly from 24 to 36 weeks at both locations. This observation underlined that time to follow up is an important parameter in evaluating the results of natural history tissue filling in experimental models. The effect of time on cartilage restoration has been well studied in experimental animal models involving defects corresponding to ICRS grade 4 (Shapiro et al. 1993). The natural history time course on ICRS grade 3b defects on the other hand is less well known. The increase in spontaneous tissue filling after 12 weeks is in line with studies from other animal models (Breinan et al. 1997, Frisbie et al. 1999, 2003). Bringing the results from the two studies of Frisbie together indicate a similar tendency of natural history filling of a defect as in our study; there was a high percentage filling at an early follow up, with a tendency towards decreasing at a medium time follow up, again increasing at a long time follow up.

Study III showed that the subchondral mineralized tissue density in the patellas with defect changed with time, in contrast to the density in MFCs with defect. The finding may indicate that the intraarticular location is a predictive factor also for subchondral bone changes related to ICRS grade 3b chondral defects in the rabbit knee. To our knowledge this issue has not been discussed in previous literature either.

A major weakness of study III was the lack of sufficient numbers of control patellas and MFCs in evaluating subchondral bone changes. However, the observation of reduced density of mineralized tissue in patellas with time, in contrast to MFCs, may be an important finding since subchondral bone changes are associated with the initiation and progression of OA (Burr 2004, Radin and Rose 1986). No consensus, however, has been reached regarding the pattern of changes and their relevance (Sniekers et al. 2008). Moreover, there are no objective criteria as to the degree of changes indicating a significant initiation or progression of OA. Whether the statistically significant changes demonstrated in study III therefore do have any clinically implications remain uncertain.

Study III demonstrated a highly significant reduction in the density of subchondral mineralized tissue from 12 to 36 weeks in the patellas with defect. The time course is in agreement with the time course of changes in corresponding variables reported in an experimental OA model

(Sniekers et al. 2008). Neither macroscopically observations nor synovial fluid proteoglycan analyses supported osteoarthritic changes in the patella. Still, the MFCs seemed to tolerate the defect better, showing no detectable changes in density with time. Thus, the intraarticular location may be a predictive factor for subchondral bone changes related to chondral defects in the rabbit knee.

A rise in synovial fluid proteoglycan content from values prior to surgery was detected in the knees of animals sacrificed at 12 weeks following surgery. There was no significant difference between knees with patella defects and knees with MFC defects. The rise observed at 12 weeks subsided with time, being non-significant at 24 and 36 weeks follow up.

Increased concentrations of proteoglycan fragments in the joint fluid have been associated with trauma and surgery in humans (Odenbring et al. 1991) and with OA in a rabbit model (Messner et al. 1993a). The significant rise at 12 weeks may reflect the initial surgery, supported by the elevated proteoglycan content gradually subsiding. Consequently, the synovial fluid proteoglycan concentrations observed in the current study did not indicate a degenerative process during the observation period, neither in the knees with a patellar defect, nor in the knees with a MFC defect.

Paper IV

The major finding of study IV was that neither of the two evaluated techniques for cartilage repair resulted in altogether good and predictable outcome regarding the chosen parameters in this long term follow-up. This is in line with the results of some of the clinical randomized studies using the same techniques (Bentley et al. 2003,Knutsen et al. 2007).

Compared to the spontaneous filling of the MFC defects in study III, considered as control defects in study IV, both cartilage repair techniques arrived at significantly better results. There was an overall significantly higher degree of total tissue filling in the defects treated with Mos compared to those treated with Mfx. Whereas the filling of defects treated with Mfx remained 30-31 % at all follow ups, the mean value of filling of Mos knees was 32% at 12 weeks, 45% at 24 and 65% at 36 weeks. However, we could not detect a significant effect of time neither on the pair wise computed difference between Mfx and Mos, nor on the timecourse of Mos separately. The reason for this was probably the lack of power due to insufficient number of sections to be analyzed at 12 and 24 weeks follow up. Although strong enough for the dependent analyses

between treatment modalities, the statistical power was too weak for comparison of independent groups over time.

A large variance in the degree of filling was noted with both treatment modalities. Such large variance in filling was also seen in the spontaneous filling of the defects in study III, and has previously been brought to attention regarding defects treated both with Mfx (Breinan et al. 2000, Kuo et al. 2006) and Mos (Hurtig et al. 1998, 2001).

Analogous to study III we considered a filling difference of more than 25 % a proper level to discard the H_{01}-hypothesis. Thus, the significantly 34% higher degree of filling following Mos compared to Mfx at the latest follow up discarded our H_{01}-hypothesis. Whereas the mean differences in tissue filling between the Mfx and control defects were less than 25% at all time points, the difference between the Mos and the untreated defects were more than 25% at both 24 and 36 weeks, resulting in significantly 45% difference between the Mos and control defects at the latest follow up.

There are to our knowledge only 2 clinical studies comparing these two techniques for cartilage repair (Gudas et al. 2005, 2009). Gudas et al. (2005) demonstrated superior results of Mos compared to Mfx clinically, macroscopically, histologically and radiologically 37 months following treatment. Tissue filling of the defects was, however, not evaluated.

There are several experimental studies evaluating tissue filling in cartilage defects following Mfx (Breinan et al. 2000, Dorotka et al. 2005, Frisbie et al. 1999, Gill et al. 2005, Hoemann et al. 2005). The majority of these studies, however, are on ICRS grade 3c and 4 defects. Few, if any, report tissue filling following Mfx of ICRS grade 3b defects in the rabbit knee, and no studies as far as we know have compared Mfx to Mos in an animal model. Although the majority of studies on Mfx report tissue filling superior to the 31% observed at 36 weeks in study IV, only a limited number of papers present an increase in filling of more than 25% compared to controls (Dorotka et al. 2005). Since discrepancies between studies may have several explanations, differences in results between studies should be interpreted with caution. Furthermore, due to large disparity between studies, experimental comparisons of different treatment modalities and techniques should preferably be performed in the same animal model.

In contrast to Mfx, tissue filling has not been a focus in evaluating Mos. The implantation of osteochondral grafts implies that there will be an initial filling. However, due to multiple circular grafts there will be interstitial space in between, the initial filling being limited to theoretically

37% in our case. Gradual filling of the interstitial space may contribute to an increased amount of tissue filling with time as observed in our study. The mean tissue filling of the Mos defects was 32% at 12 weeks, whereas it was 66% at 36 weeks follow up. The lower value of tissue filling at 12 weeks compared to the theoretically initial filling might be due to subsidence of grafts and lack of filling of the interstices in some defects. There was a great variance in the results at 36 weeks (SD 38.0%, 95% CI [39.9 – 91.0]). Since tissue filling exceeding 37% was dependent on cartilage flow and extrinsic repair, our findings are in agreement with previous studies suggesting that this kind of repair is unpredictable (Hurtig et al. 1998, 2001).

Study IV demonstrated that both Mfx and Mos resulted in new bone formation above the level of the tidemark. New bone formation following Mos comprised considerable 13% and 12% of the defect volume at 24 and 36 weeks respectively, significantly different from the 1.4% and 2.5% following Mfx. In comparison, the untreated MFC defects in study III showed no signs of new bone formation above tidemark at any follow up. New bone formation has previously been reported following Mfx (Dorotka et al. 2005, Frisbie et al. 1999, 2006) as well as Mos (Huang et al. 2004). The implications of new bone formation observed in cartilage repair are, however, not known. On the other hand, new bone formation reflected as tidemark advancement and thereby calcified tissue advancement into the non-calcified cartilage is associated with the progression of OA (Burr 2004) and therefore a matter of concern, also with regards to cartilage repair.

Study IV also showed that the mean difference in subchondral mineralized tissue density between Mos and Mfx was significant at the latest time point of follow up. Compared to the MFC defects in study III, Mos resulted in a significant reduction of the subchondral mineralized tissue density, whereas Mfx did not. To our knowledge this is the first study comparing the effect of these two cartilage repair techniques on subchondral bone. Furthermore, quantitative analyses of the subchondral mineralized tissue density following cartilage repair has not been brought to attention previously. As was mentioned above concerning study III, the implications of subchondral changes in cartilage repair is not fully understood, and whether the statistically significant changes we did observe in the studies do have any clinical implications thus remain uncertain. Nevertheless, since subchondral bone changes are associated with the initiation and progression of OA (Burr 2004, Radin and Rose 1986), they should be a matter of concern.

Mos resulted in a significantly higher rise of synovial fluid proteoglycan content compared to Mfx at 12 weeks follow up. The difference observed at 12 weeks subsided with time, being non-

significant at 24 and 36 weeks follow up. There was no effect of time for neither of the techniques. As for study III we consider the significant difference in rise seen at 12 weeks a result of the surgery, indicating that Mos is more traumatizing than Mfx. This is in agreement with how the techniques are being performed. On the other hand, synovial fluid analyses did not indicate a degenerative process in any of the groups during the observation period.

GENERAL CONCLUSIONS

1. Patients with focal cartilage defects in their knees have major problems with pain and functional impairment. Their complaints are worse than those of ACL deficient patients, and quality of life is affected to the same extent as in patients scheduled for knee replacement. Based on the literature we conclude that the treatment options for cartilage patients are still not sufficient. Considering the substantial number these patients represent and the degree of complaints they have we suggest that improved treatment methods are urgently called for.

2. Patients with a cartilage defect in their knee detected at arthroscopy may improve their knee function over the subsequent 5-8 years with or without cartilage repair. However, they cannot expect to regain normal knee function. Moreover, even in a young patient population radiological changes in the affected knee can be expected at a 6-year follow up.

3. The intraarticular location of a focal cartilage defect play a role in the natural history of defect filling and subchondral mineralized tissue changes in the rabbit knee. In evaluating new clinical techniques based on the results from animal models, the choice of location should be considered. Moreover, interpretation of previous literature should be based on the same knowledge.

4. In the rabbit knee mosaic plasty resulted in higher degree of tissue filling than microfracture technique, however, large standard deviations imply unpredictable results in the single case – even in a standardized animal model. Mosaic plasty affected the subchondral mineralized tissues more than microfracture technique, the implications, however, remain to be settled.

5. Experimental studies evaluating both natural history and cartilage repair outcome regarding tissue filling of defects and changes in subchondral mineralized tissue necessitate long term follow up to substantiate the final results.

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I

II

6-year follow up of 84 patients with cartilage defects in the knee:

Knee scores improved but recovery was incomplete

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Abstract:

Background and purpose: The natural history of focal cartilage injury is largely unknown. This study investigated 6-year outcomes in patients with arthroscopically verified, focal, full thickness cartilage injuries in the knee.

Methods: In a previous report (baseline study) of 993 knee arthroscopies, 98 patients were less than 50 years old at baseline, and showed a grade 3-4 focal cartilage injury, assessed with the International Cartilage Repair Society (ICRS) scale. In the present study, 84 of the 98 patients completed follow ups at median 6.1 years (range 5.3-7.8) after baseline assessments. At baseline the patients underwent different types of cartilage repair (n= 34) or had no treatment or only debridement (n=64) for their cartilage injury. The follow up included evaluations with the ICRS Knee Evaluation form, the Lysholm score, and other knee evaluation tests. 68 patients underwent weight bearing radiographic assessments.

Results: Improvements compared to baseline were noted in the average ICRS functional score, Visual Analog Scale pain score, and the patients' rating of the function in the affected knee compared to the contra-lateral knee. However, the average ICRS activity level had decreased from baseline. The average Lysholm score was 76 (SD 21). 19 patients exhibited Kellgren-Lawrence grades 2-3 in the affected knee, and 6 patients exhibited grades 2-3 in the contra-lateral knee and there was a statistically significant difference between affected and contra-lateral knees.

Interpretation: Patients with arthroscopically diagnosed ICRS grade 3-4 cartilage injuries in the knee may improve their knee function over the subsequent 5-8 years, with or without cartilage repair. However, knee function remained substantially affected. Further studies are needed to determine whether cartilage surgery can yield better functional outcomes than non-surgical or less invasive surgical treatments.

Introduction:

Focal cartilage and osteochondral injuries in the knee are common (Hjelle et al. 2002, Årøen et al. 2004, Widuchowski et al. 2007) and may cause quality of life impairments similar to those associated with severe osteoarthritis (OA) (Heir et al. 2009). The natural history of focal cartilage injuries is largely unknown. For example, it is not clear whether cartilage injury always leads to some degree of OA, or whether there is a critical size or depth of cartilage injury that progress to OA. Cartilage injuries are commonly associated with anterior cruciate ligament (ACL) injuries. Data from the Norwegian National Cruciate Ligament Registry show that preoperative Knee injury and Osteoarthritis Outcome Scores (KOOS) in patients that have ACL with an associated cartilage injury are similar to those of patients that have ACL with no associated cartilage injury (Hjermundrud et al. 2010) indicating that cartilage injuries do not always affect knee function. Although many cartilage injuries are asymptomatic, several randomized controlled trials on the repair of chronic focal cartilage injuries have shown that cartilage injuries may lead to severe disability (Bentley et al. 2003, Horas et al. 2003, Knutsen et al. 2004, Gudas et al. 2005, Knutsen et al. 2007, Saris et al. 2008, Saris et al. 2009). Taken together, these studies have not demonstrated that one method is superior to any other, but none have included a control group of patients that did not undergo cartilage repair. The best outcomes have been reported after autologous chondrocyte implantation (ACI) in young, active patients with a high preoperative score, a single defect, and less than 2 years of symptoms (Krishnan et al. 2006). Due to the lack of knowledge concerning the natural history of cartilage injuries, we performed a 6-year follow up study on a group of patients with a cartilage injury found at arthroscopy. Our study hypothesis was that patients with an arthroscopically diagnosed cartilage injury would exhibit no change in knee function at the 6-year follow up compared to baseline.

Patients and methods

The patients in this follow up study were part of a group of patients from a previous report (baseline study) of 993 knee arthroscopies (Årøen et al. 2004) (Figure 1). This follow up study included the following inclusion criteria: a cartilage lesion classified as a focal ICRS (International Cartilage Repair Society 1998) grade 3-4 injury, and less than 50 years old at the time of arthroscopy in the baseline study. Both traumatic and osteochondritis dissecans (OCD) lesions were included. Patients classified with

general osteoarthritis either by radiographs or at arthroscopy were excluded. 98 patients fulfilled the inclusion criteria. Of these, 2 patients had died; thus, the remaining 96 patients were asked to participate in the follow up study. 5 patients were lost to follow up. 7 patients responded, but did not attend the follow up examination, and did not return the questionnaires after repeated contact by letter and telephone. 84 patients completed the questionnaires at median 6.1 years (range 5.3-7.8) after the baseline arthroscopy. Among these, 77 patients attended a physical examination and 7 returned the questionnaires by mail. 68 patients completed the radiographical examination.

The patients were divided into 4 subgroups according to the procedures that were performed at baseline (Table 1). The surgical procedures stated were performed as a part of the arthroscopic procedure. Patients undergoing a cartilage biopsy for cell culture before autologous chondrocyte implantation (ACI) were stated as treated with ACI at baseline as the arthroscopy in this case was a part of a two stage procedure. Group 1 (n=25) had no cartilage repair and no ligament/meniscal surgery performed at baseline. Of these,13 underwent only a diagnostic knee arthroscopy, 7 underwent debridement, 3 underwent a lateral retinacular release, 1 underwent fixation of a fracture, and 1 underwent removal of an implant; Group 2 (n=39) did not undergo cartilage repair , but did undergo ligament and/or meniscal surgery at baseline; Group 3 (n=21) underwent cartilage repair at baseline, but did not undergo additional ligament/meniscal surgery; and Group 4 (n=13) underwent both cartilage repair and additional ligament/meniscal surgery at baseline. Cartilage repair was defined as ACI, microfracture, osteochondral cylinder transfer (OCT), or fixation of an osteochondral fragment. These procedures, together with a post-operative rehabilitation program have the goal to induce a repair of the cartilage defect. The aim of debridement of a cartilage lesion was to reduce mechanical symptoms and/or inflammation and was consequently not considered a cartilage repair.

In the baseline study (Årøen et al. 2004); assessments were based on the first version of the ICRS form (International Cartilage Repair Society 1998). Therefore, the same version was used in this follow up study to facilitate comparisons.

The baseline characteristics of the patients are given in Table 1. The ICRS form included a history of previous surgeries in the knee. This information was supplemented by records of surgery from our hospital and other hospitals. Surgical procedures that were performed before baseline, at baseline, and after baseline are shown in Table 2.

The primary outcome variable was change in ICRS functional level from baseline according to the ICRS form. Secondary outcome variables were change in ICRS activity level and change in ICRS patient's rating of the affected knee compared to the contra-lateral knee. The ICRS questionnaire also included a Visual Analogue Scale (VAS), where zero represented a pain free joint and 100 represented "severe pain". Changes in the outcome variables over time were calculated for all patients and for the subgroups.

Other questionnaires used at follow up included: The Tegner score (Tegner and Lysholm 1985), the Lysholm score (Lysholm and Gillquist 1982), the KOOS (Roos et al. 1998), the International Knee Documentation Committee's Subjective Knee Form (IKDC) (Hefti et al. 1993, Irrgang et al. 2001), the Cincinnati Knee Rating System (Noyes et al. 1983), and the Short Form 36 Health Survey (SF- 36) (Ware, Jr. and Sherbourne 1992). Both the Lysholm score (Kocher et al. 2004) and the KOOS score (Bekkers et al. 2009) have been validated for patients with cartilage injuries. The Tegner and Lysholm scores are not completely self explanatory, thus an examiner provided guidance for the patients as they filled out the form. The other forms were filled out by the patients without guidance.

According to the ICRS form, the patients did single leg length-jumps on the right and left legs (completed by 59 patients). The average length of 3 jumps was calculated for each leg. The average jump distance performed with the affected leg was expressed as a percentage of the average jump distance performed with the contra-lateral leg as follows: affected leg function = average jump with affected leg/average jump with contra-lateral leg × 100%. The results were classified into 4 categories: 1: > 90%, 2: 76-90%, 3: 50-75% and 4: <50%.

Weight bearing radiographs were performed according to a routine protocol: Anteroposterior view with extended knees, and a lateral view. The radiographs were examined by one of the authors (LE), who was blinded in regard to the affected side, and they were classified according to Kellgren and Lawrence criteria (Kellgren and Lawrence 1957).

Statistics:

We used Wilcoxon paired rank test for assessing changes in the following variables from baseline: the ICRS functional level, the ICRS activity level, and the ICRS rating of the knee compared to the contra-lateral knee. All these were categorical variables with 4 levels (1-4); patients served as their own controls, and p-values were adjusted for tied observations. A paired t-test was used for analyzing VAS pain scores. For each dependent outcome variable, a linear regression model was fitted to see if the following parameters were predictors: Area of the cartilage lesion, location of the cartilage lesion, cartilage repair performed at baseline and ligament or meniscal surgery performed at baseline. After univariate and multivariate analyses of these factors, additional adjustment for the following potential confounding factors; age at the start of symptoms, age at operation, sex and body mass index (BMI) was performed in a multivariate analysis. For functional assessments, we regarded an average difference of 0.5 units (17%) on the 1-4 functional level scale as a difference of clinical interest. A priori sample size calculation showed that 47 patients would be sufficient to detect a 0.5 unit average change in functional level from baseline with a standard deviation of 1.2 units, with 80 % power, and with a 5% significance level.

Radiographic assessments of affected and contra-lateral legs were compared with a Wilcoxon paired rank test. We used SPSS® version 15.0 (SPSS Inc. Chicago, Illinois, 2006) for the statistical analysis. For all analyses, significance level was set to 5 %.

Ethics

The study had a non-interventional design and was approved by the regional ethical committee (Date of issue: 11.10.2004 - registration number 517-04197). All patients provided informed written consent to participate.

Results

Change from baseline (ICRS- form):

At the follow up, patients assessments of knee defects indicated that, on average, the functional level increased, the VAS pain score decreased, the rating of knee function compared to the contra-lateral knee increased, and the activity level decreased compared to baseline (Table 3). All these changes were statistically significant ($p \leq 0.001$). All subgroups reported improvements in functional levels and in VAS pain scores, but the change was not significant in the smallest group (cartilage repair + ligament/meniscal surgery). All subgroups reported improvements in knee function compared to the contra-lateral knee. All subgroups reported a reduction in activity level, but the reduction was statistically significant only in the largest group (no cartilage repair, but with ligament/meniscal surgery).

Other test scores at follow up:

All the knee specific scores; the KOOS, Lysholm, Tegner, IKDC, Cincinnati Knee Rating System, SF-36, and the single leg length jump showed average knee function in the range of 61 to 86% of the maximum score for the total group, with the KOOS quality of life and sport/recreation being mostly affected and the KOOS activities of daily life being least affected (Table 4 and supplementary data). The average Tegner score was 4.3 (SD 1.9), which corresponded to a moderate level of non-pivoting sports. The physical components of the SF-36 general health score were in the range of 71 to 83% of the maximum score.

Linear regression analysis

For the primary outcome variable (change in ICRS functional level) there was no predictive association with the area or location of the cartilage lesion, cartilage repair performed at baseline and ligament or meniscal surgery performed at baseline in univariate or multivariate analysis (Table 5 and supplementary data). Adjustment for age at the start of symptoms, age at operation, sex and BMI did not alter these findings. The same result was found for all outcome variables, including Lysholm score, Cincinnati score, IKDC, KOOS and SF-36 (data not shown). An association was found between BMI and the Kellgren-Lawrence grade of the operated knee (multivariate analysis, $p = 0.002$).

Radiographs:

Radiographs were obtained from 68 patients. The difference between the operated and contra-lateral knee was statistically significant in the total group of patients and in subgroups 1, 2, and 3. No significant differences were found between operated knees between all subgroups (Kruskal-Walis non-parametric test for several independent samples) or between operated knees in pairs of subgroups (Mann-Witney U non-parametric test for 2 independent samples) (Table 6).

Discussion

We found improvements in knee ICRS functional scores over time. In a linear regression analysis, we did not detect any association between the type of surgery (including cartilage repair) and the functional outcome. However, these results must be interpreted with caution because they were averages from a mixed patient cohort. The most common cartilage repair procedure performed in our patients was microfracture; thus, the results may not be generally applicable to all cartilage repair patients. Our study was not originally designed to compare different treatment procedures. Groups 3 and 4 had a higher proportion of lesions that were larger than $2cm^2$ compared to groups 1 and 2. Moreover, most cartilage lesions were patellar in groups 1 and 2. Consequently, the groups should not be compared; each should be evaluated separately. The Lysholm score (average 76) and Cincinnati score (average 71) were low at follow up; this indicated that cartilage defects had severe impacts on knee function, irrespective of treatment. Another study from our group on other patient cohorts has confirmed this observation (Heir et al. 2009).

The improvements observed in knee scores may have been due to a real improvement, to the various therapeutic procedures performed, to a placebo effect of surgery, and/or to a favorable natural history. Another explanation for the observed improvement in the functional score and the reduction of the pain rating on the VAS-scale may be that the patients reduced their activity levels over the years after the initial surgery; with less physical activity, there may be less pain.

Most studies that evaluated outcomes after cartilage surgery have reported improvements in functional scores, both in case series and in randomized controlled

trials (RCT). In a review of studies that evaluated outcomes after cartilage repair (Jakobsen et al. 2005), Lysholm scores were reported in 17 of the 61 studies included in the review, with 95% confidence intervals of 78-97 for microfracture, 86-95 for OCT, and 67-99 for ACI, with no statistically significant differences between the treatment modalities. Results from 2 RCTs that evaluated Lysholm scores (Horas et al. 2003, Knutsen et al. 2007) and 1 RCT that evaluated KOOS scores (Saris et al. 2008) after cartilage surgery, showed mean scores that were in the same range at follow up as those found in our study. Results from 2 other RCTs could not be compared with our data. One, by Bentley et al (2003), used the Cincinnati score, but categorized the patients into groups without giving the mean values; the other, by Gudas et al (2005), used the ICRS questionnaire without reporting their methods for the calculations. Thus, the patients in our study had an average functional level similar to that of patients that had undergone cartilage surgery in RCTs that gathered similar data. However, patients in RCTs must fulfill strict inclusion criteria; thus, those results may not be applicable to all patients with cartilage injuries (Engen et al. 2009). Nevertheless, conclusions from RCTs are often generalized to all patients with cartilage injuries. One strength of our study was that it represented the average patient with a cartilage injury that is seen in orthopedic practice.

The Lysholm score we found was in the same range as that found in patients with ACL-injury awaiting surgery (Drogset et al. 2005). The KOOS scores we found were slightly higher compared to preoperative KOOS scores in the Norwegian National Knee Ligament Registry (Granan et al. 2008), but they were still clearly abnormal, reflecting the functional impairment of patients with cartilage injuries.

Our cohort consisted of relatively young patients (average 32 years old at baseline) with a cartilage injury that was classified as a full thickness focal injury at arthroscopy. Patients with a knee defect classified as general osteoarthritis at arthroscopy were excluded from our study. Nevertheless, the radiographical examinations showed substantial osteoarthritic changes in affected knees 6 years after baseline surgery, even in groups with isolated cartilage injuries (without additional ligament or meniscal injuries), and suggest a relationship between focal cartilage injuries and early development of osteoarthritis. This is in accordance with the findings from a study by Knutsen et al. (2007), who reported osteoarthritis in one

third of the patients 5 years after ACI or microfracturing. An association has also been shown between OCD diagnosed in patients after closure of the epiphyseal plates (average age 29 years old) and osteoarthritis later in life (average age 62 years old) (Linden 1977). In a regression analysis, we found that a high BMI was associated with a high Kellgren-Lawrence grade, consistent with results from several other studies (Spector et al. 1994, Hochberg et al. 1995, Felson et al. 1997). We did not obtain long radiographs from hip to ankle; thus, we could not measure alignment. Consequently, we could not assess a possible additional effect of malalignment on the side to side differences shown.

Our patients with cartilage defects were heterogeneous with respect to knee scoring and co-morbidites. However, this is also true in published RCTs, which have shown a wide range in knee scores, both preoperatively and postoperatively, and a substantial percentage of patients with additional co-morbidities (Horas et al. 2003, Knutsen et al. 2004, Knutsen et al. 2007, Saris et al. 2008, Saris et al. 2009). Thus, our follow up study reflects the clinical variation observed in patients with cartilage defects.

One limitation of our study is that, despite a high follow up rate (84 of 96 patients; 87%), the number of patients was low; moreover, half had additional injuries that caused difficulties in the interpretation of the results. In addition, several patients had had surgery in the same knee, before (42 patients) and/or after (32 patients) baseline arthroscopy. Another limitation is the use of the non-validated ICRS questionnaire. This had been published (International Cartilage Repair Society 1998) about the time that the baseline study was planned. At that time, no validated outcome scores existed for patients with cartilage injuries, and the ICRS questionnaire was regarded as a new and improved tool for the evaluation of patients with cartilage injuries. Therefore, to supplement the ICRS data, we included other, validated questionnaires and a general health score (SF-36) at the follow up. The categorization of patients into subgroups was intended to give a better description of the cohort, but the subgroups were too small to be compared statistically with each other. In any case, a comparison would have been of limited value due to several confounding factors. Thus, a regression analysis to detect possible predictors and to correct for confounding factors was performed.

Our results should remind surgeons involved in cartilage repair to question whether non-operative treatment modalities, like active rehabilitation, might be sufficient. At present, in our institution, all patients that are candidates for cartilage surgery studies are enrolled in a 3 months physical training program study before surgery. To date, many patients have improved on this program and decided to postpone surgery. Several RCTs have been conducted to evaluate the efficacy of surgical cartilage repair methods (Bentley et al. 2003, Horas et al. 2003, Knutsen et al. 2004, Gudas et al. 2005, Knutsen et al. 2007, Saris et al. 2008, Saris et al. 2009). In addition, many case series have been conducted. However, to our knowledge, no studies have included a control group of patients that received non-surgical or no treatment. In an RCT that compared mosaic plasty and ACI, where debridement of the lesion was performed at the time of enrollment, one third of the patients improved after the initial debridement, and further cartilage surgery was not needed (Dozin et al. 2005). In a report of 28 patients with a cartilage defect in the knee diagnosed at arthroscopy, 22 patients functioned well 14 years after diagnosis (Messner and Maletius 1996). In that study, Pridie drilling in 3 patients and cartilage shaving or removal of free bodies in some patients were performed initially. During follow up 5 of the patients underwent arthroscopy and of these 3 had removal of free bodies. These findings, together with the results from our study, suggest that non-operative treatment or less invasive surgery may be sufficient to relieve symptoms for many patients with knee cartilage defects.

In summary we found that improvements in functional scores was possible without cartilage surgery in many patients with knee cartilage defects. However, knee function remained seriously affected. This study was not designed to compare operative and non-operative treatments of cartilage lesions. Further studies are needed to determine whether cartilage surgery can yield better functional outcomes than non-surgical or less invasive surgical treatments. We suggest that a control group of patients receiving non-surgical treatment should be included in future RCTs.

Contribution of the authors:

SL, SH, LE and AÅ participated in study design and data collection. IH were the supervisor of the statistical analysis. LE classified the radiographs. All authors were

involved in the statistical analysis, the interpretation of the data and in writing the manuscript.

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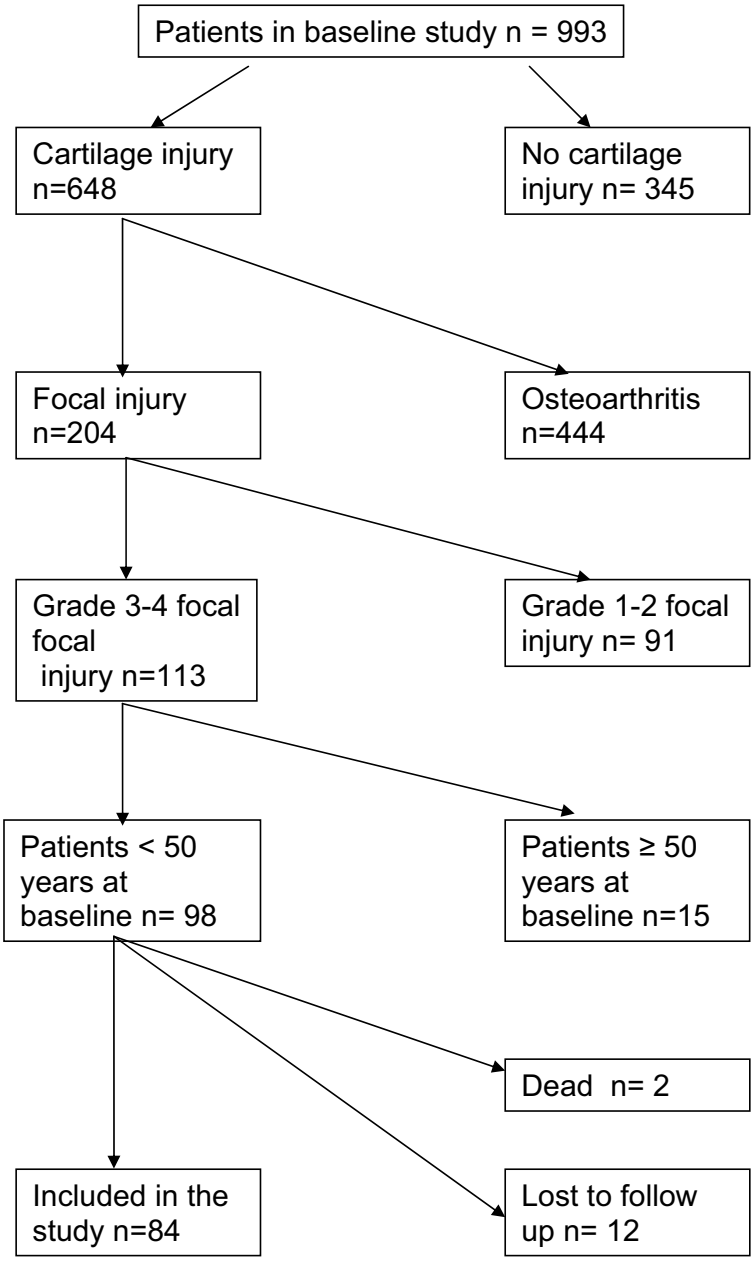

Figure 1: Flow chart showing the selection of patients from the baseline study to the follow up study.

Table 1. Baseline characteristics of patients with knee defects. Patients were grouped based on the types of procedures performed.

	Subgroup 1: No cartilage repair[a] - no ligament/meniscus surgery n=25	Subgroup 2: No cartilage repair - but with ligament/meniscus surgery n=39	Subgroup 3: Cartilage repair - no ligament/meniscus surgery n=21	Subgroup 4: Cartilage repair - and ligament/meniscus surgery n=13	All patients N=98[b]
Baseline characteristics: mean (SD)					
Age at the start of symptoms	25 (10)	31 (11)	25 (11)	32 (8.9)	28.2 (11)
Age at baseline	28 (9.4)	35 (9.4)	30 (10)	35 (8.6)	31.9 (9.7)
BMI at baseline	24 (4.4)	25 (3.8)	24 (3.3)	26 (4.5)	24.8 (3.9)
Females/males (ratio)	12/13	14/25	8/13	4/9	38/60
Area of cartilage defect [c](no. of patients)					
< 1 cm2	3	7	0	1	11
1-2 cm2	8	18	6	0	32
2-4 cm2	6	6	6	6	24
> 5 cm2	8	8	9	6	31
Sum	25	39	21	13	98
Localization of cartilage defects (no. of defects)[d]					
Lateral femoral condyle	5	5	2	5	17
Medial femoral condyle	9	21	17	8	55
Lateral tibial condyle	4	5	2	0	11
Medial tibial condyle	4	2	0	2	8
Patella	10	14	3	1	28
Sum	32	47	24	16	119[e]

[a] Cartilage surgery was defined as: an autologous chondrocyte implantation (with a preceding biopsy), an osteochondral cylinder transfer, a microfracture repair, or fixation of osteochondral fragment.

[b] All patients fulfilling the inclusion criteria were included in the table (n=98). Of these, 2 were dead, 14 were lost to follow up and 84 were included in the final follow up.

[c] In patients with more than one defect, the areas represent the sum of the areas.

[d] All injuries were rated grade 3 or 4 by the International Cartilage Repair Society scale.

[e] Some patients had more than one defect; hence, the total number of defects exceeded the number patients.

Table 2. The numbers of patients with knee defects that underwent the procedures shown. Patients were grouped based on the types of procedures performed at baseline. In some cases, more than one procedure was performed in a single knee, thus the number of procedures exceeds the number of patients.

	Subgroup 1: No cartilage repair[a] - no ligament/meniscus surgery n=25			Subgroup 2: No cartilage repair - but with ligament/meniscus surgery n=39			Subgroup 3: Cartilage repair - no ligament/meniscus surgery n=21			Subgroup 4: Cartilage repair - and ligament/meniscus surgery n=13			All patients n=98[b]		
	Before[c]	Base-line	After	Before	Base-line	After	Before	Base-line	After	Before	Base-line	After	Before	Base-line	After
Cartilage repair:															
Microfracture	1						1	11	1	1	10	2	3	21	3
Osteochondral cylinder transfer			1					2	1					2	2
Biopsy preceding ACI [d]			1			1		4	1		3	1		7	4
Fixation of osteochondral fragment								4						4	
Other procedures:															
Diagnostic arthroscopy	6	13	3	7	6		9			2			24	19	3
Debridement of cartilage Injury		7	1		3	1			3			1		10	6
Ligament reconstruction	1			2	10	1	1			2	2	2	6	12	3
Meniscal resection	1			7	24	2			2	1	5		9	29	4
Other	2	5	3			2			1			1	2	5	7
No information	3			2									5		

[a] Cartilage repair was defined as: an autologous chondrocyte implantation (with a preceding biopsy), an osteochondral cylinder transfer, microfracture, or fixation of osteochondral fragment.

[b] All patients fulfilling the inclusion criteria were included in the table (n=98). Of these, 2 were dead, 14 were lost to follow up and 84 were included in the final follow up.

[c] The time the procedures were performed, expressed relative to the baseline assessment: Before = before baseline; Baseline = at baseline; and After = after baseline (during follow up).

[d] ACI = autologous chondrocyte implantation

Table 3. Knee function assessments based on ICRS questionnaires at baseline and follow up. Patients were grouped based on the types of procedures performed. Values indicate the numbers of patients in each subgroup that agreed with the corresponding item.

	No cartilage repair[a] - no ligament/meniscus surgery n=19/25[b]		No cartilage repair - but with ligament/meniscus surgery n=35/39		Cartilage repair - no ligament/meniscus surgery n=18/21		Cartilage repair - and ligament/meniscus surgery n=12/13		Total group n=84/98	
Functional level	Baseline	Follow up	Baseline	Follow up	Baseline	Follow up	Baseline	Follow up	Baseline	Follow up
I can do everything I want do with my knee	1	6	0	6	0	4	0	1	1	17
I can do nearly everything I want to do with my knee	4	6	5	19	2	8	1	4	12	37
I am restricted and a lot of things that I want to do with my knee are not possible	8	5	20	10	10	6	7	4	45	25
I am very restricted and I can do almost nothing with my knee without severe pain and disability	9	2	11	0	7	0	4	3	31	5
Total	22	19	36	35	19	18	12	12	89	84
Wilcoxon test	p=0.05		p<0.001		p=0.002		p=0.096		p<0.001	
Activity level										
Are you a highly competitive sportsman/sportswoman?	3	1	9	4	6	1	6	2	24	8
Are you well trained and frequently sporting?	10	5	14	9	5	6	1	2	30	22
Sporting sometimes	8	10	10	17	5	9	4	5	27	41
Non sporting	2	3	3	4	4	2	2	3	11	12
Total	23	19	36	34	20	18	13	12	92	83
Wilcoxon test	p=0.05		p=0.03		p=0.2		p=0.2		p=0.001	
Patient's rating of the knee function in comparison with the contralateral knee										
90-100%	1	8	1	18	1	7	0	4	3	37
70-90%	5	4	10	15	3	7	0	3	18	29
40-70%	8	4	19	2	10	1	9	2	46	9
0-40%	9	2	5	0	5	2	3	1	22	5
Total	23	18	35	35	19	17	12	10	89	80
Wilcoxon test	p=0.009		p<0.001		p=0.009		p=0.03		p<0.001	
VAS Pain[c]										
Mean values (SD)	55.7 (23.4)	33.6 (29.2)	44.2 (20.8)	23.5 (19.4)	50.9 (23.1)	31.8 (23.0)	46.0 (24.1)	31.0 (23.3)	48.3 (22.3)	28.5 (22.9)
Change in VAS pain (SD)	22.1 (37.7)		20.6 (24.8)		19.1 (13.0)		15.0 (24.6)		19.8 (25.3)	
95% Confidence interval	[0.4 - 43.9]		[11.6 - 29.7]		[12.4 - 25.8]		[-2.6 - 32.6]		[13.9 - 25.7]	
Paired t-test	p=0.05		p<0.001		p<0.001		p=0.09		p<0.001	

[a] Cartilage repair was defined as: an autologous chondrocyte implantation (with a preceding biopsy), an osteochondral cylinder transfer, microfracture, or fixation of osteochondral fragment.

[b] Refers to the number of patients who attended follow up in relation to the number of patients at baseline.

[c] VAS = Visual Analogue Scale; zero represented a pain free joint and 100 represented “severe pain”.

Table 4. Various tests conducted at follow up to assess knee function. Patients were grouped based on the types of procedures performed.

	Subgroup 1: No cartilage repair[a] - no ligament/meniscus surgery n=19/25[b]	Subgroup 2: No cartilage repair - but with ligament/meniscus surgery n=35/39	Subgroup 3: Cartilage repair - no ligament/meniscus surgery n=18/21	Subgroup 4: Cartilage repair - and ligament/meniscus surgery n=12/13	All patients: N=84/98
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Lysholm	71 (23)	83 (16)	75 (24)	67 (24)	76 (21)
Tegner[c]	3.7 (2.2)	4.9 (1.6)	4.4 (1.6)	3.8 (2.5)	4.3 (1.9)
KOOS Pain	72 (22)	82 (19)	80 (21)	72 (29)	78 (22)
KOOS Symptoms	72 (22)	78 (20)	76 (17)	67 (24)	75 (21)
KOOS Activities of daily life	83 (20)	89 (17)	87 (16)	79 (26)	86 (19)
KOOS Sport and recreation	55 (34)	68 (28)	63 (33)	48 (39)	61 (33)
KOOS Knee-related quality of life	55 (27)	64 (27)	64 (24)	54 (28)	61 (26)
IKDC	60 (24)	70 (21)	69 (24)	59 (29)	66 (23)
Cincinnati	67 (27)	76 (17)	71 (25)	62 (28)	71 (23)
SF36 Physical Function	71(22)	83 (17)	80 (23)	70 (28)	78 (22)
SF36 Role Physical	75 (25)	86 (22)	92 (14)	73 (32)	83 (24)
SF36 Bodily Pain	62 (24)	78 (24)	73 (26)	65 (33)	71 (26)
SF36 General Health	72 (20)	81 (19)	75 (21)	77 (24)	77 (20)
SF36 Vitality	58 (20)	71 (18)	66 (22)	61 (24)	65 (21)
SF36 Social Function	88 (14)	91 (15)	93 (15)	84 (23)	90 (16)
SF36 Role Emotional	94 (12)	93 (14)	97 (6)	91 (17)	94 (13)
SF36 Mental Health	86 (10)	84 (12)	87 (7)	83 (15)	85 (11)
Single leg jump[d]:					
1. > 90 %	9	15	6	5	35
2. 76-90%	1	7	3	2	13
3. 50-75%	1	4	1	1	7
4. < 50%	0	3	0	1	4

[a] Cartilage repair was defined as: an autologous chondrocyte implantation (with a preceding biopsy), an osteochondral cylinder transfer, microfracture, or fixation of osteochondral fragment.

[b] Refers to the number of patients who attended follow up in relation to the number of patients at baseline.

[c]Tegner activity score: Categorical variable 1-10 (10= highest level).

[d]Single leg jump: Categorical variable 1-4 (average one-leg jump distance with affected knee/distance with contra-lateral knee × 100%). All other scores are numeric variables 0-100 (100 = best score).

Abbreviations: KOOS= Knee injury and Osteoarthritis Outcome Score; ADL= activities of daily life; QOL=quality of life; IKDC= International Knee

Documentation Committee's Subjective Knee Form; Cincinnati= Cincinnati Knee Rating System; SF36= Short Form 36 Health Survey.

Table 5: Linear regression analysis shown for the main outcome variable: "Change in ICRS functional score". First a univariate regression was made for the following possible predictors at baseline: area of the cartilage lesion, location of the cartilage lesion, additional ligament or meniscus surgery and cartilage surgery. Then a multivariate analysis was made for these 4 variables combined. Finally, a second multivariate analysis was made adjusting for age at the start of symptoms, age at baseline, sex and BMI.

	Univariate					Multivariate (first 4 variables)					Multivariate (all variables)				
	B	95% CI		p-value	R^2	B	95% CI		p-value	R^2	B	95% CI		p-value	R^2
Possible predictors:															
Area of cartilage lesion	-0.038	-0.27	0.20	0.7	0.001	-0.075	-0.33	0.18	0.6	0.015	-0.052	-0.34	0.24	0.7	0.017
Location of cartilage lesion	-0.026	-0.16	0.11	0.7	0.002	-0.011	-0.15	0.13	0.9		0.016	-0.14	0.17	0.8	
Ligament or meniscus surgery	-0.014	-0.50	0.47	1.0	<0.001	0.012	-0.50	0.52	1.0		-0.012	-0.61	0.58	1.0	
Cartilage repair	0.205	-0.29	0.71	0.4	0.009	0.256	-0.30	0.82	0.4		0.204	-0.40	0.81	0.5	
Possible confounding factors:															
Age at the start of symptoms											-0.007	-0.05	0.04	0.7	
Age at baseline											0.008	-0.04	0.06	0.7	
Sex											-0.169	-0.75	0.41	0.6	
Body Mass Index											0.018	-0.06	0.09	0.6	

Table 6. The numbers of patients with knee defects graded 0-3 on radiographs at follow up. Weight bearing radiographs were graded according to Kellgren and Lawrence. Patients were grouped based on the types of procedures performed.

	Subgroup 1: No cartilage repair[a] - no ligament/meniscus surgery N=15/25[b]		Subgroup 2: No cartilage repair - but with ligament/meniscus surgery N=27/39		Subgroup 3: Cartilage repair -no ligament/meniscus surgery N=17/21		Subgroup 4: Cartilage repair -and ligament/meniscus surgery N=9/13		All patients: N=68/98	
	Operated knee	Contra-lateral knee	Operated knee	Contra-lateral knee	Operated knee	Contra-lateral knee	Operated knee	Contra-lateral knee	Operated knee	Contra-lateral knee
Grade 0	10	15	8	22	8	15	3	5	29	57
Grade 1	2		9	4	6		3	1	20	5
Grade 2	1		8	1	3	2	2	1	14	4
Grade 3	2		2				1	2	5	2
Wilcoxon test for difference between operated and contra-lateral knee	p=0.041		p<0.001		p=0.033		p=0.78		p<0.001	

[a] Cartilage repair was defined as: an autologous chondrocyte implantation (with a preceding biopsy), an osteochondral cylinder transfer, microfracture, or fixation of osteochondral fragment.

[b] Refers to the number of patients with radiographs at follow up in relation to the number of patients at baseline.

III

Heir S et al.

Intra-articular location predicts cartilage filling and subchondral bone changes in a chondral defect

A randomized, blinded, long term follow up trial in 82 rabbit knees

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ABSTRACT

Background and purpose

The natural history and predictive factors for outcome of cartilage restoration in chondral defects are poorly understood. We investigated the natural history of cartilage filling and subchondral bone changes, comparing defects at two locations in the rabbit knee.

Animals and Methods

In New Zealand rabbits aged 22 weeks, a 4 mm pure chondral defect (ICRS grade 3b) was created in patella in one knee and in the medial femoral condyle in the other. A stereomicroscope was used to optimize the preparation of the defects. The animals were sacrificed 12, 24 and 36 weeks after surgery. Defect filling and the density of subchondral mineralized tissue was estimated using Analysis Pro® software on micrographed histological sections.

Results

The mean filling of the patellar defects was more than twice that of the medial femoral condylar defects at 24 and 36 weeks follow up. There was a statistically significant increase in filling from 24 to 36 weeks after surgery at both locations.

The density of subchondral mineralized tissue beneath the defects subsided with time in the patellas, in contrast to the density in the medial femoral condyles which remained unchanged.

Interpretation

The intraarticular location is a predictive factor for spontaneous filling and subchondral bone changes of chondral defects corresponding to ICRS grade 3b. Disregarding location, the spontaneous filling increased with long term follow up. This knowledge should be considered when evaluating aspects of cartilage restoration.

Introduction

Focal articular cartilage injuries in the knee are common (Hjelle et al. 2002, Aroen et al. 2004), and may impair quality of life equally to that of severe osteoarthritis (Heir et al. 2010). The literature concerning natural history of focal cartilage defects in patients, and the intrinsic factors affecting it, is limited (Linden 1977, Messner and Gillquist 1996, Drogset and Grontvedt 2002, Shelbourne et al. 2003, Loken et al. 2010). In experimental studies evaluating cartilage restoration in general, the importance of intrinsic factors such as the depth and size of the lesion and the time from the lesion was made to evaluation have been emphasized (Shapiro et al. 1993, Hunziker 1999, Lietman et al. 2002). Which part of the joint being affected and whether the defect is weight bearing or not, is also of interest (Hurtig 1988, Frisbie et al. 1999). Most of these studies, however, are on defects penetrating the subchondral mineralized tissues corresponding to ICRS grade 4 (Brittberg and Winalski 2003). Access to bone marrow elements in these defects might be one of the strongest predictive factors for filling of the defect, making the importance of other factors difficult to evaluate (Hunziker 1999).

In experimental studies on pure chondral defects not penetrating the subchondral mineralized tissues corresponding to ICRS grade 3b (Brittberg and Winalski 2003), the type of animals studied, the size of the lesion, and the location of the defects vary, and there is limited data on the influence of these parameters in relation to outcome (Breinan et al. 2000). The information on spontaneous filling is mainly from observations of untreated defects serving as controls (Grande et al. 1989, Brittberg et al. 1996, Breinan et al. 1997, 2000, Frisbie et al. 1999, 2003, Dorotka et al. 2005), the information on subchondral bone changes being even more limited (Breinan et al. 1997, Frisbie et al. 1999). Although most human focal cartilage lesions are located on the medial femur condyle (Aroen et al. 2004), experimental studies involving untreated ICRS grade 3b defects on the medial femur condyle are rare (Dorotka et al. 2005). The rabbit knee is the most widely used experimental animal model for cartilage restoration according to a PubMed search (Årøen 2005). Locations of ICRS grade 3 chondral defects in the rabbit knee evaluated for spontaneous changes include patella (Grande et al. 1989, Brittberg et al. 1996) and in one study defects at the distal surface of the femur (Mitchell and Shepard 1976). The latter study, however, do not contain quantitative data.

To our knowledge the influence of the intraarticular location on the natural history outcome of cartilage restoration and subchondral bone changes has not been thoroughly studied. Thus, the primary purpose of our study was to test the hypothesis that the intra-articular location

influences the spontaneous filling of a chondral defect not penetrating the subchondral bone. Secondly, the purpose was to evaluate whether the intraarticular location would influence changes in the subchondral bone and degenerative changes evaluated by macroscopic appearance and synovial fluid proteoglycan content (Messner et al. 1993a).

Methods

We used our established experimental animal model (Aroen et al. 2005). Adult New Zealand rabbits were included in a randomized study where circular lesions, diameter 4 mm, were created in the patella of one knee and pair wise compared to identical lesions in the medial femoral condyle of the contralateral knee. The lesions were pure chondral – down to, but not penetrating the calcified layer – corresponding to ICRS grade 3b. The follow up was 12, 24, and 36 weeks after initial surgery.

The main endpoint was difference in degree of tissue filling between the defects in patella and the condyles at each follow up. Secondary endpoints were difference in changes of the density of subchondral mineralized tissue with time and difference in degenerative changes evaluated by macroscopic appearance and joint fluid proteoglycan content between the two locations at each follow up.

Animal care

The animal environment, diet, application of sterile perioperative conditions, capturing of synovial fluid for proteoglycan analyses, anesthesia, analgesia and the procedure of killing have all been described previously (Aroen et al. 2005). Throughout the follow up period, the experimental animals gained weight obtaining a similar weight at follow up as control animals at corresponding age (Figure 1). The animals were allowed to move freely in their cages, and all animals were able to bear weight on both extremities immediately after surgery. The surgeons were experienced orthopedic surgeons and certified according to the rules of animal care and experimental surgery, and the study was performed according to the guidelines for animal research at the University of Oslo and approved by the Norwegian Government Committee for Experimental Animal Care.

Experimental groups

41 adult New Zealand rabbits were studied. The animals had defects created in both knees at age 22 weeks – in patella of one and in the medial femoral condyle of the other. To avoid "learning curve" as a bias, the animals were block randomized for killing at the follow up time points. 12 animals were planned to be killed at 12 weeks, 12 at 24 weeks, and 17 at 36 weeks. However, due to great loss of animals during follow up, the decision was made to spare animals to secure a sufficient number at 36 weeks follow up since that was considered the most important follow up time point of the study. Partly because of this shift in group sizes, and partly because of other complications throughout the study, the final numbers of patellas / condyles available for evaluation were 8 / 8 at 12 weeks, 9 / 9 at 24 weeks, and 17 / 18 at 36 weeks follow up. The numbers of animals available for paired analyses (sections from both knees available for evaluation) were 8 at 12 weeks, 7 at 24 weeks, and 17 at 36 weeks.

Additionally, 20 knees from 10 animals without initial surgery were evaluated by the same methods as the experimental knees. The mean value of the 2 knees of each animal was used for calculations. 3 animals were killed at age 22 weeks, 3 at age 22 + 12 weeks, 2 at 22 + 24 weeks, and 2 at 22 + 36 weeks. The intention was to add these knees to a larger number of control specimens; harvesting patella as a control specimen from knees with condylar defects and visa versa. However, since surgery had been performed on another joint surface within the same knee, the latter control specimens were excluded. Thus, due to the low number of "control" specimens, the data extracted were used for observational purpose only and were not included in statistical comparisons.

Surgical technique

Through a medial parapatellar incision, a defect (φ = 4 mm) was created in patella in one knee (randomized to left or right) and in the medial femur condyle of the contralateral knee as previously described (Aroen et al. 2005) (Figure 2). A stereomicroscope and small instruments were used (Figure 3), and care was taken to avoid any damage to the rims of the defects or to the underlying calcified cartilage. The defects were left untreated, the joint irrigated, hemostasis obtained, and wound closure performed as previously described (Aroen et al. 2005).

Killing of animals, macroscopic evaluation and preparations for histological analyses

The animals were killed and synovial fluid obtained as previously described (Aroen et al. 2005). The patellas and the femoral condyles were dissected free and gross morphologic grading was performed. Changes to the cartilage corresponding to ICRS grade 1-2 and / or small osteophytes were characterized as minor changes, whereas changes exceeding that were characterized as major. The specimens were immersed in phosphate-buffered 4% paraformaldehyde for 1 week and decalcified in 20 % formic acid until the bone was soft enough for sectioning. Cubes, measuring 8 by 8 mm containing the defect at one side were harvested from the specimens, dehydrated in graded alcohol and embedded in an epoxy resin. The patellas and condyles with no defects were handled in the same manner as the experimental specimens. To define the region for histological analysis, an area corresponding to the location of the experimental defects was outlined using the same kind of 4 mm biopsy punch as used when creating the defects in the experimental knees.

Histology

The cubes were sectioned from one longitudinal surface, the anterior-posterior and medial-lateral orientation of the defect at random (Gundersen et al. 1988). From the point where the rim of the defect was reached, sections – each 1-2 µm thick – were captured at 5 levels, each level 700 µm further into the defect. This technique ensured that one of the sections would represent a level at a maximum distance of 350 µm from the very centre of the defect (Figure 4). The sections were stained with tolouidin blue and micrographed at 40x magnification using a digital camera mounted on the microscope. An interactive semiautomatic image analysis program (Analysis Pro®, Olympus Soft Imaging Solutions, Münster, Germany) was used for all measurements. The section closest to the centre of each defect (largest diameter) was included in the statistical analyses. The observer was blinded to the location of the defects.

Estimation of tissue filling

The borders of the defects were identified by the interfaces between presumed original cartilage and newly formed fibrocartilaginous / fibrous repair tissue on both sides. The midpoint along the tidemark of the defect was defined. From the midpoint, sectors of 0.5 mm length were marked along the tidemark to each side until 2 mm from midpoint was reached on

each side, representing the tidemark at the base of the "shoulders" of the 4 mm defect. Cartilage height was measured at these two shoulder points, as was tissue height at the 7 intersection points of the 0.5 mm length sectors (Figure 5). Tissue filling was estimated by relating the mean height of tissue at the 7 defined points to the mean cartilage height of the 2 shoulders of the same defect, expressed as percentage filling. Whenever one of the shoulders was technically not measurable, the one shoulder left served as the reference. This occurred in sections from 1 patellar defect and 1 condylar defect.

Estimation of the density of subchondral mineralized tissue

The density of the subchondral mineralized tissue was estimated in an area immediately 1.5 mm deep to the tidemark of the defect, the shape of the region of interest depending on the curvature of the tidemark (Figure 5). The morphometry was performed by point counting (Gundersen et al., 1988) using computer software. A grid with 0.3 mm between test lines was superimposed on the micrograph. The intersections between the test lines served as test points. Subchondral mineralized tissue density was expressed as the number of test points overlying mineralized tissue relative to the total number of test points within the region of interest. The counting was repeated twice for each defect with the orientations of the grid randomly selected at both occasions. The mean value of the 2 measurements was used for statistical analysis. A similar technique was used by Løken et al. (2008) demonstrating a variance less than 10% between measurements and between observers.

Analysis of synovial fluid

41 animals had initial surgery at age 22 weeks. From 60 of the knees a wash out sample of the synovial fluid containing a minimum of 0.75 mL collected before surgery (time zero) was available for proteoglycan content analyses using standard ELISA technique (Messner et al., 1993b). A second wash out sample, collected at killing, was available for analyses from 69 of the knees. The discrepancy was mainly due to dry taps. 58 knees had samples available for analyses both at time zero and at killing, whereas 24 animals had samples available from both knees at both time zero and at killing. The samples collected from the experimental animals were all analyzed as one batch.

Statistics

Based on previous experimental studies (Aroen et al., 2006) a filling difference of more than 25% was considered as a proper level to discharge the H_{01}-hypothesis of no difference in tissue filling between the patellae and the condyles. Since the experimental animals all underwent surgery in both knees, with patella defects in one and defects in the MFC of the other, they would serve as their own control regarding tissue filling of the defects, and thus a paired Student t-test could be applied. Preexperimental analysis to detect sample size using a power of 0.80 and a significance level of 0.05 and a standard deviation for the differences of less than 24 % indicated a need of 9 animals in each group for this purpose. To evaluate the difference in tissue filling from one follow up time point to another regarding each location separately, the need of animals was estimated to 12 for each of the 3 follow up time points - due to an unpaired experimental situation. Power and sample size estimations were not performed for the secondary endpoints. To cover up for possible loss of animals during follow up, 41 rabbits were to be included in the study.

The difference in tissue filling between patella defects and MFC defects was calculated and evaluated by Oneway ANOVA and paired Student t-tests. Tissue filling with time was evaluated by Oneway ANOVA and unpaired t-tests. Differences in mineralized tissue density between patellae and condyles – and the changes with time – were analyzed by Oneway ANOVA and paired and unpaired t-tests. The change in synovial fluid proteoglycan content from time zero to follow up, named delta (Δ), was evaluated by ANOVA and paired T-tests. Interactions between time and location were investigated; dependent observations on the individual level were accounted for by computing pair wise the difference between the deltas of the knees, the results applied in an ANOVA model with time to follow up as group factor. The rise in proteoglycan content (delta) for each location at each follow up was further analyzed by paired T-tests, the level of significance corrected according to Bonferroni. SPSS statistical package version 14 (Chicago, Illinois, USA, 2005) was used for statistical analysis.

Results

Complications

No animals died during surgery. Postoperatively, visual observations did not detect any harmful effects on gait among the animals and no differences in level of activity or motion

pattern between the legs were observed. 5 of the 41 experimental animals died unexpectedly during follow up, the reason being unknown. The knees were all unaffected, the deaths were all within 2 weeks from a follow up time point and the specimens therefore maintained in the study. Additionally 4 animals had to be killed during follow up due to impaired general health conditions. 1 of these was killed 2 weeks after initial surgery and excluded from the study. Among all animals, complications related to the knee were observed in 14 of the knees: 7 knees were excluded due to patellar dislocation, 1 knee because of infection and 6 knees due to technical failures in histology preparation.

Macroscopic changes

Among the knees with defects but no complications, none had major degenerative changes at any follow up. Some minor degenerative changes were observed in 8 of 69 experimental knees (Table 1). There were no statistically significant differences in the number of knees with minor degenerative changes related to neither the location of the defect nor time. Furthermore, neither changes in subchondral mineralized tissue density ($p=1.0$) nor changes in synovial fluid proteoglycan content ($p=0.6$) were correlated to the minor degenerative changes observed.

Filling of the chondral defects

There was a significantly higher degree of tissue filling in patellar defects compared to condylar defects at all follow ups (Table 2). The difference in tissue filling between the 2 locations remained similar with time ($p=0.2$). Both the patellar and condylar defects showed a change in filling with time ($p=0.02$ and $p=0.02$ respectively) (Figure 6), the change being apparent at the interval from 24 to 36 weeks ($p=0.003$ for patellar defects and $p<0.001$ for condylar defects).

Subchondral mineralized tissue

The subchondral mineralized tissue density of the patellas with defect decreased with time ($p=0.01$), whereas the density of condyles with defect remained similar with time ($p=0.9$). The reduction in patella density mainly occurred in the interval between 12 and 24 weeks ($p=0.01$), contributing to a major reduction in density between 12 and 36 weeks ($p<0.001$)

(Figure 7). Although the descriptive statistics (95% CI) and oneway ANOVA applied on the pair wise computed differences between patellar and condylar defects showed a higher density in patellas with defect than in condyles with defect at all time points, the difference was reduced in the interval from 24 to 36 weeks (p=0.02). These findings were supported by paired Student t-tests comparing patellar with condylar defects, being statistically highly significant at 12 and 24 weeks, whereas the difference was borderline significant at 36 weeks – and non-significant if Bonferoni corrected.

Synovial fluid proteoglycan content

A higher proteoglycan content was detected at 12 weeks compared to time zero both for knees with patellar defects (p=0.007) and those with condylar defects (p=0.003), whereas the values at 24 and 36 weeks were similar to those at time zero (Figure 8). There was no interaction between defect location and time to follow up, and there was no effect of location on the change in proteoglycan content.

Discussion

Effect of intraarticular location on defect filling

We found a higher degree of filling in the patellar defects than in the medial femur condylar defects at 24 and 36 weeks follow up. At 36 weeks the mean difference in filling was 28 % with a standard deviation of 27 % (p=0.001). The large variance in spontaneous filling of ICRS grade 3b defects has previously been emphasized by other authors (Breinan et al., 1997). Tissue filling is to some extent assumed to be crucial in cartilage restoration of a chondral defect. However, the critical amount of tissue filling necessary to discriminate one clinical outcome from the other regarding joint function, pain, disability and reduced risk of osteoarthritis, is not well understood. According to our power and sample size estimation, a filling difference of more than 25 % was considered as a proper level to discard the H_{01}-hypothesis of no difference in tissue filling between the locations in patella and the medial femoral condyle. The mean differences at 12 and 24 weeks were both below 25%. At 36 weeks, however, the mean difference was 28 %, thus the H_{01}-hypothesis could be discarded.The finding indicates that the intraarticular location influences the natural history filling of a pure chondral defect ICRS grade 3b in a long term follow up. To our knowledge this issue has not previously been thoroughly investigated. The potential effect of the

intraarticular location on the outcome of surgical repair of chondral defects ICRS grade 3b has been discussed in previous papers (Breinan et al. 1997, 2000). Breinan et al. (1997) compared autologous chondrocyt implantation (ACI) in trochlear defects in dogs to controls. They did not detect any significant effect of the ACI on the amount of defect filling, in contrast to Brittberg et al. (1996) who did the same comparison of ACI vs controls in patellar defects in rabbits. Breinan et al. (1997) suggested the intraarticular location of the defect (trochlea vs patella) being one possible explanation for the discrepancy in results. In a later study (Breinan et al. 2000), using the same dog model with 2 chondral defects ICRS grade 3b in trochlea, they found a difference between proximal and distal defects in the percentage of reparative tissue that was fibrocartilage ($p=0.02$), suggesting that the intraarticular location may play a role in cartilage restoration. A discrepancy of results after cartilage repair possibly related to the intraarticular location has been noted in clinical studies as well, the femoral condyle being the most favourable location (Brittberg et al. 1994, Hangody and Fules 2003, Krishnan et al. 2006).

We found an effect of the intraarticular location as a predictive factor for the outcome of natural history tissue filling of an ICRS grade 3b defect in the rabbit knee. We believe that knowledge of intrinsic factors influencing the outcome is essential in evaluating different aspects of cartilage restoration.

Effect of time on defect filling

We observed a change in tissue filling of both the patellar and condylar defects with time. This difference remained similar with time, indicating that the changes in tissue filling were similar at the two locations. At both locations the amount of filling increased from 24 to 36 weeks. This observation underlines that time to follow up is an important parameter in evaluating the results of natural history tissue filling in experimental models. The effect of time on cartilage restoration has been well studied in animal models involving defects corresponding to ICRS grade 4 (Shapiro et al. 1993, Lietman et al. 2002,). The natural history time course of ICRS grade 3 defects is on the other hand less well known. In their untreated control defects Grande et al. (1989) observed 17 % spontaneous filling of a 3 mm “full thickness” defect (through all chondral layers into the calcified zone – corresponding to ICRS grade 3c) in the rabbit patella at 6 weeks follow up. Brittberg et al. (1996), using the same rabbit model as Grande, increased the follow up time and reported 29 % spontaneous filling at 12 weeks, similar to the 32 % filling of patellar defects obtained at 12 weeks in our study. However, in our study the amount of tissue filling increased to 49 % at 36 weeks follow up.

The increase in spontaneous tissue filling after 12 weeks is in line with studies from other animal models. Breinan et al. (1998) reported 35 % spontaneous filling of a 4 mm defect corresponding to ICRS grade 3b in the knee trochlea of dogs at 3 months. In a separate study, using the same dog model, they obtained 41 % spontaneous filling at 12 months, increasing to 76 % at 18 months (Breinan et al. 1997).

In our study, for both locations the amount of filling tended to decline from 12 to 24 weeks, and then increased from 24 to 36 weeks. This observation is probably not in conflict with those of Frisbie et al. (1999) investigating 1 cm^2 "full thickness" defects (removing all the calcified cartilage, but preserving the bone plate – corresponding to ICRS grade 3c) in a horse model. Due to merging of the data from the groups in various ways, the presented results are not easy to interpret, but the mean filling of the treated defects and the control defects together was 44 % at 4 months and 54 % at 12 months – the filling of the controls merging the 2 time points together being lower than the filling of the treated defects. In another study the same authors, using the same model, presented 52 % filling of control defects at 8 weeks follow up (Frisbie et al. 2003). The combined results from these 2 studies seem to give the same natural history for filling of a defect as we found; there was a high percentage filling at an early follow up, with a tendency towards decreasing at a medium time follow up, and again increasing at a long time follow up. We observed such a time course for both locations, but we can not explain the phenomenon. We believe, however, that the knowledge of the natural history time course in a given model is essential in evaluating different aspects of cartilage restoration.

Subchondral mineralized tissue density

The subchondral mineralized tissue density in the patellas changed with time, in contrast to the density of the condyles. This indicates that the intraarticular location is a predictive factor also for subchondral bone changes related to chondral defects ICRS grade 3b in the rabbit knee. To our knowledge this issue has not been discussed in previous literature.

A weakness of our study is the lack of sufficient numbers of control patellas and condyles for evaluation of subchondral bone changes. The intention was to use patellas from knees with condylar defects, and femur condyles from knees with patellar defects as controls. Additional animals were added only to make up a sufficient number of control specimens. We learned, however, that there is a potential effect of chronic cartilage lesions in one articular surface to cause changes to the subchondral bone of other articular surfaces within the same joint (Marijnissen et al. 2002, Sniekers et al. 2008). The contralateral patella / condyle of the

experimental animals were therefore excluded – leaving us with a number of "controls" too small for statistical analysis. However, the observation that the density of mineralized tissue in patellas changed with time – whereas in condyles it did not, may be an important finding. Subchondral bone changes are associated with the initiation and progression of osteoarthritis (OA) (Radin and Rose 1986, Burr 1998, 2004,). There are, however, no objective criteria as to the degree of changes indicating a substantial initiation or progression of OA. Thus, no power or sample size estimations were carried out considering the changes in the density of subchondral mineralized tissue. Whether the statistical significant changes we found have any clinically implications therefore remains uncertain.

Some authors emphasize that changes in the calcified cartilage, being part of the subchondral mineralized tissue, may play a role in the pathogenesis of OA (Burr 2004). Thus, defects involving iatrogen damage to the calcified cartilage (ICRS grade 3c) probably should be distinguished from pure chondral lesions (ICRS grade 3b) in evaluating the natural history of the subchondral mineralized tissue. The impairment of a well defined pure chondral defect (ICRS grade 3b) on the subchondral bone is less well known. Breinan et al. (1997) reported resorption of the subchondral bone leading to moderate to severe bone loss in 3 of 14 untreated ICRS grade 3b defects in the trochlea of dogs by merging data from 12 and 18 months follow up. They offered no explanation of the changes, but raised the possibility of having caused damage to the calcified cartilage during surgery, made evident by studying fresh defects in cadaver dogs. To reduce the risk of damage to the tidemark and the underlying calcified cartilage in our study, we used a stereomicroscope and small instruments in preparing the defect.

We found a reduction in the density of subchondral mineralized tissue from 12 to 36 weeks in the patellas with defect. The time course is in agreement with that of changes in corresponding parameters reported in experimental OA models. In their "groove" OA model in dogs, Sniekers et al. (2008) reported a 6 % increase in bone volume fraction at ten weeks, subsiding to a 13 % loss at 20 weeks. The finding of decreased bone volume fraction combined with thinning of the subchondral bone plate as well, in early degenerative joint disease, is also in agreement with other studies (Dedrick et al. 1993).

Although the filling of the defects in the condyles was lower at all time points, the subchondral mineralized tissue in the condyles seemed to tolerate the defect better, showing no detectable changes in density with time. Thus, the intraarticular location may be a predictive factor for subchondral bone changes related to chondral defects ICRS grade 3b in the rabbit knee.

Synovial fluid proteoglycan content

A rise in synovial fluid proteoglycan content was detected in the knees of animals killed 12 weeks after surgery. There was no statistically significant difference between knees with patellar defects and knees with condylar defects. The rise observed at 12 weeks subsided with time, being similar to time zero values at 24 and 36 weeks follow up.

Increased concentrations of proteoglycan fragments in the joint fluid have been associated with trauma and surgery in humans (Lohmander et al. 1989, Odenbring et al. 1991), and with increasing knee OA in rabbits (Messner et al. 1993a). In their rabbit OA model Messner et al. found an increase at 3 months which they explained by the effect of surgery, the values decreasing to normal at 6 months, and then increasing again at 12 months which they explained as possibly related to initial degeneration. These findings are not in conflict with the observations in our study; there was a rise in proteoglycan content at 12 weeks subsiding to values similar to time zero at 24 and 36 weeks follow up. Consequently, the synovial fluid proteoglycan concentrations in our study did not indicate a degenerative process during the observation period.

Weaknesses

The sample size estimation indicated a need of 12 animals for each of the three follow ups. A sufficient number was obtained at 36 weeks follow up whereas the numbers at 12 and 24 weeks were below that due to a high number of complications and failures in histological preparation. The data obtained at 36 weeks, however, represent the most valuable information. Complications and loss of animals related to the rabbit model have previously been described by other authors as well (Brittberg et al. 1996).

Strength

The importance of defining the depth of experimentally produced defects in relation to the different layers of the joint organ – and evaluating the results in relation to that – has been emphasized by others (Brittberg et al. 1996, Breinan et al. 1997, Frisbie et al. 1999, 2003, Hunziker 1999, Burr 2004). Even the bias of not removing all the tissue of a layer as intended, or causing damage to the layer beneath, has been a topic in discussing the results of cartilage restoration (Breinan et al. 1997, Frisbie et al. 1999). Methodologically our study was strengthened by the use of a stereomicroscope which ensured removal of all the cartilage

without causing damage to the calcified layer and the tissue beneath in preparing the defects. Additionally, we used the most common applied animal model in cartilage repair.

Contributions of authors:

All authors contributed to planning of the study, study design, interpretation of the data and writing the manuscript. SH, AÅ, SL and SS were involved in animal care and surgery. SH, AÅ, SL, SS and FPR contributed to specimen and tissue handling. SH and FPR did the histological analyses and SH the statistics guided by our statistical advisor.

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No affiliations or conflicting interests declared.

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Tables:

Table 1: The fraction of knees with minor degenerative changes observed macroscopically at follow up.

	12 weeks	24 weeks	36 weeks
Knees with a patellar defect	3 / 8	2 / 9	1 / 17
Knees with a MFC defect	2 / 8	0 / 9	0 / 18

Table 2: The percentages (SD) of tissue filling in defects. The two locations are pair wise compared at each time point of follow up, giving the mean differences, the 95% CI and the p-values obtained by Student t-tests. ([a]Not significant with Bonferoni correction).

	12 weeks (n=8)	24 weeks (n=7)	36 weeks (n=17)
Patellar defects	32 (11)	21 (4.5)	49 (26)
Condylar defects	17 (14)	6.2 (3.0)	21 (11)
Mean difference	15 (17)	14 (5.9)	28 (27)
95% CI of difference	[1.3 – 30]	[8.8 – 20]	[14 – 42]
p-value	0.04[a]	0.001	0.001

Illustrations (Graphics):

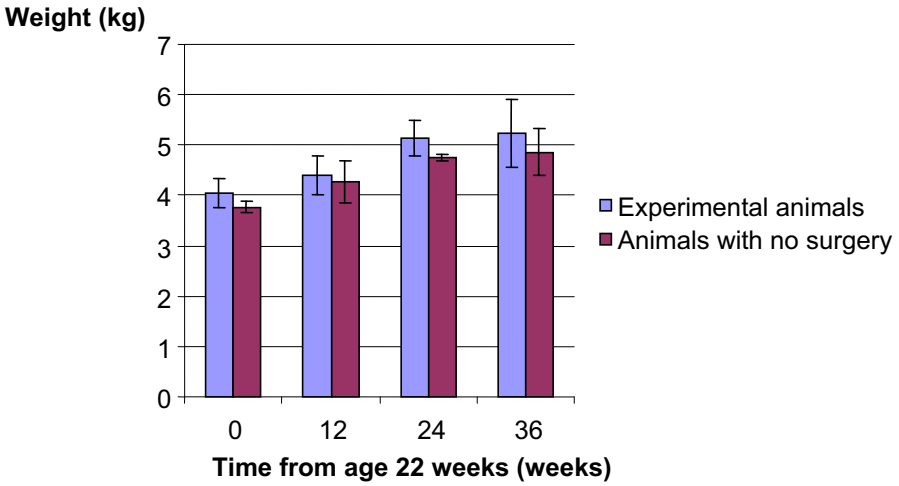

Figure 1: The experimental animals obtained weight gain throughout the experimental period similar to that of the control animals. The number of experimental animals at time zero (age 22 weeks) equalized the total number of animals evaluated at the follow ups (37) – since they were all measured preoperatively. The number of experimental animals at 12, 24 and 36 weeks were 8, 11 and 18 respectively. The number of animals without surgery at time 0, 12, 24 and 36 weeks were 3, 3, 2 and 2 respectively.

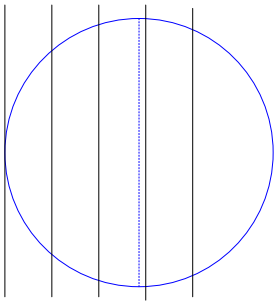

Figure 4: Starting at the rim of the defect, five levels of sectioning, each 700 µm further into the 4 mm defect, ensured a central section to be analysed.

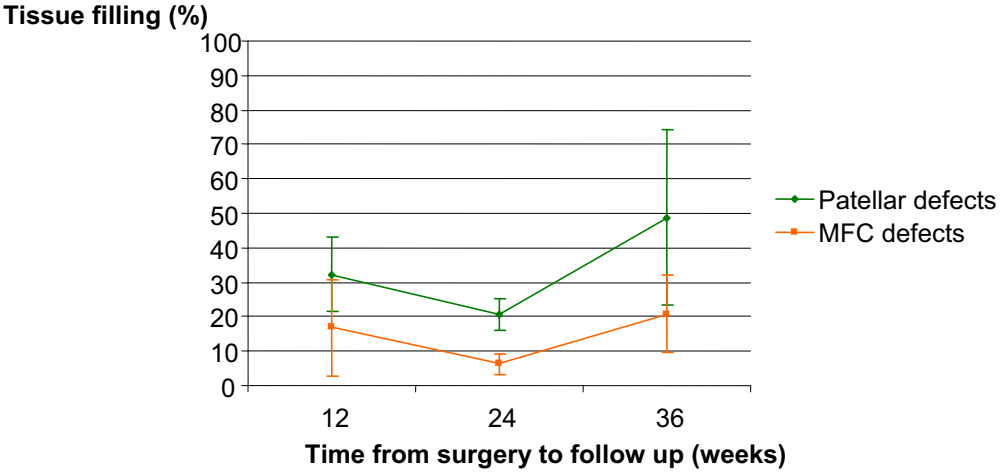

Figure 6: The percentage filling of the patellar and MFC chondral defects at the different time points of follow up. For pair wise comparison, the number of animals at 12, 24 and 36 weeks follow up were 8, 7 and 17 respectively.

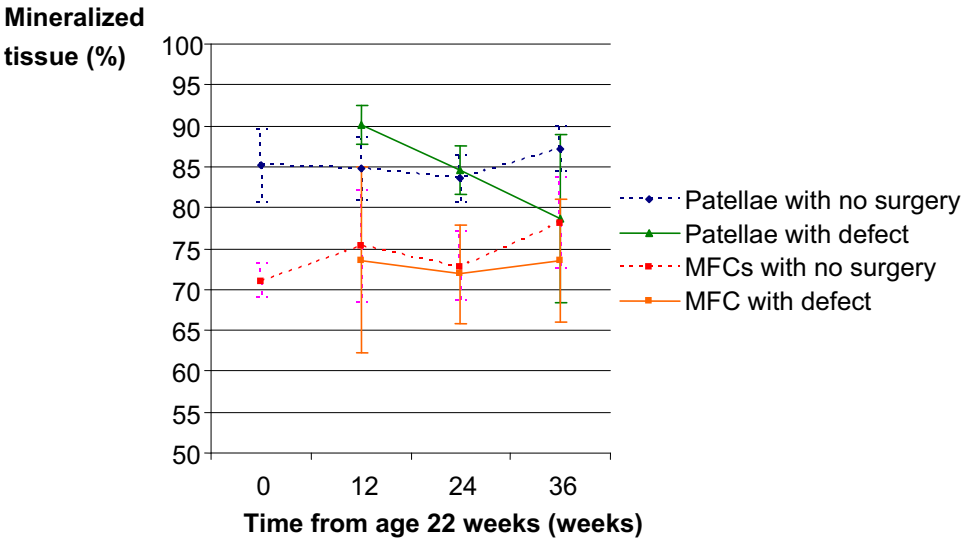

Figure 7: The percentage of subchondral mineralized tissue with time in patellar and MFC specimens. For pair wise comparison, the number of experimental animals at 12, 24 and 36 weeks follow up were 8, 7 and 17 respectively. The number of animals with no surgery sacrificed at time point 0, 12, 24 and 36 weeks were 3, 3, 2 and 2 respectively.

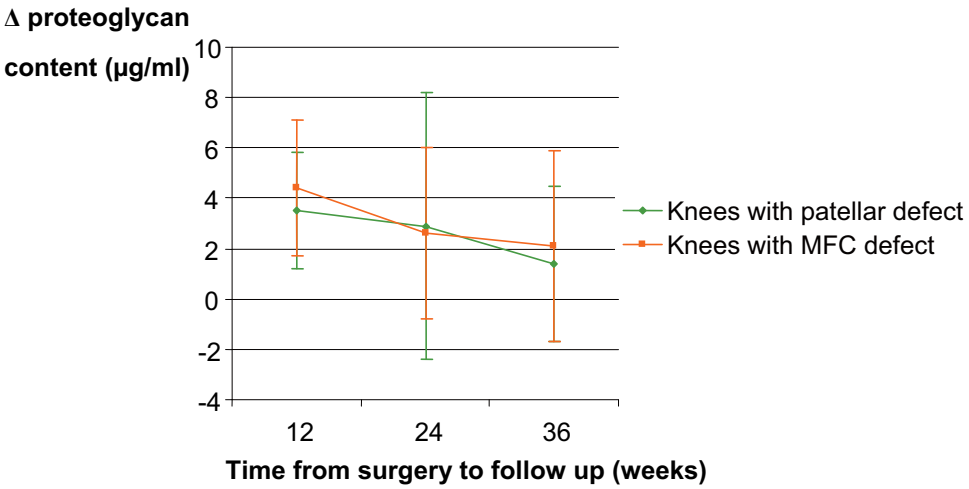

Figure 8: The values of change (δ) in synovial fluid proteoglycan content from time zero to the different time points of follow up in knees with patellar and MFC defects. The number of animals at 12, 24 and 36 weeks follow up were 8, 7 and 17 respectively.

Illustrations (Photos)

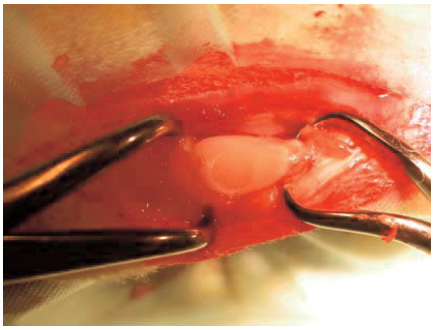

Figure 2a: The 4 mm chondral defect in the rabbit patella.

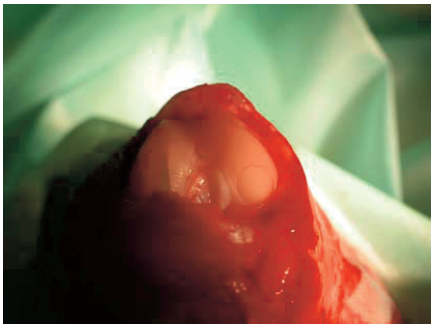

Figure 2b: The 4 mm chondral defect in the rabbit MFC.

Figure 3: The set-up for surgery. A stereomicroscope ensured the complete removal of all cartilage above the tidemark without harming underlying tissue.

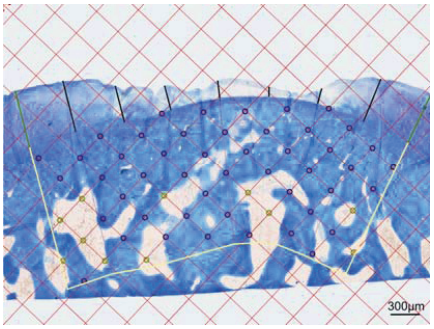

Figure 5: Histological section of a defect in the patella stained with tolouidin blue and micrographed at 40x magnification. The section is modified high-lightening some of the graphics superimposed by the software Analysis Pro®. The two green lines outline the height of the shoulders measured. The seven black lines indicate the height of the tissue filling the defect. The yellow lines together with the tidemark frame the area of interest for subchondral mineralized tissue evaluation. The intersections of the red grid are marked with blue circles whenever overlying mineralized tissue, and green circles whenever not.

IV

Cartilage repair in the rabbit knee: Mosaic plasty resulted in higher degree of tissue filling but affected subchondral bone more than microfracture technique

A blinded, randomized, controlled, long term follow up trial in 88 knees

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Abstract

Discrepancies and variances in outcome following different surgical techniques for cartilage repair are poorly understood. Successful repair rely on proper tissue filling without initiating degenerative processes in the cartilage-bone unit. Consequently, the objective of the current study was to compare two available techniques for cartilage repair, i.e. microfracture technique (Mfx) and mosaic plasty (Mos), regarding tissue filling and subchondral bone changes in an experimental model.

A 4 mm pure chondral defect (ICRS grade 3b) was created in the medial femoral condyle of both knees in New Zealand rabbits, aged 22 weeks. A stereomicroscope was used to optimize the preparation of the defects. In one knee (randomized) the defect was treated with Mfx, whereas in the other with Mos. The animals were sacrificed 12, 24 and 36 weeks after surgery. Defect filling, new bone formation above the level of the tidemark and the density of subchondral mineralized tissue were estimated by histomorphometry.

Mos resulted in a significantly 34% higher degree of tissue filling than Mfx at 36 weeks, SD of mean difference being 34%. Mos resulted in significantly more new bone formation and reduced subchondral mineralized tissue density compared to Mfx. The differences between the two techniques were apparent mainly at the long term follow up.

Tissue filling is a limiting factor regarding Mfx when compared to Mos, whereas Mos resulted in more bone changes than Mfx – the implications of the latter remain to be settled. This study underlines the difficulty in predicting outcome in the single case with any of these two techniques, particularly in a long-term perspective.

Key words: Cartilage, Chondral defect, Microfracture, Mosaic, Filling, Subchondral bone, Knee, Rabbit, Surgery.

Introduction

Focal articular cartilage injuries in the knee are common [3, 28], and the complaints they cause may impair quality of life as much as severe osteoarthritis [27]. Injured articular cartilage has a limited capacity for complete spontaneous healing [12], and a number of therapeutic measures have been published [9, 26, 44, 58]. However, no treatment modality has so far proved to be superior compared to the others [5, 6, 21-23, 30, 36, 37, 45, 48, 57, 59, 61], and the results of each technique demonstrate large variances [35]. Two inexpensive and commonly used techniques for cartilage repair reported from clinical practice are the microfracture technique (Mfx) and mosaic plasty (Mos) [26, 58]. Although each of them has been studied in separate animal models [19, 25], their discrepancies have not been evaluated by randomized comparison in standardized animal models. The amount of tissue filling reported from experimental Mfx studies vary [7, 16, 19, 20], whereas from previous experimental Mos studies tissue filling has not been reported. Moreover, the majority of experimental studies concerning these two techniques for cartilage repair are on defects penetrating into the subchondral mineralized tissues [19, 20, 25, 29, 39, 53] corresponding to ICRS grade 3c or grade 4 defects [11]. Access to bone marrow elements might be one of the strongest predictive factors for filling of the defect itself – thus being a bias in evaluating other factors [32]. In addition, iatrogen damage to the calcified layer may cause moderate to severe subchondral bone loss at a long term follow up [8] and new bone formation above the level of the tidemark [17]. The consequences of such changes regarding cartilage repair are not clear, but abnormalities in subchondral mineralized tissue remodeling and bony advancement above the level of tidemark are associated with the progression of degenerative joint disease [14].

The primary purpose of the current study was to test the hypothesis that the choice of Mfx vs Mos would influence on the amount of filling of a chondral defect not penetrating the subchondral mineralized tissues. Secondly, the purpose was to evaluate whether the choice of cartilage repair technique would influence on new bone formation and changes in the subchondral mineralized tissues.

Methods

Design / Animal model

Our established experimental animal model was used [2]. Adult New Zealand rabbits were included in a randomized study where circular lesions, diameter 4 mm, were created in the medial femoral condyle (MFC) of both knees. The lesions were pure chondral – down to the tidemark, not penetrating the calcified layer – corresponding to ICRS grade 3b [11]. By random, the defect in one of the knees was treated with Mfx and compared to the defect in the other knee treated with Mos. The age of the animals at surgery was 22 weeks. The follow up was either 12, 24 or 36 weeks following initial surgery.

The main endpoint was difference in degree of tissue filling between the defects treated with Mfx and the defects treated with Mos at each follow up. Secondary endpoints were differences in new bone formation within the defects, changes of the density of subchondral mineralized tissues, and difference in degenerative changes evaluated by macroscopic appearance and joint fluid proteoglycan content between the defects treated with Mfx and those treated with Mos at each follow up.

Animal care

The animal environment, diet, application of sterile perioperative conditions, capturing of synovial fluid for proteoglycan analyses, anaesthesia, analgesia and the procedure of sacrifice have been described previously [2]. Throughout the follow up period, the experimental animals showed normal weight gain (Fig. 1).
The animals were allowed to move freely in their cages, and all animals were able to bear weight on both extremities immediately after surgery.
Surgery was performed by experienced orthopaedic surgeons certified according to the rules of animal care and experimental surgery. The study was carried out according to the guidelines for animal research at the University of Oslo and approved by the Norwegian Government Committee for Experimental Animal Care.

Experimental groups

Forty adult New Zealand rabbits as planned, and additionally four to compensate for early loss to follow up were included as experimental animals. They all had defects created in both knees at age 22 weeks – in one knee treated with Mfx by randomization, whereas in the other knee treated with Mos. To avoid "learning curve" as a bias, the animals were block randomized for sacrifice at the follow up time points. Twelve animals were supposed to be sacrificed 12 weeks post surgery, 12 at 24 weeks and 16 at 36 weeks. Due to loss of animals during follow up and other complications throughout the study, the final numbers of experimental knees treated with Mfx / Mos available for histological examination were 7 / 8 at 12 weeks, 8 / 10 at 24 weeks and 13 / 13 at 36 weeks follow up. The numbers of animals available for paired analyses (sections from both knees available for evaluation) were 7 at 12 weeks, 8 at 24 weeks, and 11 at 36 weeks.
Among the 44 animals (88 knees) undergoing cartilage repair, a wash out sample of the synovial fluid containing a minimum of 0.75 ml collected prior to surgery (time zero) was available for proteoglycan content analyses from 86 knees. Due to loss of animals and exclusion of knees due to either patella dislocation or infection, the number of knees with samples both from time zero and at sacrifice was reduced to 70. A total of 26 animals (52 knees) had samples available for analyses from both knees at both time zero and at sacrifice.

Surgical technique

Through a medial parapatellar incision, a defect (φ = 4 mm) was created in the MFC of both knees as previously described for patella [2]. A stereomicroscope and small instruments were used, and care was taken to avoid any damage to the rims of the defects or to the underlying calcified cartilage. By randomization, the defect in one of the knees was treated with Mfx using a specially designed awl and a mallet, the awl being tapped through the subchondral cortical bone plate establishing 4 equally spaced penetrating channels to the blood filled lacunas of the subchondral miscellaneous bone (Fig. 2). The diameter of the channels was proximally 0.8mm. The defect of the other knee was treated with Mos, using a 1.4mm cylindrical motorized chisel harvesting osteochondral plugs close to the intercondylar notch. In the defect, three recipient sockets were made using a regular 1.4mm burr. The grafts were transferred to the sockets and left with press fit fixation flush to the cartilage surrounding the defect (Fig. 3). Due to the fragility and consequently breakage of some grafts, sometimes 4 and occasionally 5 grafts were harvested. The joint was irrigated, haemostasis applied, and wound closure performed as previously described [2].

Sacrifice of animals, macroscopic evaluation and preparations for histological analyses

The animals were sacrificed and synovial fluid obtained as previously described [2]. The femoral condyles were dissected free and gross morphologic grading was performed. Changes to the cartilage corresponding to ICRS grade 1-2 and / or small osteophytes were characterized as minor changes, whereas changes exceeding that were characterized as major. The specimens were fixed by immersion in phosphate-buffered 4% paraformaldehyde for one week and decalcified in 20 % formic acid until the bone was soft enough for sectioning. Cubes, measuring 8 by 8 mm containing the defect at one side were harvested from the specimens, dehydrated in graded alcohol and embedded in an epoxy resin (Agar 100®, Agar Scientific, Stanstead, UK).

Histology

The cubes were sectioned from one longitudinal surface, the anterior-posterior and medial-lateral orientation of the defect at random [24]. From the point where the rim of the defect was reached, sections – each 1-2 µm thick – were captured at 5 different levels, each level 700 µm further into the defect. Sections from the three most central of the five levels were used for evaluation. This technique ensured that those three sections would all be within a maximum distance of 1050 µm from the very centre of the defect (Fig. 4). The sections were stained with tolouidin blue and micrographed at 40x magnification using a digital camera mounted on the microscope (Color View III®, Olympus Soft Imaging Solutions, Münster, Germany). An interactive semiautomatic image analysis program (Analysis Pro®, Olympus Soft Imaging Solutions, Münster, Germany) was used for all measurements. The mean values of the three sections closest to the centre of each defect (selected by the largest diameters) were included in the statistical analyses. The observer was blinded to the technique used for treatment of the defects.

Estimation of tissue filling

The borders of the defects were identified by the interfaces between presumed original cartilage and newly formed fibrocartilaginous / fibrous repair tissue on both sides. The tidemark was identified. If the tidemark was partially disrupted, the level of tidemark between identifiable fractions was estimated taking the curvature of the condyle into account. The midpoint along the tidemark of the defect was defined. From the midpoint, sectors of 500 µm length were marked along the tidemark to each side until 2 mm from midpoint was reached on each side, representing the tidemark at the base of the “shoulders” of the 4 mm defect. Cartilage height was measured at these two shoulder points, as was the height of new bone formation above the level of the tidemark and total tissue height at the 7 intersection points of the 500 µm length sectors (Fig. 5). New bone formation and total tissue filling was estimated by relating the mean height of tissue at the 7 defined points to the mean cartilage height of the 2 shoulders of the same defect, expressed as percentage filling. Whenever one of the shoulders was technically not measurable, the one shoulder left served as the reference. This occurred in 15 sections from nine knees treated with Mfx and in 9 sections from six knees treated with Mos.

Estimation of the density of subchondral mineralized tissue

The density of the subchondral mineralized tissue was estimated in an area immediately 1500 μm deep to the tidemark of the defect, the shape of the region of interest depending on the curvature of the tidemark (Fig. 5). Morphometry was performed by point counting [24] using computer software. A grid with 300μm between test lines was superimposed on the micrograph. The intersections between the test lines served as test points. Subchondral mineralized tissue density was expressed as the number of test points overlying mineralized tissue relative to the total number of test points within the region of interest. The counting was repeated twice for each section with the orientations of the grid randomly selected at both occasions. The mean value of the two measurements was used for further statistical analysis. A similar technique was used by Løken et al. [42] demonstrating a variance less than 10% between measurements and between observers.

Analysis of synovial fluid

Proteoglycan content analyses was performed using standard ELISA technique [50]. The samples were all analysed as one batch. Only knees with samples from both time zero and at sacrifice were included in the statistical analyses. The value of proteoglycan content at time zero was subtracted from the value at sacrifice, and the mean change, named delta (Δ), was compared between groups.

Statistics

Based on previous experimental studies [1] a filling difference of more than 25% was considered being a proper level to discard the H_{01}-hypothesis of no difference in tissue filling between the knees treated with Mfx and those treated with Mos. Since the experimental animals all underwent cartilage repair in both knees, with Mfx in one and Mos in the other, they would served as their own control regarding tissue filling of the defects, thus a paired Student t-test could be applied. Preexperimental analysis to detect sample size using a power of 0.80 and a significance level of 0.05 and a standard deviation for the differences of less than 24% indicated a need of 9 animals in each group for this purpose. To evaluate the difference in tissue filling from one follow up time point to another regarding each treatment separately, the need of animals was estimated to 12 due to an unpaired experimental situation. Power and sample size estimations were not performed for the secondary endpoints. To cover up for loss during follow up, we initially included 40 rabbits in the study. However, due to early loss of animals during surgery and follow up, the experimental group was expanded to 44 rabbits.

The differences in tissue filling, new bone formation and subchondral mineralized tissue density between defects treated with Mos and those treated with Mfx – and the changes with time – were evaluated by Oneway ANOVA, post hoc Bonferroni and paired Student t-tests. The change in synovial fluid proteoglycan content from time zero to follow up, named delta (Δ), was evaluated by Oneway ANOVA, post hoc Bonferroni and paired Student t-tests as well. Interactions between time and treatment technique were investigated; dependent observations on the individual level were accounted for by computing pair wise the difference between the deltas of the knees, the results applied in an Oneway ANOVA model with time to follow up as group factor. The rise in proteoglycan content (delta) for each location at each follow up was further analyzed by paired Student t-tests,

the level of significance corrected according to Bonferroni. Results are given as mean and standard deviations (SD). SPSS statistical package version 14 (Chicago, Illinois, USA, 2005) was used for statistical analysis.

Results

Macroscopic changes

Among the knees with cartilage repair but no complications, none had major degenerative changes at any follow up. Some minor degenerative changes were observed in twelve of the 70 treated knees available for evaluation (Table 1). There was no difference in the frequencies of changes between Mfx and Mos, and there was no effect of time to follow up. Furthermore, none of the variables new bone formation ($p=0.847$), subchondral mineralized tissue density ($p=0.426$) or change in synovial fluid proteoglycan content ($p=0.634$) were correlated to the minor degenerative changes observed.

Tissue filling of the chondral defects

The values of percentage filling comparing the two treatment techniques at all time points are shown in Table 2. Overall, Mos resulted in a significant higher degree of total filling of the defects than did Mfx ($p=0.010$), the standard deviations of mean difference however varying from 22.5 to 40.2. There was no significant effect of time on the difference between Mos and Mic. However, from being similar at 12 weeks the difference in filling increased to significantly 34% at 36 weeks follow up (Fig. 6).

New bone formation within the chondral defects

The values of percentage new bone formation above the level of the tidemark comparing the two treatment techniques at all time points are shown in Table 3. Overall there was significantly more new bone formation within the defects treated with Mos compared to Mfx ($p=0.010$), the difference being significantly apparent at 24 and 36 weeks follow up. There was no significant effect of time, neither for Mos and Mfx separately, nor for the difference between them.

Subchondral mineralized tissue density

The values of subchondral mineralized tissue density comparing the two treatment techniques at all time points are shown in Table 4. Overall, the density of Mos treated condyles was significantly lower than that of Mfx treated condyles ($p=0.001$). Although there was no significant effect of time, the difference between Mfx and Mos appeared significant only at 36 weeks follow up (Fig. 7).

Synovial fluid proteoglycan content

The mean values for the changes (Δ) in synovial fluid proteoglycan content from time zero to follow up, comparing the two treatment techniques at all time points are shown in Table 5. There was a significant rise in synovial fluid proteoglycan content from the time of surgery to follow up in the knees treated with Mfx ($p=0,004$) as well as in those treated with Mos ($p<0,001$), with no significant effect of time to follow up. There

was an overall tendency of a higher rise of proteoglycan content in knees treated with Mos than in those treated with Mfx (p=0.051), the difference, however, being significant at 12 weeks follow up only (Fig. 8).

Complications

No animals died prior to surgery. Two of the animals died during surgery, the reason being unknown. They were both replaced. Among the others, visual observations did not detect any harmful effects on gait postoperatively and no difference in level of activity or motion pattern between the two legs was observed. Six of the animals sustained sudden unexpected death during follow up, the reason being unknown. Two of these animals died within 3 weeks after surgery and were replaced. The other four deaths were within two weeks from a follow up time point, and except one knee with patellar dislocation, the knees were all unaffected and the specimens therefore maintained in the study. Three animals had to be sacrificed during follow up due to impaired general health conditions. In two of these the knees had to be excluded, whereas in one rabbit both knees were unaffected, the time of sacrifice was within 2 weeks from a follow up time point, and the specimens therefore maintained in the study. Among the total population of animals, complications related to the knee were observed in 24 % of the knees: Three knees (3.4%) were excluded due to patellar dislocation, six knees (6.8%) because of infection and twelve knees (13.6%) due to technical failures in histology preparation.

Discussion

The major finding of the current experimental study was that neither of the two evaluated techniques for cartilage repair resulted in altogether good and predictable outcome regarding the parameters observed in this long term follow-up. This is in line with some of the clinical publications from randomized studies using the same techniques [6, 36].

Effect of Mfx and Mos on tissue filling of the defect

The present study demonstrated an overall higher degree of total tissue filling in the defects treated with Mos compared to those treated with Mfx. The difference was 0.2% at 12 weeks, 15.3% at 24 weeks and significantly 34% at 36 weeks follow up, the filling of Mfx defects remaining unchanged. We did not, however, have enough power in the study to detect this increase as a significant effect of time, neither separately for Mos filling (p=0.097) nor for the computed difference between Mos and Mfx (p=0.127).

A large variance in the degree of filling was noted with both treatment modalities. Such large variance in filling of ICRS grade 3b chondral defects has previously been brought to attention regarding both untreated defects (Heir et al., unpublished data) [8] as well as defects treated with Mfx [7, 39]. The variability in filling of the interstitial space between the grafts in Mos has been underlined as well [33, 34].

Tissue filling is considered to be an important variable in restoration of cartilage defects. However, the critical amount of tissue filling necessary to significantly discriminate one clinical outcome from the other concerning joint function, pain, disability and reduced risk of osteoarthritis , is not well understood. Moreover, the correlation between tissue filling and other histological findings on one hand, and functional outcome on the

other, is not obvious [37, 43]. According to our power and sample size estimation, a filling difference of more than 25 % was considered as a proper level to discard the H_{01}-hypothesis of no difference in tissue filling between treatment modalities. Thus, the 34% higher degree of filling following Mos compared to Mfx at the latest follow up discarded our H_{01}-hypothesis.

We further compared the data from the Mfx and Mos repairs in this study to data from 35 identical control defects (no treatment) observed at corresponding follow ups in a previous study (Heir et al., unpublished data). Generally there was a significant effect of surgical repair compared to natural history on total filling of the chondral defects ($p<0.001$). However, the mean differences in tissue filling between the Mfx and control defects were less than 25% at all time points, whereas the difference between the Mos and the untreated defects were more than 25% at both 24 and 36 weeks, resulting in a 45% difference between the Mos and control defects at the latest follow up.

There are to our knowledge only two clinical [23, 23] and no experimental studies comparing these two techniques for cartilage repair. In their clinical studies Gudas et al demonstrated superior results of Mos compared to Mfx clinically, macroscopically, histologically and radiologically 37 months following treatment. Tissue filling of the defects was, however, not evaluated.

In animal models on the other hand, tissue filling has been studied following Mfx [7, 16, 19, 20, 29]. However few, if any, studies report tissue filling following Mfx of ICRS grade 3b defects in the rabbit knee. Although the majority of studies on Mfx report tissue filling superior to the 31.4% observed at 36 weeks in the present study, only a limited number of papers present an increase in filling of more than 25% compared to controls [16]. Since discrepancies between studies may have several explanations, differences in results between studies should be interpreted with care. Besides parameters generally influencing the result of defect filling such as the anatomical and biomechanical differences between species [13], the intraarticular location (Heir et al., unpublished data) [7, 9, 20, 38], the depth of the lesion [17, 32] and the size of the defect [40], the technique of which the penetrations to the bone marrow is performed may play a role [15]. In the current study bleeding from the Mfx channels varied, both from one pick hole to another within a defect and from one animal to another. This phenomenen has been described by other authors as well [7].

Due to large disparity between studies, experimental comparisons of different treatment modalities and techniques should be performed in the same animal model. Although rabbits are the most widely used animal model for cartilage restoration in general [4, 20], this is the first study applying the MFC of the rabbit knee as a model for Mos.

At 36 weeks follow up the filling of the Mos defects was 65.5%, more than twice that of Mfx and significantly 45% higher than the 20.5% observed in untreated defects (Heir et al., unpublished data). In previous literature concerning Mos the evaluation of tissue filling has not been a focus of attention. Mos implies the transplantation of several circular osteochondral autografts to a chondral defect where the grafts are implanted in precisely drilled recipient holes to resurface the defect by press fit graft to wall friction – composing a “mosaic”. Accuracy of graft delivery such as perpendicularity of the grafts, adequate press fit fixation to insure sufficient stability, and the grafts not being left prone to the surrounding surface is associated with success – avoiding graft subsidence and overgrowth with fibrous tissue [26, 33, 53]. The use of circular grafts initially leaves some denuded interstitial space in between the cartilage of the grafts and towards the surrounding cartilage. These gaps are supposed to heal by “cartilage flow” and extrinsic repair [33]. The gradual filling of the interstitial spaces

may contribute to an increased amount of tissue filling with time – not in conflict with the current study. In the present study we used three 1.4 mm grafts in a 4 mm defect, covering 37% of the defect – thus corresponding to an initial 37% tissue filling – if the delivery was appropriate. The mean tissue filling of the defects was 31.8% at 12 weeks, compared to 65.5% at 36 weeks follow up. The lower value of tissue filling at 12 weeks compared to the theoretically initial filling might be due to subsidence of grafts and lack of filling of the interstices in some defects. The 65.5% filling achieved at 36 weeks is likely due to filling of the interstices and/or overgrowth of the grafts with fibrous tissue.

There was a great variance in the results at 36 weeks (SD 38.0%, 95% CI [39.9 – 91.0]). Since tissue filling exceeding 37% was dependent on cartilage flow and extrinsic repair, our findings are in agreement with previous studies suggesting that this kind of repair is unpredictable [33, 34].

Quantitative measures of tissue filling following Mos add valuable information on the overall consequences of both graft subsidence and interstitial filling. Moreover, evaluating defect filling by measuring the height of tissue above the level of the tidemark – as in the present study – reflects one of the main proposed advantages of Mos; namely preserving the joint surface congruity. To our knowledge, the present study is the first to evaluate Mos by quantitative measure of tissue filling, making it possible to compare the results to other cartilage repair techniques such as Mfx.

Effect of Mfx and Mos on new bone formation within the defects

The current study demonstrated that both Mfx and Mos resulted in new bone formation above the level of the tidemark. New bone formation following Mos comprised considerable 13.2 and 12.0 % of the defect volume at 24 and 36 weeks respectively, significantly different from the 1.4 and 2.5 % following Mfx. In comparison, untreated defects previously reported showed no signs of new bone formation above tidemark at any follow up (Heir et al., unpublished data). New bone formation in defects treated with Mfx has been reported previously [16-19] as well as in defects treated with Mos [31]. The implications of new bone formation observed in cartilage repair are, however, not known. On the other hand, new bone formation reflected as tidemark – and thereby calcified tissue – advancement into the non-calcified cartilage has been associated with the progression of OA [14] and therefore is a matter of concern – also in regards to cartilage repair.

Effect of Mfx and Mos on subchondral mineralized tissue density

The current study showed an overall significant lower subchondral mineralized tissue density in Mos treated defects compared those treated with Mfx, the significance being apparent at the latest time point of follow up. Compared to untreated defects reported previously (Heir et al., unpublished data) Mos resulted in a significant reduction of the subchondral mineralized tissue density (p=0,002), whereas Mfx did not (p=0,269). To our knowledge this is the first study comparing the effect of these two cartilage repair techniques on subchondral bone. Subchondral bone changes are associated with the initiation and progression of OA [14, 56]. However, no consensus has been reached regarding the pattern of changes or regarding the degree of changes at which initiation and/or progression of OA could be anticipated. Whether the statistical significant changes we did observe in the present study do have any clinical implications thus remain uncertain, however, they still are a matter of concern. Although grading of subchondral bone changes have been included in different scoring

systems for cartilage restoration [46, 47, 51, 54, 55, 60], the scores are semi quantitative. To our knowledge quantitative analyses of the subchondral mineralized tissue density following cartilage repair has not been brought to attention previously.

Synovial fluid proteoglycan content

Mos resulted in a significantly higher rise of synovial fluid proteoglycan content compared to Mfx at 12 weeks follow up. There was no difference at 24 and 36 weeks follow up. Increased concentrations of proteoglycan fragments in the joint fluid have been associated with trauma and surgery in humans [41, 52], and correlated to increasing OA in a rabbit model [49]. In their rabbit model, Messner and coworkers demonstrated values being elevated at 3 months, decreasing to normal values at 6 months, and then increasing again at 12 months. The rise at 3 months was explained as possibly surgery related in line with clinical findings [52] whereas the rise at 12 months was explained as possibly related to initial degeneration. Their observations are not in conflict with the current study. The significant difference in rise seen at 12 weeks may reflect that Mos is more traumatizing to the joint than is Mfx. On the other hand, synovial fluid analyses did not indicate a degenerative process in neither of the groups during the observation period.

Weaknesses of the current study

The sample size estimation for the current study indicated a need of 12 animals for each of the three follow ups. The numbers achieved for histological analyses at 12, 24 and 36 weeks were 7, 8 and 11 respectively. The insufficient numbers left us with a power problem in evaluating the effect of time. The material suffered from a high number of complications and failures in histological preparation. Two animals died during surgery and six animals died unexpected in the follow up. However, the data obtained at 36 weeks represent the most valuable information from the current study. Complications and loss of animals related to the rabbit model have previously been described by our group (Heir et al., unpublished data) as well as by other authors [10].

Strength of the current study

The importance of defining the depth of experimentally produced defects in relation to the different layers of the joint organ – and evaluating the results in relation to that – has been emphasized [8, 10, 14, 32]. Even the bias of not removing all the tissue of a layer as intended, or causing damage to the layer beneath, has been a topic in discussing the results of cartilage restoration [8, 19]. Methodologically this study was strengthened by the use of a stereomicroscope during surgery. Without that the procedure demands great care and is hard to perform without leaving residual cartilage or damage to the calcified cartilage [7, 8, 29]. Additionally, a commonly applied animal model was used to systematically investigate discrepancies in two techniques widely used for cartilage repair. The study was randomized, controlled and the histological sections for analyses were blinded to the investigator. The main outcome measures were all quantitative.

Scientific relevance

Cartilage repair techniques possess three goals: 1. Complete filling of the defect, 2. Restoration of normal cartilage morphology and 3. Restoration/preservation of the subchondral mineralized tissues. The current study

demonstrates that Mos resulted in a higher degree of tissue filling but affected the subchondral bone more than Mfx. Insufficient filling occurred following both techniques. The study illustrates the possible shortcomings in two of our current techniques in cartilage repair. The rabbit knee is a useful experimental model for cartilage restoration and repair, and currently there is no other animal model proved to be superior. The study demonstrated experimentally that Mos and Mfx both do have unpredictable results in the single case – even in a standardized animal model. Further clinical studies with optimized set up are needed before firm recommendations can be given as to which – if any – of the techniques to be used.

The current study suggests that experimental studies evaluating cartilage repair necessitate long term follow up to reveal relevant results, an aspect emphasized by other authors as well [39].

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TABLES

Table 1 The fraction of knees with minor degenerative changes observed macroscopically at follow up.

	12 weeks	24 weeks	36 weeks
Knees treated with microfracture technique	1 / 10	2 / 10	1 / 15
Knees treated with mosaic plasty	1 / 11	3 / 10	4 / 14

Table 2 Total amount of tissue filling in defects treated with Mos and Mfx, pair wise compared at the different follow up time points.

	12 weeks	24 weeks	36 weeks
Mosaic plasty	31,8 % SD 17,6 95% CI [15,5 – 48,1] (n=7)	45,5 % SD 30,3 95% CI [20,1 – 70,8] (n=8)	65,5 % SD 38,0 95% CI [39,9 – 91,0] (n=11)
Microfracture technique	31,6 % SD 17,7 95% CI [15,2 – 47,9] (n=7)	30,2 % SD 17,4 95% CI [15,7 – 44,7] (n=8)	31,4 % SD 13,4 95% CI [22,4 – 40,4] (n=11)
Mean paired difference Mosaic-Microfx	0,2 % SD 22,5 95% CI [-20,5 – 21,0]	15,3 % SD 40,2 95% CI [-18,4 – 48,9]	34,0 % SD 33,9 95% CI [11,3 – 56,8]
p-value	0,978	0,319	0,008

Table 3 New bone formation within defects treated with Mos and Mfx, pair wise compared at the different follow up time points.

	12 weeks	24 weeks	36 weeks
Mosaic plasty	2.0 % SD 3.0 95% CI [-0.8 – 4.7] (n=7)	13.3 % SD 12.8 95% CI [2.5 – 24.0] (n=8)	12.0 % SD 10.6 95% CI [4.9 – 19.1] (n=11)
Microfracture technique	4.9 % SD 7.4 95% CI [-1.9 – 11.7] (n=7)	1.4 % SD 2.6 95% CI [-0.7 – 3.6] (n=8)	2.5 % SD 3.3 95% CI [0.3 – 4.7] (n=11)
Mean paired difference Mosaic-Microfx	-2.9 % SD 9.2 95% CI [-11.4 – 5.6]	11.8 % SD 14.0 95% CI [0.1 – 23.5]	9.5 % SD 10.9 95% CI [2.2 – 16.8]
p-value	0.433	0.048	0.016

Table 4 Subchondral mineralized tissue density immediately deep to the chondral defects treated with Mos and Mfx, pair wise compared at the different follow up time points.

	12 weeks	24 weeks	36 weeks
Mosaic plasty	68,6 % SD 6,9 95 % CI [62,3 – 75,0] (n=7)	69,4 % SD 3,4 95 % CI [66,5 – 72,3] (n=8)	63,9 % SD 7,3 95 % CI [58,9 – 68,8] (n=11)
Microfracture technique	74,4 % SD 8,6 95 % CI [66,5 – 82,4] (n=7)	70,1 % SD 5,1 95 % CI [65,8 – 74,4] (n=8)	70,4 % SD 7,9 95 % CI [65,4 – 74,5] (n=11)
Mean paired difference Mosaic-Microfx	-5,8 % SD 6,9 95 % CI [-12,2 – 0,6]	-0,7 % SD 2,4 95 % CI [-2,7 – 1,3]	-6,5 % SD 7,2 95 % CI [-11,3 – -1,7]
p-value	0,068	0,434	0,013

Table 5 Rise (Δ) in synovial fluid proteoglycan content (µg/ml) from time zero to follow up in knees treated with Mos and Mfx, pair wise compared at the different follow up time points.

	12 weeks	24 weeks	36 weeks
Mosaic plasty (Δ)	4,4 SD 3,1 95 % CI [2,1 – 6,5] (n=10)	2,9 SD 5,5 95 % CI [-1,1 – 6,8] (n=10)	2,9 SD 2,2 95 % CI [1,7 – 4,1] (n=14)
Microfracture technique (Δ)	0,2 SD 2,7 95 % CI [-1,7 – 2,2] (n=10)	2,0 SD 3,3 95 % CI [-0,4 – 4,3] (n=10)	2,4 SD 3,2 95 % CI [0,7 – 4,1] (n=14)
Mean paired difference Mosaic-Microfx	4,2 SD 4,6 95 % CI [0,9 – 7,5]	0,9 SD 5,9 95 % CI [-3,3 – 5,1]	0,5 SD 4,1 95 % CI [-1,8 – 2,9]
p-value	0,018	0,643	0,620

ILLUSTRATIONS (Graphics and Photos):

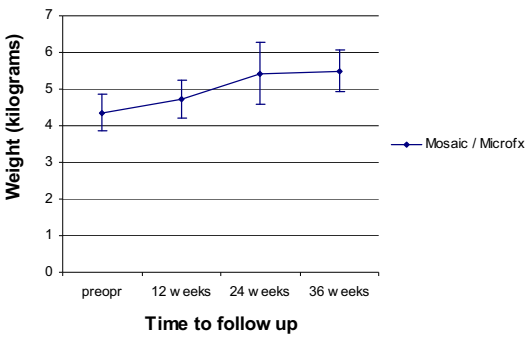

Fig. 1 The experimental animals gained weight throughout the experimental period. Mean weight at time zero was 4.35 kg. The number of animals at time zero (age 22 weeks) equalized the total number of animals evaluated at the follow ups (37) – since they were all measured preoperatively. The number of experimental animals at 12, 24 and 36 weeks were 11, 10 and 16 respectively.

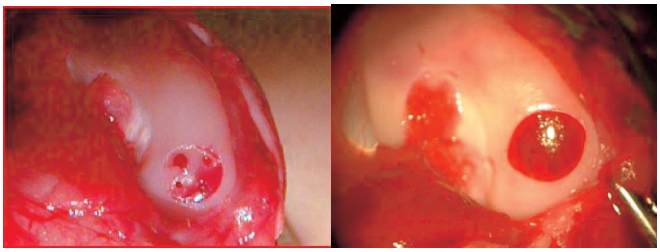

Fig. 2 Defect in MFC with the Mfx performed

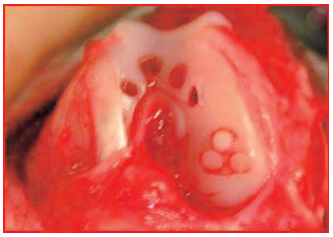

Fig. 3 Defect in MFC with the Mos performed

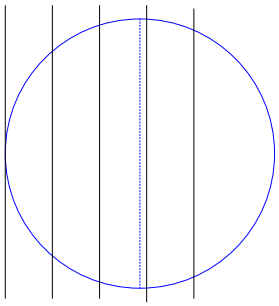

Fig. 4 Starting at the rim of the defect, five levels of sectioning each 700 µm further into the 4 mm defect ensured central sections to be analysed.

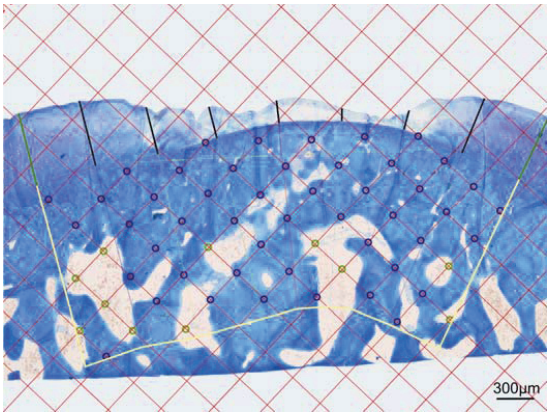

Fig. 5 Histological section of a defect stained with tolouidin blue and micrographed at 40x magnification. The section is modified high-lightening some of the graphics superimposed by the software Analysis Pro®. The two green lines outline the height of the shoulders measured. The seven black lines indicate the height of the tissue filling the defect. The yellow lines together with the tidemark frame the area of interest for subchondral mineralized tissue evaluation. The intersections of the red grid are marked with blue circles whenever overlying mineralized tissue, and green circles whenever not.

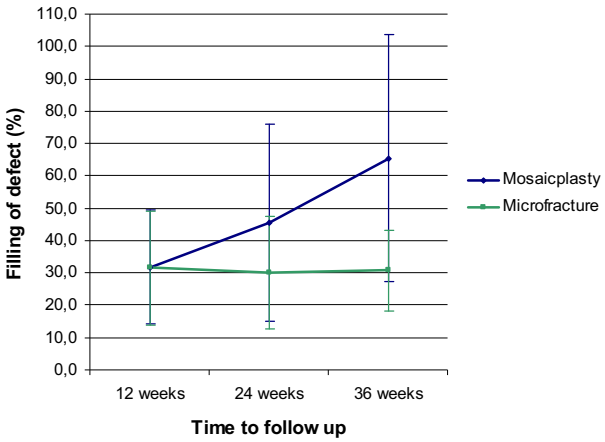

Fig. 6 Percentage filling of the defects treated with mosaic plasty and microfracture technique at the different time points of follow up. For pair wise comparison, the number of animals at 12, 24 and 36 weeks follow up were 7, 8 and 11 respectively.

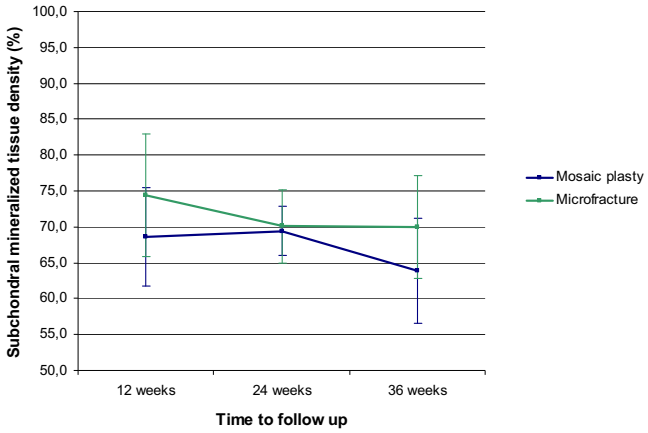

Fig. 7 Subchondral mineralized tissue density immediately deep to the defects treated with mosaic plasty and microfracture technique at the different time points of follow up. For pair wise comparison, the number of animals at 12, 24 and 36 weeks follow up were 7, 8 and 11 respectively.

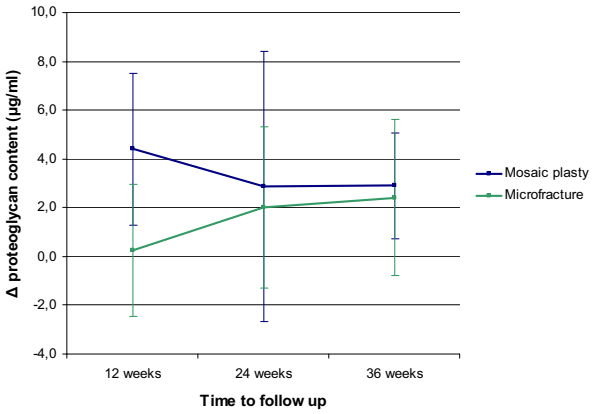

Fig. 8 Change (Δ) in synovial fluid proteoglycan content (µg/ml) from time zero to the different time points of follow up in knees treated with mosaic plasty and microfracture technique. For pair wise comparison, the number of animals at 12, 24 and 36 weeks follow up were 10, 10 and 14 respectively.