Cartilage Injuries in the Knee – Natural History and Surgical Repair

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II. Løken S, Heir S, Holme I, Engebretsen L, Årøen A. Six-year follow up of 84 patients with cartilage defects in the knee: Knee scores improved, but recovery was incomplete. Submitted to Acta Orthopaedica.

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IV. Løken S, Jakobsen RB, Årøen A, Heir S, Shahdadfar A, Brinchmann JE, Engebretsen L, Reinholt FP. Bone marrow mesenchymal stem cells in a hyaluronan scaffold for treatment of an osteochondral defect in a rabbit model. Knee Surgery Sports Traumatology and Arthroscopy. 2008 Oct; 16(10):896-903.

ABBREVIATIONS

ACI	Autologous Chondrocyte Implantation
ACI-C	Autologous Chondrocyte Implantation covered with a collagen membrane
ACL	Anterior Cruciate Ligament
ACP	Auto Cross-linked Polysaccharide Polymer
BMI	Body Mass Index
BMP	Bone Morphogenic Protein
CaReS®	Cartilage Repair System
CPM	Continuous Passive Motion
FGF	Fibroblast Growth Factor
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
MRI	Magnetic Resonance Imaging
MSC	Mesenchymal Stem Cell
OA	Osteoarthritis
OCD	Osteochondritis Dissecans
RCT	Randomized Controlled Trial
SD	Standard Deviation
SEM	Scanning Electron Microscopy
SF-36	Short Form 36
TEM	Transmission Electron Microscopy
TGF	Transforming Growth Factor
VAS	Visual Analogue Scale
Wnt	Wingless + induction

INTRODUCTION

Chondral and osteochondral injuries of the knee are common. This has been shown both in cross sectional MRI studies (Ding et al. 2005), in studies of asymptomatic athletes (Kaplan et al. 2005) and in patients undergoing arthroscopy of the knee (Årøen et al. 2004, Hjelle et al. 2002). These injuries are often seen in young and active individuals, and unfortunately, the joint cartilage has a limited capacity for healing. In the long term perspective osteoarthritis may develop (Linden 1977, Drogset and Grøntvedt 2002), even after cartilage surgery (Knutsen et al. 2004) with serious consequences for work ability and quality of life. In elderly patients, good pain relief and restored function are often achieved by joint replacement. However, in young and middle aged patients this is not a satisfactory solution. The seriousness of this problem is reflected in the fact that cartilage patients enrolled for surgery have reduced quality of life to the same extend as patient enrolled for total knee replacement (Heir et al. 2009).

Several methods have been used to treat cartilage injuries. Modern methods like autologous chondrocyte implantation were introduced in the 1990ties with promising primary results (Brittberg et al. 1994, Browne et al. 2005, Drobnic et al. 2002, Marcacci et al. 2005, Micheli et al. 2001, Peterson et al. 2000, Peterson et al. 2003). However, still Messner and Gilquist's editorial in Acta Orthopaedica from 1996 (Messner and Gillquist 1996) stays firm; no method has been proven to be better than others (Bentley et al. 2003, Horas et al. 2003, Jakobsen et al. 2005, Knutsen et al. 2007, Knutsen et al. 2004, Saris et al. 2008). The natural history of cartilage injuries has also largely been unknown and it has not been proven if, or how, surgery influences the natural history. Undoubtedly, there is need for improvement of the techniques to repair cartilage, and extensive research is performed all over the world with the goal to restore the morphology and function of normal joint cartilage.

MORPHOLOGY

Hyaline cartilage

The bony surfaces of the joints are covered by hyaline cartilage. The surface is smooth and enhanced by the lubrication from the synovial fluid the friction is extremely low. The cartilage has the ability to withstand compression and distribute load, and thereby protect the subchondral bone (Suh et al. 1997). The cells producing the extracellular matrix of the hyaline cartilage are the chondrocytes, differentiated from mesenchymal stem cells.

Mesenchymal stem cells

Mesenchymal stem cells (MSCs) are multipotent cells that may differentiate along several cell lineages. They can be isolated from many different tissues: bone marrow, trabecular bone, muscle, fat, periosteum, synovial membrane, articular cartilage, and peripheral blood (Tuan et al. 2003, Chen and Tuan 2008). MSCs have theoretical advantages compared to chondrocytes regarding potential for healing. These cells have the ability to proliferate without loosing their ability to differentiate into mature chondrocytes producing collagen II and aggrecan, or osteoblasts producing osteoid (Tuan et al. 2003). Thus, MSCs may induce repair of both bone and cartilage in an osteochondral defect (Wakitani and Yamamoto 2002, Yan and Yu 2007).

Differentiation from mesenchymal stem cells to chondrocytes

Adult MSCs may differentiate into several cell types: chondrocytes, osteoblasts, adipocytes, myocytes, fibroblasts and bone marrow stromal cells (Tuan et al. 2003). MSCs can be differentiated into chondrocytes *in vitro* with the use of growth factors (table 1). Several growth factors have been proven to facilitate this differentiation (Chen et al. 2006, Gelse et al. 2003). Successful chondrogenic differentiation *in vitro* is characterized by upregulation and production of cartilage-specific matrix components, including type II collagen and aggrecan. Many of the transforming growth factors-βs (TGF-β) have been shown to induce

chondrogenic differentiation of MSCs *in vitro* (Chen and Tuan 2008), where TGF-β1, TGF-β2 are most potent for human MSCs. Bone morphogenic proteins (BMPs) of different subtypes (BMP 2, 4, 6, 7, 9 and 13) are also involved in chondrogenic differentiation as well as fibroblast growth factors (FGFs 2 and 18) and insulin like growth factor 1. Other growth factors shown to play a role are: growth differentiation factor 5 and signaling proteins of the Wnt family. The effect and importance of the different factors and the role of the combinations of them vary between the tissues according to where the MSCs are derived from and also between species (Chen et al. 2006).

Physiological factors are also important in the differentiation (table 1): oxygen tension and oxidative pressure, mechanical loading (deformation, hydrostatic pressure, fluid flow, shear stress) and the electrical potential of the cells (Chen et al. 2006). Mechanical loading is a key factor in the formation of joint cartilage (Wong and Carter 2003).

Table 1. Major factors regulating cartilage homeostasis and differentiation of mesenchymal stem cells. Adapted from Chen (Chen et al. 2006).

Growth factors, cytokines and signaling molecules
Transforming growth factor-βs 1, 2 and 3
Bone morphogenetic proteins 2, 4, 6, 7, 9 and 13
Insulin-like growth factor 1
Fibroblast growth factors 2 and 18
Growth differentiation factor 5 (also known as cartilage-derived morphogenetic protein 1)
Wnt glycoproteins* (signaling molecules)

Environmental factors
Oxygen tension and oxidative pressure
Mechanical loading
Deformation
Hydrostatic pressure
Fluid flow
Shear stress
Electrical potential

* Wnt (abbreviation: wingless + induction) – first detected to cause a wingless mutation

Chondrocytes

Chondrocytes are the cells producing hyaline cartilage. The chondrocyte differs from most other cells in the body in being usually without direct contact with its neighboring cells, and being organized in a tissue lacking direct blood supply and peripheral nerves. The chondrocyte produces its own extracellular matrix responsible for the biomechanical properties of the tissue (Archer and Francis-West 2003).

Chondrocyte nutrition and oxygen transport

Glucose is the major energy source in chondrocytes and a precursor for glycosaminoglycan synthesis (Archer and Francis-West 2003). Glucose transport in chondrocytes is mediated by glucose transporter proteins. 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.

Extra cellular matrix

The extracellular matrix is produced by the chondrocytes with mechanical loading as an important stimulus. It is composed of a collagen network, which consists of type II collagen fibrils interacting with types IX and XI collagens providing tensile strength and contributing to the retention of proteoglycans. The large aggregating proteoglycan aggrecan attached to hyaluronic acid (HA) polymers resists compressive forces (Goldring 2006). Collagen type VI is the major collagen type in the pericellular matrix (the narrow layer encapsulating the chondrocytes), and has been shown to play an important role in physiology of the chondrocytes and in the biomechanical properties of the cartilage (Alexopoulos et al. 2009). A large number of other components, including small proteoglycans and other non-collagenous proteins contribute to the properties of the matrix. Once the cartilage is formed in the adult, the chondrocytes maintain a low turnover rate of replacement of cartilage matrix proteins with a collagen half-life of more than 100 years. Glycosaminoglycans and other cartilage matrix constituents are also synthesized by chondrocytes at low rate under steady state. There are regional differences, and matrix turnover is more rapid in the immediate pericellular zones.

Layers in the articular cartilage

The superficial zone consists of tightly packed collagen fibers parallel to the articular surface and flattened chondrocytes. Type IX collagen is located between collagen type II bundles that provide resistance to shear. It is thought that the superficial zone limits passage of large molecules between synovial fluid and cartilage. The transitional layer, or middle zone, is composed of spherical chondrocytes, proteoglycans, and obliquely oriented collagen fibers that primarily resist compressive forces but also serve as a transition between the shearing forces on the surface and the compressive forces placed in the deeper layers. The deep zone consists of collagen fibers and chondrocytes oriented perpendicular to the articular surface in order to resist compressive loads. The calcified zone is separated from the deep zone by the tidemark and is characterized by the extracellular matrix being calcified. In addition, to constitute a stiffness gradient towards the subchondral bone the calcified zone also provides adhesive properties (Tyyni and Karlsson 2000). The zones are illustrated in figure 1.

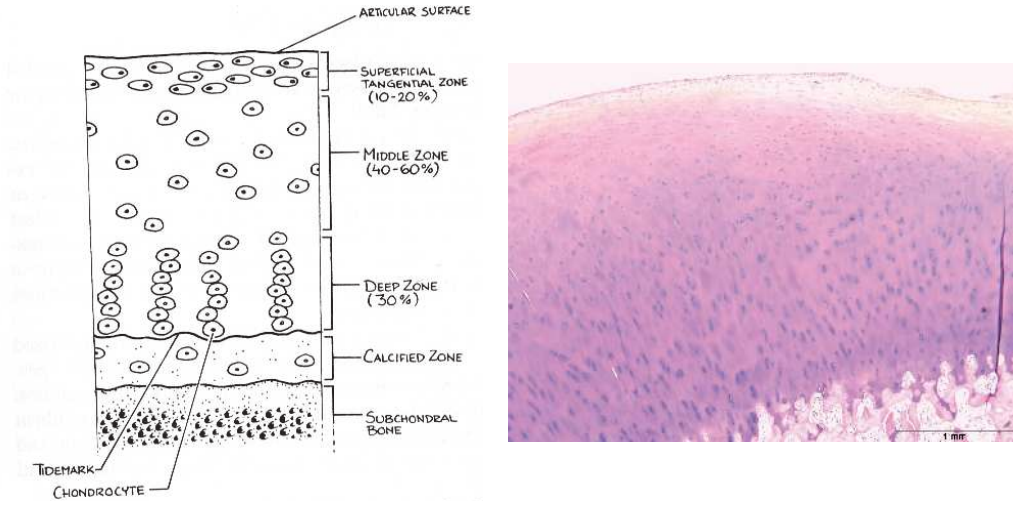

Figure 1. Layers in the articular cartilage (left: drawing from (Tyyni and Karlsson 2000)), right: image of human knee cartilage.

CARTILAGE INJURIES

This thesis focuses on focal cartilage injuries and defects following a detached osteochondritis dissecans fragment. The term focal injury is used to describe a limited lesion where the surrounding and opposing cartilage are considered normal or nearly normal. Usually a focal lesion is either a defect following an osteochondritis dissecans (OCD) or a traumatic lesion, but sometimes the term focal degenerative lesion is also used.

Osteochondritis dissecans

The term osteochondritis dissecans (OCD) was introduced by König in 1887 (König F. 1887). This is primarily a condition affecting the subchondral bone and later the articular cartilage. OCD is seen in many joints and typically on the convex joint surfaces. If the lesion does 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 free fragments in the joint cavity leaving an osteochondral defect in the joint surface. The ending ”-itis” indicates that the condition originally was believed to be inflammatory. Later, several causes have been postulated, including inflammation, genetics, ischemia, defective ossification, and repetitive trauma. Still the etiology of OCD is not unequivocally settled. Experimentally an OCD-like condition has been created in growing pigs by cutting the blood supply to the growing cartilage (Ytrehus et al. 2004), and repetitive trauma may also induce the lesion (Cahill 1995).

Classification

OCD can be classified as juvenile or adult, depending on the occurrence before or after the closure of the growth plates. The condition can be graded from radiographs (Milgram 1978), arthroscopically (AS) according to Guhl (Guhl 1979), or by MRI findings (Nelson et al. 1990). The arthroscopic and MRI gradings (table 2) have been shown to be highly correlated (O'Connor et al. 2002). The classification of Guhl is very similar to OCD classification proposed by ICRS (Brittberg and Winalski 2003).

Table 2. Arthroscopic (Guhl 1979) and MRI-classification (Nelson et al. 1990) of OCD

	Arthroscopic:	MRI:
Grade 1:	Softening of cartilage, no fissure	Thickening of cartilage, no breakage of cartilage
Grade 2:	Fissure in cartilage, but fragment not displaceable	Fissure in cartilage – signal behind fragment
Grade 3:	Displaceable fragment, but still attached	Cartilage breached, high signal on T2 behind fragment
Grade 4:	Loose fragment - osteochondral defect	Loose body – osteochondral defect

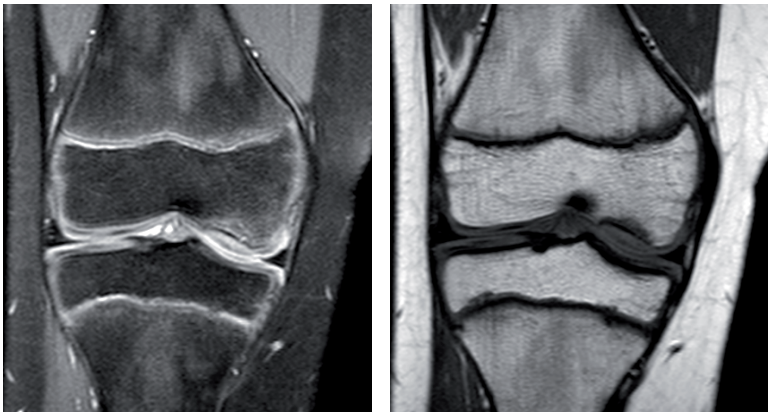

Figure 2. MRI of grade a 2 OCD lesion of the medial femoral condyle in a 12-year-old boy.

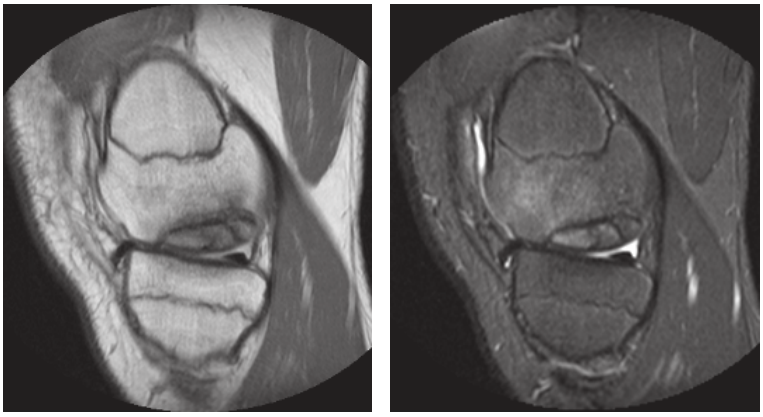

Figure 3. MRI of a large grade 3 OCD lesion of the medial femoral condyle of a 14-year-old boy. This OCD has broken into three fragments.

Incidence

The incidence (new cases per year) in the population under 50 years of age has been calculated to be between 5 and 15 per 100 000 with a peak between 10 and 20 years of age, and with a male/female ratio of 2/1 (Linden 1976). However, these numbers are from the period 1965 to 1974 in Malmø, Sweden and the incidence was increasing during the observation period. The incidence today and in other communities is unknown. OCD is seldom found in patients under 10 and over 50 years of age (Linden 1977). In the knee, the medial femoral condyle is most often affected (80% of OCD in the knee). Bilateral and familiar cases are seen, suggesting a genetic disposition. Histologically, OCD resembles a stress fracture. Males (Bohndorf 1998) and more physically active persons (Aichroth 1971) show higher prevalence. Typical idiopathic OCD must be differentiated from similar-appearing osteochondral lesions resulting from avascular necrosis associated with chemotherapy, hemoglobinopathy, steroid medication or immunosuppressive treatment (e.g. following organ transplantation).

Clinical presentation of OCD

The main presenting symptom of OCD is pain. The first presentation is usually poorly localized knee pain at/or following activity. Later in the course, the pain may increase and swelling, stiffness and finally locking caused by a loose fragment may occur. At clinical examination, the patient may be limping or walking with an externally rotated leg. Local tenderness over the affected area is often found.

Natural history of OCD

The natural history of juvenile OCD is different from the adult type. Linden observed that patients diagnosed with juvenile OCD seldom developed osteoarthritis (OA), while 80 % of adult OCD patients developed OA during a 30 years observation period (Linden 1977). Stable OCD in skeletally immature patients will heal in > 90 % of the cases without surgical intervention (Williams, Jr. et al. 1998). For patients close to, or passed epiphyseal closure, surgical treatment is recommended. Without surgical intervention, the prognosis is poor (Williams, Jr. et al. 1998). Most adult OCDs are probably unhealed juvenile OCD, but OCD development after closure of the growth plates has been reported (Garrett 1991).

Focal cartilage defects

Traumatic cartilage injuries

These injuries are caused by a traumatic event and are often seen in combination with anterior cruciate ligament (ACL) injuries (Granan et al. 2008, Shelbourne et al. 2003). Injuries of the patella and lateral femoral condyle are often seen after patellar dislocation (Elias et al. 2002), and often include the subchondral bone. Frequently the cause of a cartilage defect is unknown and the cartilage defect is discovered at arthroscopy or by MRI. In these cases, the appearance of the lesion decides whether it is classified as traumatic or degenerative. Localized lesions with sharp edges and normal surrounding cartilage will be regarded as a traumatic injury.

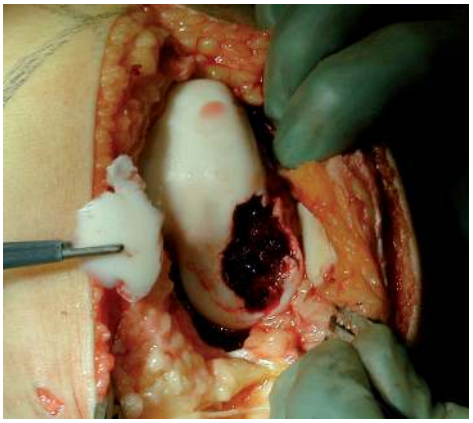

Figure 4. Acute osteochondral injury of the lateral femoral condyle after a patellar dislocation

Degenerative cartilage injuries

Larger defects with rounded and/or irregular edges, which also affect the surrounding and opposing cartilage, are usually classified as degenerative lesions. These injuries may represent the start of OA, and there is no clear distinction between a degenerative cartilage injury and OA. Sometimes terms like localized OA or one compartment OA are used.

Classification of focal cartilage injuries

Outerbridge classification (Outerbridge 1961)

This 4-graded classification was first developed and used to classify chondromalacia of the patella. Grade 1: softening and swelling of the cartilage. Grade 2: fragmentation and fissuring of an area less than ½ inch in diameter. Grade 3: as grade 2, but more than ½ inch in diameter. Grade 4: erosion of cartilage down to bone.

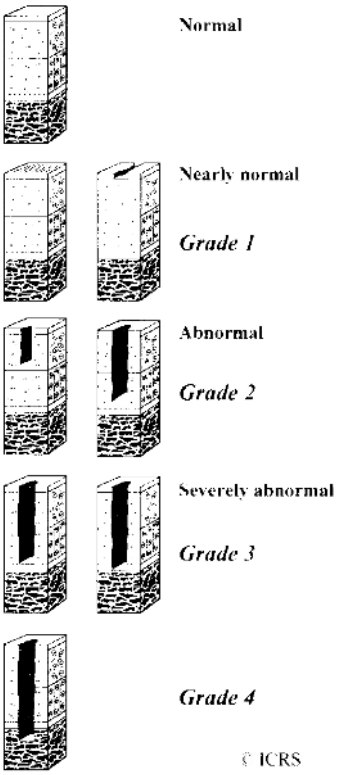

Figure 5. ICRS classification system of depth of cartilage injuries (version 1998, used in the study included in this thesis). Grade 1: nearly normal (superficial fissuring). Grade 2: abnormal (deep fissures/defect, but not down to bone). Grade 3: severely abnormal (fissures/defect down to bone). Grade 4: severely abnormal (fissures/defect extending into the subchondral bone). Reprinted with permission from International Cartilage Repair Society.

Most researchers have now replaced the Outerbridge classification system by the International Cartilage Repair Society (ICRS) classification introduced in 1998 (International Cartilage Repair Society 1998). There is also a later revision of this system (Brittberg and Winalski

2003). The main difference is that in the new version, grade 2 lesions are defined as involving less than 50% of the cartilage thickness, while grade 3 lesions are involving more than 50% of the cartilage thickness, but not extending into the subchondral bone. Blisters are also defined as a subgroup of grade 3 in the revised version.

Prevalence of focal cartilage defects

In a cross sectional MRI study the prevalence of cartilage defects were 31% in individuals under the age of 45 and 54% in those above the age of 45 (Ding et al. 2005). A high prevalence in asymptomatic high-level basketball players have also been reported with 31 MRI detected lesions in 19 of 40 players (Kaplan et al. 2005). These studies also report small lesions that may not be clinically relevant, and the sensitivity of the MRI may play a role: In a study with 1.0 Tesla MRI in the earlier days of MRI, cartilage lesions were found in 3 of 54 asymptomatic subjects (age 19-39) (LaPrade et al. 1994), while in a recent study with 3.0 Tesla MRI 9 of 20 asymptomatic subjects (age 25-45) showed cartilage lesions (Stahl et al. 2009).

The prevalence in patients undergoing arthroscopy has been investigated more extensively. In a US database of 31 516 arthroscopies (Curl et al. 1997) Outerbridge grade 4 lesions where found in 20 % of the patients and in 5 % of patients under 40 years of age. In a report of 1000 knee arthroscopies (Hjelle et al. 2002), 61 % of the patients showed a cartilage lesion, and in 19 % this was classified as focal. 7.1 % of the patients under 50 years of age had an ICRS grade 3-4 lesion more than 1 cm^2. In a study of 25 124 knee arthroscopies (Widuchowski et al. 2007), similar findings were reported showing chondral lesions in 60 % of the patients. Localized lesions, ICRS grade 3-4, were found in 9 % of patients under 50 years of age.

Clinical presentation of focal cartilage defects

Knee pain is the main symptom in patients with cartilage defects. The patients may also experience swelling and mechanical symptoms. Often a cartilage defect is diagnosed at arthroscopy or MRI in combination with ACL or meniscal injuries. They may present as an acute injury, but usually the symptoms start vaguely increasing with time and finally make the patient seek medical help. Patients undergoing cartilage surgery show lower preoperative Lysholm score compared to patients undergoing ACL reconstruction (Aarseth L. et al. 1999)

and they have similar KOOS scores as patients eligible for total knee replacement (Heir et al. 2009).

Natural history of cartilage defects

The natural history of focal cartilage defects is largely unknown. Thus, it is not known to what extent a cartilage injury leads to OA, or if there is a critical size or depth limit predicting progression to OA.

A favorable outcome was reported in a long term follow up of 28 patients with isolated severe chondral damage in the weight-bearing area of the knee joint diagnosed at arthroscopy (Messner and Maletius 1996); 14 years later 22 of the patients showed excellent or good knee function. This is the only study reporting long term results in untreated isolated cartilage lesions.

From ACL-reconstructed patients with concomitant cartilage lesions some information is available: Cartilage injuries are seen in 26% of ACL reconstructed patients (Granan et al. 2009). Shelbourne et al 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 (Shelbourne et al. 2003). These findings are supported by data from the Norwegian Cruciate Ligament Registry showing no difference in preoperative KOOS score in ACL patients with or without cartilage injury (Hjermundrud et al. 2009).

Drogset et al reported that in patients undergoing ACL-reconstruction within 2 weeks after injury the prevalence of OA after 16 years was 11% (Drogset et al. 2006), while the same group showed in another study that the prevalence of OA was 50 % after 8 years in patients undergoing ACL reconstruction at average 3.5 years after injury (Drogset and Grøntvedt 2002). They also reported that patients with a cartilage injury detected at the ACL reconstruction were more likely to develop OA later. Data from the Norwegian National Cruciate Ligament Registry demonstrate increasing prevalence of cartilage and meniscal injuries with increasing time from injury to reconstruction (Granan et al. 2009).

TREATMENT OF CARTILAGE INJURIES

Treatment of OCD (intact fragment)

Skeletally immature patients are treated non-surgically with restricted weight bearing for a period of 6 to 8 weeks followed by activity modification. More than 90% of the lesions will heal within 3-6 months (Williams, Jr. et al. 1998). Surgical intervention is recommended in failed conservative treatment and in patients close to skeletal maturity or older. A knee arthroscopy is performed, and if the fragment is stable by probing, drilling through the fragment and into the subchondral bone is performed (Williams, Jr. et al. 1998, Kocher et al. 2006). If the fragment is unstable, curettage and fixation of the fragment is recommended. Some authors recommend additional bone grafting in all cases. With this treatment algorithm, 80-90% of the patients will achieve a good or excellent result (Williams, Jr. et al. 1998).

Treatment of focal cartilage defects

Training

The effect of strength training and other training modalities have been investigated in OA patients. In a recent Cochrane report, the authors conclude that there is at least a short term benefit from exercise in terms of reduced knee pain and improved physical function for people with knee OA. The magnitude of the treatment effect is small, but comparable to the effect of non-steroidal anti-inflammatory drugs (Fransen and McConnell 2008). This has not been investigated in patients with focal cartilage lesions, but in an ongoing RCT at our institution where patients underwent a 3 months physical training program before cartilage surgery, the majority of the patients improved their subjective knee function and wanted to postpone surgery. Regarding the direct effect of training on the cartilage tissue, 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, adult cartilage will not become thicker after increased load such as intensive running and similar exercises (Eckstein et al. 2006). A study on dogs running with a weight jacket 75 km/day, five days a week for ten years did not alter cartilage morphology/thickness compared

to controls; neither did they develop OA or cartilage injuries (Newton et al. 1997). To what degree, if any the morphology of injured cartilage can be influenced by exercise is unknown (Salter et al. 1980).

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 medication. Glucosaminoglycans and chondroitin sulphate have been introduced as possible modulators of OA. A metaanalysis have concluded that there was no effect from chondroitin sulphate on pain and function (Reichenbach et al. 2007), and a Cochrane report concludes that there is a possible effect of glucosamine sulphate, but only for one particular brand (Rotta-preparation), and no effect of glucosamine hydrochloride (Towheed et al. 2005). Whether these drugs have any symptomatic effect in patients with focal cartilage defects is unknown.

Intraarticular injections

Intraarticular injections with corticosteroids have long been used to treat the synovitis that oftentimes follows the initial OA. Hyaluronan and hylan (HA) products have also been developed for intraarticular injections, so called viscosupplementation in moderate OA. 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 non-steroid anti-inflammatory drugs and intraarticular effect of corticosteroids (Bellamy et al. 2006a, Bellamy et al. 2006b). Whether viscosupplementation has any symptomatic effect on focal cartilage defects in patients is unknown. Rabbit experiments have shown that hyaluronan injections may improve the repair of osteochondral defects (Miyakoshi et al. 2005), partial thickness defects (Jansen et al. 2008) and repair after microfracture (Strauss et al. 2009).

Surgery

The spectrum of surgical alternatives for treating articular cartilage defects range from simple lavage and debridement to replacement of the knee joint. Choice of treatment depends on

multiple factors: the patient's symptoms, the number of defects, the location, size and depth of the defects, 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 given therapy.

The surgical treatment options can be divided into four categories:

I. Symptomatic treatment
II. Bone marrow stimulating techniques
III. Transplantation of osteochondral grafts
IV. Induction of chondrogenesis

I. Symptomatic treatment

Lavage

One of the most basic traditional methods of treating articular cartilage injuries is lavage. The clinical improvement following arthroscopic lavage was discovered by Robert Jackson (Jackson 1974). A possible mechanism behind the effect is that the procedure removes articular debris and inflammatory mediators known to be generated by the synovial lining of damaged joints (Jackson and Dieterichs 2003). In addition, reduced loading and activity following surgery may relieve symptoms. The limitations are that the clinical results obtained are generally insufficient for athletic or young patients, the relief of pain is short-term, and the underlying pathology is not addressed. The explanation of the effect has also been claimed to be a pure placebo effect (see debridement below).

Debridement

This is an arthroscopic surgical technique used to remove cartilaginous loose flaps/fragments, osteophytes and loose bodies that may cause mechanical irritation. 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). The doubtful effect of debridement is supported by the results of a randomized controlled trial where arthroscopic

debridement was compared to sham operation to treat OA. There was no difference between the groups (Moseley et al. 2002). Due to criticism of this study, a similar study with an improved research methodology was later conducted with identical result (Kirkley et al. 2008). Thus, at least in OA the effect of debridement seems primarily to be a placebo effect.

However, 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 be justified as a first-line therapy before more extensive procedures are performed. Not the least due to the fact that the procedure also provides valuable diagnostic information.

II. Bone marrow stimulating techniques

General considerations

In most instances, traditional wound healing requires the presence of blood. Articular cartilage lacks its own blood supply as the subchondral bone plate separates the cartilage layer from the rich vascular plexus of the bone marrow. By opening up the subchondral bone plate, hemorrhage is induced; delivering growth factors, leukocytes and MSCs, necessary to induce a fibrocartilaginous repair of a chondral lesion. Drilling, microfracture and abrasion arthroplasty, are all based on the infiltration of blood products to form a fibrin clot in the lesion that will eventually produce a fibrocartilage repair tissue. The fibrin clot is replaced within days by a granulation tissue followed by ossification of the areas closest to the bone, while the rest is transformed into fibrocartilage (Shapiro et al. 1993). The fibrocartilage differs from hyaline cartilage in several aspects (table 3): The dominating collagen is type 1 in contrast to collagen type 2 in hyaline cartilage (Mandelbaum et al. 1998, Furukawa et al. 1980). The collagen orientation is random in contrast to hyaline cartilage that has specific orientation (Kaab et al. 1998) and thickness (Hedlund et al. 1993) in the different layers. The cells are flat resembling fibroblasts.

A major concern with the bone marrow stimulating techniques is how long the fibrocartilage repair tissue will be able to withstand the stress and wear placed on an active knee joint. Mow (Mow et al. 1991) refers to fibrocartilage as being an inherently weak tissue. It is a repair

tissue consisting of a mixture of type 1 and type 2 collagen, which is unorganized and poorly integrated into the adjacent cartilage. The biomechanical properties of the repair tissue are inferior to those of the adjacent normal cartilage. Consequently, shear stresses are increased along the interface between the repair and surrounding normal tissues (Suh et al. 1997). The quality is claimed to be improved by properly performed microfracture (see below) followed by a strict rehabilitation procedure (Steadman et al. 2001).

Another concern with methods involving injury to the subchondral bone plate is what effect the procedure will have on the elastic properties of the bone. Due to its inherent elastic properties, the subchondral bone acts as a shock absorber. If the bone plate is traumatized, the bone remodels and becomes stiffer (Radin and Rose 1986). A similar finding with thickening of the subchondral bone after microfracture has been shown experimentally (Årøen et al. 2006).

Table 3. Characteristics of fibrocartilage compared to hyaline cartilage

	Type I collagen	Type II collagen	Proteo-glycan	Matrix	Cells	Collagen orientation
Fibrocartilage	++	+	+	Non-homogenous	Flat	Random
Hyaline cartilage	0	+++	+++	Homogenous	Rounded	Organized

Drilling into the subchondral bone

Pridie (Pridie KH 1959) was the first to induce the concept of drilling into the subchondral bone to produce a repair tissue capable of filling a chondral defect. With this technique, multiple drill holes were made through the subchondral bone and into the trabecular bone to create hemorrhage as basis for the formation of a repair tissue. Symptomatic pain relief has been reported by a number of investigators following this procedure (Childers, Jr. and Ellwood 1979, Dzioba 1988, Insall 1967). Another technique of penetrating through the subchondral bone was introduced by Ficat (Ficat et al. 1979), a technique called spongialization. With this technique, the entire bone plate is removed from the underlying cancellous bone. The technique showed 79 % success rate with two year follow-up, however; a positive effect like this has not been reported by others.

Abrasion Arthroplasty

Abrasion arthroplasty involves debriding the articular cartilage defect back to normal edges. The surface of the subchondral bone is then exposed, and with the use of a 1- 2 mm motorized burr the surface is removed, keeping most of the bone plate intact, but advancing deep enough to induce bleeding. Clinically, Johnson (Johnson 1986) reported a success rate of 77% in 95 patients after a two year follow-up. Other investigators reported worse results with this method compared to arthroscopic debridement alone (Bert and Maschka 1989). The use of the abrasion technique evokes the same concern with regards to disturbing the elastic properties of the subchondral bone plate as discussed above.

Microfracture

A similar technique to drilling is microfracture, an approach in which the subchondral bone plate is exposed and adjacent cartilage is debrided back to healthy cartilage. The subchondral bone is then perforated with an awl to induce hemorrhage. After the procedure, the patient follows a rehabilitation program of protective weight bearing and continuous passive motion to simulate differentiation of the repair tissue into cartilage. Clinically, at seven year follow-up, this technique showed a success rate of approximately 65% (Blevins et al. 1998). However, this patient group was mixed, also including meniscus surgery and ACL surgery, which makes the interpretation difficult. Steadman (Steadman et al. 2003) claims that the advantages of microfracture technique compared to drilling are that the subchondral bone plate is largely preserved, and the awls do not produce heat necrosis. However, concern about disturbing the elastic properties of the subchondral bone plate will be the same as for the other approaches. The method has in a meta-analysis been reported with a 95% confidence interval for Lysholm score between 78 and 97 (Jakobsen et al. 2005). Microfracture has similar clinical results as autologous chondrocyte implantation (ACI), and when histology is evaluated, similar or slightly inferior results to ACI in RCTs (Knutsen et al. 2007, Knutsen et al. 2004, Saris et al. 2008).

Today, microfracture is often used as a primary treatment option, and if not successful, more invasive cartilage repair methods are performed at a later stage. There has been a concern with the microfracture procedure whether it can hamper the result of a future alternative

procedure. In a recent study patients subjected to bone marrow stimulating procedures showed equal improvement following ACI as patient who had undergone debridement alone (Zaslav et al. 2009). On the other hand, in another recent report of 321 patients treated with ACI, previous bone marrow stimulating procedures were associated with poorer outcome (Minas et al. 2009). Three or more previous surgeries to the knee have also been associated with a less favorable outcome following ACI (Krishnan et al. 2006a).

III. Transplantation of osteochondral grafts

Allografts

This procedure uses cadaveric allografts to reconstruct the knee joint. The allograft from the cadaver knee with cartilage attached is trimmed, and press fitted into a prepared hole or attached with screws. For this technique to be successful 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. The attachment relies on bone to bone contact and a bony thickness and realignment procedures are frequently used to remove stress from the grafted area (Gross et al. 1975).

There are two types allograft in use - fresh or frozen. Fresh is defined as harvested less than 12 hours after death (Gross et al. 1975). Good long term results with up to 25 years graft survival have been reported (Gross et al. 2008). In a case report, 60% donor chondrocyte viability has been demonstrated after 29 years (Jamali et al. 2007). The concerns of the fresh allografts are the risk of immunological reactions and disease transmission, but after 5 years, no instances of tissue rejection were reported (Langer et al. 1978). Frozen allografts are reported to show decreased cell-viability with time, and to yield inferior results compared to fresh allografts (Branam and Johnson 2007).

Autografts

The use of autografts were first reported by Matsusue (Matsusue et al. 1993) who harvested autologous osteochondral grafts as cylinders from the lateral wall of the patellar groove to treat osteochondral lesions. Later, Hangody have reported that 92% of the patients achieved a good or excellent result (Hangody et al. 1997, Hangody et al. 1998) following this procedure.

Good clinical results have also been reported in RCTs (Gudas et al. 2005, Horas et al. 2003), while another RCT questioned if the method could be justified (Bentley et al. 2003).

The advantages of this technique are elimination of the concerns for rejection and the transmission of diseases, as well as a graft (although limited in amount) always being available. It is a one step procedure that in many cases can be done arthroscopically. The main concerns with this method are the donor site morbidity (LaPrade and Botker 2004), failure of ingrowth of the plugs (Huntley et al. 2005), and the limited treatment options if the pain persists. Even though the method show similar results compared to other methods, its use has declined over the last years.

IV. Induction of chondrogenesis

Periosteum transplantation

The periosteum consists of two layers: an outer fibrous layer and the deeper cambium layer containing undifferentiated mesenchymal stem cells. These cells may differentiate into chondrocytes that may produce hyaline cartilage. This has been shown in different experimental models both *in vitro* and *in vivo*, mainly in rabbits (O'Driscoll and Fitzsimmons 2001). The formation of hyaline cartilage has been shown by histological assessment of quality and quantity and by the demonstration of collagen type II.

The ability to produce cartilage is dependent on age. In rabbits, the capability to synthesize hyaline cartilage is at maximum at the age of 2 months, and from that age, there is a linear decline up to 12 months. After 12 months, there is hardly any cartilage formation from periosteum explants (O'Driscoll et al. 2001). The rabbits in these studies were skeletally mature at 6 months of age. The ability to form cartilage is also proportional to the thickness and the number of cells in the cambium layer (O'Driscoll et al. 1986). The formation of hyaline cartilage is stimulated by joint movement. Rabbits treated with continuous passive motion achieved a better cartilage repair than those immobilized. O'Driscoll also demonstrated that with periosteal transplantation to osteochondral defects both the subchondral bone and the cartilage regenerated (O'Driscoll and Fitzsimmons 2001). The technique of periosteal transplant is to cover the base of a cartilage defect with a periosteal

flap. The flap can be placed with the cambium layer up, facing the joint as recommended by O'Driscoll (O'Driscoll and Fitzsimmons 2001) or with the cambium layer down, facing the bone as recommended by Lorentzon and co-workers (Lorentzon et al. 1998). In rabbit studies, the best results were achieved with the cambium layer facing the joint (O'Driscoll and Fitzsimmons 2001).

The periosteal flap is usually secured to the base of the defect by a combination of sutures and fibrin glue. This is technically different from ACI (see below) where the periosteum flap is attached with sutures to the rim of the defect as a cover with the cells injected into the space under the flap.

In clinical case series, the results have been good to excellent in 70-80% of the cases with the most promising results on the patella (Alfredson and Lorentzon 1999). On the other hand, in a Danish study of 18 patients treated with periosteal transplant for OCD defects the results were inferior and they concluded that the method was not justified (Madsen et al. 2000). The method has not been studied in any RCT in comparison to other methods. In a Danish RCT where periosteal cover was performed with cells (=ACI) or without cells the clinical results were similar in the two groups with a tendency for a better histological result in the cell group (Haugegaard M et al. 2006). However, in this study the periosteum was attached as a roof over the defect in both groups. In recent years, little clinical research on periosteal transplantation has been conducted and the method is less utilized.

Autologous chondrocyte implantation (ACI)

First generation ACI

Experimental studies on autologous chondrocyte implantation in rabbits were first published in 1989 (Grande et al. 1989), and the first clinical results from the repair of focal cartilage injuries using this method in the human knee were published in 1994 (Brittberg et al. 1994) and led to renewed interest in research aiming at repairing or restoring injured articular cartilage. With this method an arthroscopy is performed, to harvest 200-300 mg of healthy cartilage. The harvested cartilage is treated by enzymes and expanded *in vitro*, usually in

autologous serum. The number of cells increases from 300 000 to 10-60 millions. 2-3 weeks later an arthrotomy is performed. The defect is debrided, periosteum is sutured over the defect and sealed with fibrin glue, and the cultured chondrocytes are injected under the flap.
Until July 2004, 61 studies including 3987 surgeries had been published (Jakobsen et al. 2005). Most studies have been published with short term results of ACI in single series (Brittberg et al. 1994, Drobnic et al. 2002, Erggelet et al. 2000, Fu et al. 2005, Micheli et al. 2001). Only a few long term studies have been published (Peterson et al. 2000, Peterson et al. 2003, Peterson et al. 2002). In general, the study designs have been poor with only five randomized controlled trials (RCTs) available (Bentley et al. 2003, Horas et al. 2003, Knutsen et al. 2007, Knutsen et al. 2004, Saris et al. 2008, Dozin et al. 2005). These RCTs compare ACI to other methods such as osteochondral plug transfer or bone marrow stimulating procedures, and they also include histological evaluations. Generally, the clinical results are promising with 80-90% excellent to good results in the single series studies. The results from RCTs vary and taking all these studies together, no method has proven to be superior to others.

Second generation ACI

Second generation ACI includes the use of a biomaterial or a so-called scaffold as a carrier for the cells. Some authors have defined the use of scaffolds as a cover or cells carried on the surface as a second generation ACI, and the use of scaffolds where the cells grow in a three-dimensional scaffold as a third generation ACI (Brittberg 2008), while others, as the current presentation, define all use of scaffolds seeded with cells as second generation ACI (Kon et al. 2008). One advantage with the use of scaffolds is that the cells will be contained in the biomaterial, limiting the possibility of leakage of the cells into the joint. Another advantage is that the chondrocytes with some of these scaffolds will grow in a three dimensional framework in which the cells have been shown to maintain more of their original properties like synthesis of collagen type II. Finally, the implantation may be performed arthroscopically with some of the scaffold types. With both first and second generation approaches the chondrocytes are harvested from the joint and then expanded *in vitro*. In this process, the cells dedifferentiate and loose their ability to produce collagen type II. Before implantation, the cells are transferred to the scaffold. *In vitro* studies have shown that growth in such an environment allows the cells to redifferentiate and resume synthesis of collagen type II (Grigolo et al. 2002, Shahdadfar et al. 2008).

Commercially available scaffolds

Several scaffolds have been developed of which some have been approved for clinical use (Kon et al. 2008, Iwasa et al. 2009).

Hyalograft C

This scaffold is based on the benzylic ester of hyaluronic acid (HYAFF 11, Fidia Advanced Biopolymers Laboratories, Padova, Italy) (Solchaga et al. 2005, Campoccia et al. 1998) and is derived from roosters. The cells harvested from the patient are expanded in monolayer, and then seeded onto the scaffold. The commercially available product (HYAFF 11 seeded with cells) is named Hyalograft C. According to the provider and publications, the product is sticky and will adhere to the bottom of the defect without the use of glue or sutures. However, for larger lesions the use of fibrin glue is recommended. A system for arthroscopic implantation has been developed and is in clinical use.

Good to excellent results in case series have been published. In a comparative study the results were better than with microfracture (Kon et al. 2009), and similar to ACI in another comparative study (Manfredini et al. 2007). No RCTs have been conducted.

Matrix-induced chondrocyte implantation (MACI)

This collagen type I/III membrane is derived from pork. The membrane may also be used as an alternative to periosteum as in the first generation ACI, but with the MACI technique, the cells are cultured for 4 weeks and then seeded on the matrix and cultured with autologous serum for 3 days before implantation. Fibrin glue is usually sufficient to secure the implant in the defect.

Good to excellent results have been reported in case series (Cherubino et al. 2003). No clinical difference was detected in an RCT where this scaffold loaded with cells was compared to the same biomaterial used as a cover over the cells (Bartlett et al. 2005b).

Bioseed C

Bioseed C is a polyglactin/poly-p-dioxanon fleece with predetermined sizes. Autologous chondrocytes are expanded *in vitro* and then loaded onto the porous scaffold using fibrin glue to distribute the cells. The graft is fixed in the corners with reabsorbable sutures placed transosseously. A technique for arthroscopic implantation and suturing has been developed.

Good to excellent results was reported after 4 years in one case series (n=40) (Kreuz et al. 2009). No RCTs have been conducted so far.

CaReS

CaReS (Ars Arthro, Esslingen, Germany) is composed of autologous chondrocytes seeded on 3-dimensional collagen type I gel. The cells are harvested, mixed with the collagen gel, and following 2 weeks expansion in autologous serum, the chondrocyte-loaded gel is ready for transplantation performed through a mini-open surgery technique with fibrin glue used for fixation.

Good to excellent results have been shown in case series (Maus et al. 2008).

Cartipatch

Cartipatch (TBF Banque de tissues, France) is a hydrogel composed of agarose and alginate with autologous chondrocytes added. This scaffold is implanted through a mini-open surgery technique with specially designed instruments to debride and shape the defect into an osteochondral defect. The implant is secured with a press fit configuration. Promising clinical results have been reported in a small series of 17 patients. Eight of 13 biopsies showed predominately hyaline like cartilage after a minimum of two years follow up (Selmi et al. 2008).

Novocart 3D

This is an autologous chondrocyte implant on a collagen-based biphasic scaffold (TETEC Tissue Engineering Technologies AG, Reutlingen, Germany) with a protective dense layer on the top. The transplantation is performed through a mini-open surgery technique using specially designed instruments. Reabsorbable minipins are used for graft fixation.

Significant improvement was shown in 22 OCD patients after 6-36 months (Ochs et al. 2007).

Fibrin glue

There are reports on the use of fibrin glue as a scaffold for autologous chondrocytes.

There was a statistically significant improvement compared to abrasion arthroplasty in an RCT (Visna et al. 2004). The same group also reported good results in case series (Visna et al. 2003).

Atelocollagen gel

There are some case reports on the use of autologous chondrocytes cultured on

Atelocollagen gel (Koken, Tokyo, Japan). Case reports with promising results have been reported by Japanese investigators (Adachi et al. 2006, Adachi et al. 2007).

As referred above several scaffolds are approved for clinical use with autologous chondrocytes. So far, the results have not been proven better than first generation ACI (Iwasa et al. 2009). However, easier and simpler implantation and the possibility for arthroscopic implantation are obvious advantages compared to first generation ACI. One concern is that many of the scaffolds are derived from animals. Although the tissue source is highly purified, there may still be a risk of disease transmission or immunological responses.

Mesenchymal stem cell implantation

In theory, MSCs have several advantages compared to chondrocytes in cartilage repair: Firstly, donor site morbidity from the joint for cartilage harvest will be avoided. A possible donor site morbidity after harvesting cartilage from a healthy knee for ACI in the ankle have been shown (Whittaker et al. 2005). MSCs may be isolated in humans from sources with little or no donor site morbidity, such as bone marrow, adipose or synovial tissue (Yoshimura et al. 2007). Secondly, dedifferentiation during expansion is avoided. Promising preliminary results have also been shown in the regeneration of injured tissue in organs such as heart, central nervous system, liver, kidney, and others (Brooke et al. 2007).

Experimental studies

Extensive basic laboratory research has been performed on mesenchymal stem cells on molecular and cellular level. The following discussion will be limited to experimental studies on cartilage repair in rabbits with the use of MSCs. Wakitani et al studied the repair of osteochondral defects in rabbits with the use of MSCs derived from periosteum and bone marrow. MSCs were seeded in a collagen gel. They found that MSCs repaired the defect with bone in the bony part of the defect and with hyaline like cartilage in the cartilage part of the defect (Wakitani and Yamamoto 2002). This is in contrast to previous studies on chondrocytes from the same group where the whole defect was filled with cartilage. Other investigators have later confirmed the difference between MSC and chondrocytes in this respect (Yan and Yu 2007). Various scaffolds have been studied in rabbits in combination with MSCs over the last 10 years. The usual experimental design has been to implant the

scaffold with cells in one knee and without cells in the other knee. In many experiments, the effect of different growth factors has also been studied. Some authors report better filling or higher score with cells in a scaffold than without, both for MSCs (Guo et al. 2004, Uematsu et al. 2005) and for chondrocytes (Frenkel et al. 1997, Willers et al. 2005). Radice et al (Radice et al. 2000) did not find any difference between the hyaluronan scaffold with or without MSCs after observation periods of 8 and 16 weeks. Kayakabe (Kayakabe et al. 2006) observed a better filling compared to empty defects (with no scaffold) only when fibroblast growth factor-2 (FGF-2) was added to the MSC-loaded hyaluronan scaffold. In a study in adult rabbits, osteochondral defects treated with MSCs appeared to have better cell arrangement, subchondral bone remodeling, and integration with surrounding cartilage than did repair tissues generated by chondrocyte implantation, while chondrocytes induced a thicker cartilage layer than MSC (Yan and Yu 2007).

Although MSCs from different sources share, several characteristics they also show different repair properties. For example MSCs from the synovial membrane have been reported to have a greater chondrogenic potential than bone marrow and periosteum derived cells, which again are superior to muscle tissue and adipose tissue derived cells in this respect (Sakaguchi et al. 2005).

Hybrid scaffolds combining hyaluronan with other components have shown promising results as well (Fan et al. 2006, Frenkel et al. 2005). An injectable synthetic extracellular matrix composed of chemically modified hyaluronic acid and collagen loaded with MSCs induced complete filling and excellent integration in osteochondral defects in rabbits after 12 weeks (Liu et al. 2006). According to these authors, this matrix may be implanted arthroscopically in patients.

MSC cell density

In the first publication on ACI in humans (Brittberg et al. 1994) the number of cells implanted was 2,6 million to 5 million cells in 50-100 microliter suspension (= cell density from 2,6 – 10 x 10^7/ml). The cell density needed for MSC implantation is not known. In a rabbit study, implantation of MSC at a higher density (5×10^7 cells/ml vs 1×10^6 cells/ml) induced a better repair tissue (Koga et al. 2008). In an *in vitro* study (Iwasa et al. 2003) on chondrocytes in agarose gel, there was a tendency towards better cartilage formation with a cell density of 2 x

10^7 cells/ml compared to 2 x 10^6 and 2 x 10^5 cells/ml. This may indicate that the cell density needed for MSC implantation is in the same range as for ACI.

Studies on MSCs in cartilage repair in humans

The clinical reports on mesenchymal stem cells in cartilage repair are limited.
In a recent report, 28 patients treated with MSC to knee cartilage defects were compared to a previous group of 100 patients treated with ACI. There were no significant differences in knee function scores between the two treatment modalities (Nejadnik H et al. 2009).

Wakitani et al compared the implantation of MSCs in a collagen gel covered with periosteum in patients undergoing high tibial osteostomy. Twelve patients underwent this treatment while 12 patients served as controls. Arthroscopic and histological evaluation was better in the experimental group, but there were no clinical difference (Wakitani and Yamamoto 2002).

In a report of 9 patients treated with MSCs under a periosteal flap to defects on the talus 8 patients showed good or very good clinical results, supported by MRI (Jancewicz et al. 2004).

Rehabilitation after surgery

The research on the effect of rehabilitation after cartilage repair is limited, and rehabilitation programs are mostly based on clinical experience. Postoperative rehabilitation programs following articular cartilage repair procedures vary greatly among patients and need to be individualized (Reinold et al. 2006). Basic animal research has shown better healing of a cartilage injury with the use of continuous passive motion (CPM) (Salter et al. 1980). Steadman et al have advocated the use of CPM 8 hours a day for 8 weeks following microfracture procedure (Blevins et al. 1998). Many surgeons have replaced this demanding program by low load stationary bicycling. 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). Most centers recommend a partially weight bearing period of 6-8 weeks. The range of motion is usually not restricted with the exception of patellofemoral lesions where flexion is usually restricted for the first 4 to 6 weeks.

GOALS OF THE PRESENT THESIS

The overall purposes of the studies included in this thesis were to increase the knowledge of the epidemiology, natural history and surgical treatment of cartilage injuries

The specific goals were:

1. To establish the prevalence of cartilage injuries in patients undergoing arthroscopy of the knee:

What are the type, size, depth and localization of the injuries?

What are the number of osteochondritis dissecans lesions, traumatic lesions and degenerative lesions in relation to each other?

What is the relationship to other injuries?

2. To investigate the natural history of cartilage injuries:

What can patients with a known cartilage injury in their knee expect with respect to knee function?

How does other injuries and cartilage repair affect the long term knee function in patients with cartilage injuries?

3. To investigate the quality of the cartilage repair tissue after autologous chondrocytes implantation (ACI):

Do morphometric methods and transmission electron microscopy give additional information on the quality of the repair tissue?

4. To investigate the functional long term outcome after ACI:

How is the knee function as evaluated with standard evaluation forms?

How is the isokinetic muscle force affected in the long term after ACI?

Can measurement of isokinetic muscle force be a useful tool in the evaluation of cartilage treatment?

5. To evaluate if mesenchymal stem cells is a feasible alternative to chondrocytes in the repair of cartilage injuries:

Can Mesenchymal stem cells (MSCs) be harvested, cultivated and reimplanted in an osteochondral model in rabbits?

Will filling of the defect be obtainable with this model?
Will the degree of filling and the quality of the repair tissue be better with MSCs than without?

Hypotheses:
With the aim to answer the above questions, the following hypotheses were tested:

1. Cartilage injuries are commonly detected in arthroscopy of the knee (paper I).
2. Knee function in patients with a cartilage defect in the knee remains stable over a 6-7 years observation period (paper I)
3. The repair tissue following autologous chondrocytes implantation is mainly fibrous cartilage (paper II)
4. Muscle strength is permanently impaired in patients with cartilage defects treated with ACI (paper III)
5. Mesenchymal stem cells can be implanted in a hyaluronan scaffold and induce cartilage repair in an osteochondral defect in a rabbit model (paper IV)

SUMMARY OF THE PAPERS

Paper I

Articular cartilage lesions in 993 consecutive knee arthroscopies

Background: Traumatic articular cartilage injuries heal poorly and may lead to development of osteoarthritis in young age. This study estimates the number of patients who may be a candidate for one of the surgical methods of cartilage repair.

Material and methods: All patients undergoing knee arthroscopy during a 6-month period at three collaborating hospitals were consecutively evaluated according to the International Cartilage Repair Society (ICRS) knee form. The material consists of 993 consecutive knee arthroscopies in patients with median age of 35 years.

Results: Preoperative radiographs demonstrated degenerative changes in 13% of the knees. Articular cartilage pathology was found in 66% and a localized cartilage defect was noted in

20% of the knees. A localized full-thickness cartilage lesion (ICRS grade 3 and 4) was observed in 11% of the knees. Of the localized full-thickness lesions, 55% (6% of all knees) were larger than 2 cm^2.

Conclusion: Eleven percent of all knee arthroscopies show cartilage defects that may be suitable for cartilage repair procedures. However, the natural history of these lesions untreated and the number of patients that will benefit from a cartilage repair procedure are so far unknown.

Paper II

Six-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 injuries is unknown, and despite a high number of cartilage repairs performed, we do not know if surgery improves the long term outcome. This study investigated six-year outcomes in patients with arthroscopically verified, focal, full thickness cartilage injuries in the knee.

Material and methods: The patients in this study are a subgroup of patients in the previous report of 993 knee arthroscopies (paper 1). Patients younger than 50 years and with a focal ICRS grade 3-4 injury at the time of the baseline study were included (n=98, 13.8% of patients < 50 years) in a 6 year follow-up. Of these, 2 patients were dead, 12 were lost to follow up/did not meet and 84 patients completed the follow up study. Sixty-four patients had no cartilage surgery of the defect performed at baseline, except debridement, while 34 patients had cartilage surgery performed at baseline (microfracture n=21, autologous chondrocyte implantation n=7, osteochondral cylinder transfer n=2, fixation of osteochondral fragment n=4). During the follow-up period additional nine patients had cartilage surgery performed (microfracture n=3, autologous chondrocyte implantation n=4, osteochondral cylinder transfer n=2). At follow up a clinical examination including one leg jumps were performed. The patients completed the following questionnaires: ICRS, Lysholm, Tegner, KOOS, Cincinnati,

IKDC 2000, SF-36. Weight-bearing radiographs were obtained from 68 patients and classified according to Kellgren and Lawrence.

Results: The average ICRS functional level, the VAS pain score, and knee self-assessment all improved from baseline ($p < 0.001$), while ICRS activity level decreased ($p< 0.001$) from baseline. A linear regression analysis showed that these changes were independent of age, sex, BMI, size and localization of the lesion, additional meniscal or ligament surgery or whether the patient had undergone a cartilage repair or not.
At follow up the average Lysholm score was 75 (SD 20), Cincinnati score was 73 (SD 22), IKDC score 66 (SD 23). The KOOS subscores were: pain: 78 (SD 22), symptoms 75 (SD 21), activities of daily living (ADL) 86 (SD 19), sport and recreation 61 (SD 33) and quality of life 61 (SD 26). Nineteen of the knees with cartilage defects showed Kellgren-Lawrence grade 2 or 3 changes compared to six of the contralateral knees ($p<0.001$).

Conclusion: Patients with arthroscopically diagnosed ICRS grade 3-4 cartilage injuries in the knee can expect a stable or improved knee function over the subsequent 6-7 years. Further comparative studies are needed to determine whether cartilage repair methods yield better results than non-surgical treatments.

Paper III

Autologous chondrocyte implantation to repair knee cartilage injury: ultrastructural evaluation at 2 years and long term follow up including muscle strength measurements

Background: Autologous chondrocyte implantation usually results in improvement as measured by clinical scores. However, long term isokinetic muscle strength measurements have not been reported. Biopsies from the repair tissue have shown variable proportions of hyaline-like cartilage.

Material and methods: Twenty-one consecutive patients were treated with autologous cartilage implantations in the knee in the years 1997-1998. Mean size of the lesions was 5.5 cm^2. Follow up arthroscopy with biopsy was performed at 2 years in 19 patients. The biopsies were examined by both light microscopy and transmission electron microscopy including

immunogold analysis for the detection of collagen type I. Patient function was evaluated with modified 10 point scales of the Cincinnati knee rating system obtained preoperatively and at 1 and 8.1 years. Isokinetic quadriceps and hamstrings muscle strength testing was performed at 1, 2 and 7.4 years.

Results: Light and transmission electron microscopy both showed predominately fibrous cartilage. The immunogold analysis showed a high percentage of collagen type I. At 7.4 years the total work deficits compared to the contra-lateral leg for isokinetic extension were 19.1% and 11.4%, and for isokinetic flexion 11.8% and 8.5% for 60º/sec and 240º/sec, respectively. Mean pain score improved from 4.3 preoperatively to 6.3 at one year ($p=0.031$) and 6.6 at 8.1 years ($p=0.013$). Overall health condition score improved from 4.1 preoperatively to 6.1 at one year ($p=0.004$) and 6.5 at 8.1 years ($p=0.008$). Three patients have later been reoperated with other resurfacing techniques and are considered failures.

Conclusions: The formation of fibrous cartilage following autologous chondrocyte implantation was confirmed by transmission electron microscopy with immunogold histochemistry. Although the functional scores were generally good, strength measurements demonstrated that the operated leg remained significantly weaker at all time points.

Paper IV

Bone marrow mesenchymal stem cells in a hyaluronan scaffold for treatment of an osteochondral defect in a rabbit model

Background: Donor site morbidity, hypertrophy of the graft and dedifferentiation of the chondrocytes during the culturing process are concerns related to ACI. The use of MSC in a scaffold will eliminate the need for harvesting cartilage from the joint, and the dedifferentiation of the cells. The use of a scaffold may reduce the risk of graft hypertrophy. The purpose of this study was to evaluate the efficiency of using MSCs in a hyaluronan scaffold for repair of an osteochondral defect in rabbit knee.

Material and methods: Bone marrow was harvested from the posterior iliac crest in 11 New Zealand White rabbits. MSCs were isolated and cultured in autologous serum for 28 days and

transferred to a hyaluronan scaffold 24 hours prior to implantation. A 4 mm diameter and 1.5 mm deep defect was created in the medial femoral condyle of both knees and the scaffold with MSCs was implanted in one knee, while an empty scaffold was implanted in the contra-lateral knee. After 24 weeks, the rabbits were killed and histological sections were subjected to semiquantitative and quantitative evaluation by observers blinded regarding treatment modality.

Results: A high degree of filling was obtained, but there was no statistically significant difference between the two treatment modalities. However, there was a tendency for a better quality of repair in the MSCs treated knees. No hypertrophy was observed by either method.

Conclusion: MSCs in a hyaluronan scaffold is a promising treatment approach, but further studies are needed to determine the best combination of scaffold and cells.

GENERAL DISCUSSION

Material

Clinical studies (paper I, II and III)

Patient selection: The patients in paper I were all patients undergoing arthroscopy of the knee. The aim was to find the frequency of patients eligible for cartilage repair techniques. Obviously, this study is not a study of prevalence of cartilage injuries in the general population, but rather the prevalence of lesions in a surgical population with knee pain or instability. The patients may not be fully representative for the general orthopaedic practice. The main contributor of patients to the study was a university hospital with a known interest in cartilage patients, while the other two hospitals had a more general patient panorama. Thus, the prevalence of cartilage injuries in this material may be somewhat higher than in a general orthopaedic hospital.

The patients in paper II were a subgroup of patients in paper I re-examined after 6 years. These patients represent a mixture of patients with isolated cartilage lesions and patients with injuries in addition to their cartilage lesion. Some underwent treatment for their cartilage

injury and some did not. Thus, this was a heterogeneous group of patients. Ideally the natural history of cartilage lesions should be studied in a group of patients with both symptomatic and non-symptomatic cartilage injuries, and without additional injuries who where followed without treatment. Such a study would be very difficult to perform. Many symptomatic patients would probably not accept non-surgical treatment for several years in a time where new methods of surgical treatment are continuously being developed. Ideally, in RCTs comparing cartilage treatments, a group of patients with non-surgical treatment should be included since the natural history of these injuries is not known.

The patients in paper III constitute a group of patients treated by ACI. Two thirds of these were patients having OCD sequelae, while the rest presented focal lesions of traumatic or unknown origin. This is not a randomized study and the number of patients is limited. In essence, they represent a pilot study prior to an RCT started in 1999 (Knutsen et al. 2007, Knutsen et al. 2004). Therefore, one must be careful in interpreting the clinical outcome after ACI from this study. However, it is of value to study the biopsy results from such a cohort and use this biopsy material to evaluate the use of morphometric evaluation of the biopsies and to investigate the usefulness of transmission electron microscopy (TEM). Such a small cohort is also well suited for a pilot study of isokinetic strength with the contralateral leg as a control, with the aim to test if this method should be used in larger series or in RCTs.

Experimental study (paper IV)

Rabbits have been widely used in experimental cartilage repair studies (Radice et al. 2000, Kayakabe et al. 2006, Årøen et al. 2006, Årøen et al. 2005, O'Driscoll et al. 1986, O'Driscoll et al. 1988, Yan and Yu 2007, Salter et al. 1980, Solchaga et al. 2005). Some scientists claim that a large animal model is to be preferred, but there is agreement on the use of rabbit as the first model when new methods are introduced, and some recommend the use of a larger animal in later stages before clinical trials (Reinholz et al. 2004). The rabbit model has also been used previously by our group in experimental cartilage surgery (Årøen et al. 2005, Årøen et al. 2006). When looking specifically at studies on MSCs in animal cartilage repair studies, the rabbit is often used. It is well known that adolescent rabbits are associated with better cartilage repair than adult rabbits. New Zealand White rabbits, which were used in the current study, reach skeletal maturity between 4 and 6 months (O'Driscoll 2001). Some

authors claim that this occurs with weight more than 3.2 kg (Messner et al. 1993). In our study, the rabbits were 24 weeks old at the time of surgery, weighing average 3.3 kg. Another issue is the inter-individual variance in healing response of the animals. In the current study this was handled by bilateral surgery, using one surgical method in one knee compared to the control method in the contra-lateral knee.

Methods

Registration of arthroscopic findings (paper I and III)

ICRS surgeons form (paper I)

This system was first published by ICRS (International Cartilage Repair Society 1998) with later revisions (Brittberg and Winalski 2003). The 1998 version was used in paper 1. The surgeon maps the cartilage lesions in the joint according to depth (figure 5), size and localization (figure 6). The accuracy of estimating size of cartilage defects at arthroscopy has been shown to be poor in a study on a plastic model, but accuracy improved when using specially designed measurement tools (Oakley et al. 2002, Oakley et al. 2003). In a study on arthroscopic videos of cadaveric knees, arthroscopic Outerbridge grading showed reasonable accuracy (Cameron et al. 2003). The inter observer variability in locating the cartilage lesion within the knee joint was shown to be satisfactory in a mapping system similar to the ICRS mapping (Hunt et al. 2001). In an on-going study from our group the size of the lesion measured at arthroscopy were similar to the size measured at later knee arthrotomy (Årøen et al, unpublished data). In the current study, a standard arthroscopic probe was used and we did not investigate the accuracy of the surgeons' measurements.

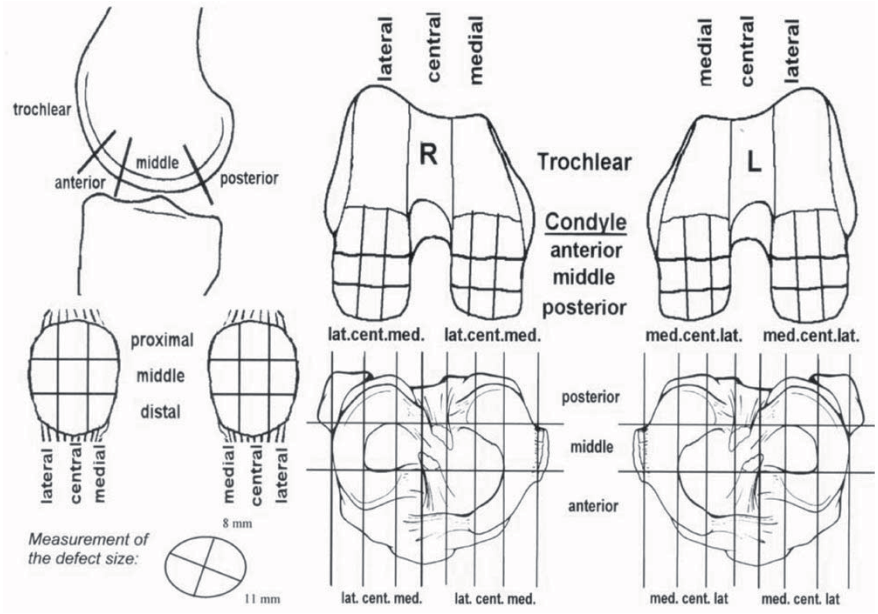

Figure 6. ICRS system (1998 version) for mapping cartilage injuries (reprinted with permission from International Cartilage Repair Society).

ICRS arthroscopic assessment of cartilage repair (paper III)

This evaluation system is based on the macroscopic appearance of the repair tissue at arthroscopy and includes an evaluation of defect fill, integration with surrounding cartilage, and surface mechanical characteristics (Brittberg and Winalski 2003). The system is shown in table 4. This assessment system has been found to be reproducible (Smith et al. 2005, van den Borne et al. 2007).

Table 4: ICRS assessment of cartilage repair (From: Brittberg and Winalski, 2003).

Filling	
In level with surrounding cartilage	4
75% repair of the defect	3
50% repair of the defect	2
25% repair of the defect	1
0% repair of the defect	0
Integration to border zone	
Complete integration with surrounding cartilage	4
Demarcating border < 1 mm	3
3/4 of graft integrated, 1/4 with a notable border > 1 mm width	2
1/2 of graft integrated with surrounding cartilage, 1/2 with a notable border > 1 mm	1
From no contact to 1/4 of graft integrated with surrounding cartilage	0
Macroscopic appearance	
Intact smooth surface	4
Fibrillated surface	3
Small, scattered fissured or cracks	2
Several, small or few but large fissures	1
Total degeneration of grafted area	0

Functional outcome scores (paper I, II and III)

Several functional outcome scores have been used. A functional score is an instrument developed to measure the functional status for an individual. It may be used to compare groups or individuals, and to detect changes over time in a group or in an individual. Different aspects of a score are evaluated to estimate the usefulness of a specific score for a specific condition (Fitzpatrick et al. 1998).

- Appropriateness indicates how well the measurement tool is designed to answer the research questions in the study. This property of an outcome measure cannot be calculated or measured, but has to be based on a sound judgment by the investigators. As an example, it is not appropriate to use a shoulder score to evaluate knee function.

-Reliability is a measure of the internal consistency and reproducibility of an instrument. Internal consistency can be measured by randomly dividing the items in a scale into two groups and to assess the degree of agreement between the two halves. The two halves should correlate well. For example, in several questions concerning pain, the answers from one

patient should be in the same area of the scale for all questions. On the other hand, if the answers are completely similar it may indicate that the questions are too similar. The correlation of the agreement should be between 0.7 and 0.9. Reproducibility can be measured by test – retest in a stable patient group.

-Validity expresses to what extent an instrument measures what it is meant to measure. This can be evaluated qualitatively by examination of the content of the instrument or quantitatively by factor analysis. It can also be validated by comparison to other related instruments. If a "gold standard" exists, (which it seldom does) the validity of a new instrument can be compared to this.

-Responsiveness expresses how well the instrument detects changes over time that matter to the patients. There is no universal agreement of how to evaluate the responsiveness of an instrument and several approaches are used. Generally, a responsive instrument requires statistically significant change of observations at separate time points when there is reason to believe that clinically important changes have occurred. For example, an instrument evaluating outcome after a particular surgical procedure should reflect the improvement from early postoperative phase to one or two years follow up.

-Precision describes how well the instrument reflects true differences. Precision will include responsiveness, but also other changes or differences.

ICRS functional score (paper I and II)

The ICRS form was first published by the ICRS in 1998 (International Cartilage Repair Society 1998) and represented a new standard of how to evaluate and map cartilage lesions of the knee and how to evaluate the result after cartilage repair at arthroscopy. The patients give general information about sex, age, weight, height, mechanism of injury, previous surgery etc. It also contains a grading of activity level and a functional score. When planning our arthroscopy registration we regarded this as a modern and up to date tool for our study. Although the questionnaire may be a useful checklist in a surgeon's practice, it has not been used widely in research, and moreover, it has not been validated for any patient group. When performing our follow up study (paper II) we obviously had to repeat the use of the ICRS

form, but in addition, we included other more common scoring systems that have been validated for other knee conditions and later also for cartilage patients.

Lysholm and Tegner score (paper II)

The Lysholm score was first published in 1982 (Lysholm and Gillquist 1982) for ACL patients and has later been widely used. In 1985 a modification of the Lysholm score together with the Tegner activity score was published (Tegner and Lysholm 1985). The activity score was intended to be used as a supplement to the Lysholm score. These scores have been used in several publications on different knee conditions, also in cartilage patients. The Lysholm score is a set of subscores (each with 5-25 points of maximum score) and the maximum total score is 100. The score has been validated for cartilage conditions (Kocher et al. 2004). In a recent study of patients regarding their knee function as normal the average Lysholm score was 94 (range 43-100) (Briggs et al. 2009).

The Tegner score is an activity scale where the patients state the most demanding activity he or she does. 0 is a patient on sick leave and 10 is a top league soccer player. The score gives complementary information to the Lysholm score. It was developed as an improvement to the less precise "return to sport". Tegner score is not intended to be used as a single score and has therefore not been validated as such. It has not been compared to other activity scores.

IKDC (paper II)

The first version was developed by International Knee Documentation Committee (Hefti et al. 1993), and later a revised version has been published (Irrgang et al. 2001). The score has been validated for ACL patients (Irrgang et al. 1998, Risberg et al. 1999a) and for knee patients in general (Higgins et al. 2007). In a study where outcome measures were ranked according to the patients evaluation of the importance of the questions in the score IKDC came out best (see below) (Hambly and Griva 2008).

KOOS (paper II)

Knee injury and Osteoarthritis Outcome Score was first validated for ACL patients (Roos et al. 1998) and has later been validated for OA knee patients (Roos and Toksvig-Larsen 2003).

Recently the score has also been validated for cartilage patients (Bekkers et al. 2009). The score has five subscales, which each has a maximum score of 100. In other scoring system, there is always a question of how the relative proportion of each subscore should be in relation to the total score. This is usually decided by the designer of the score. The developers of the KOOS score have omitted this problem by presenting all subscores without summing up a total score. Reference values for men and women in different age groups have been published (Paradowski et al. 2006).

Cincinnati Knee Rating System (Paper II and III)

The Cincinnati Knee Rating System was published in 1983 by Noyes et al (Noyes et al. 1983b, Noyes et al. 1983a) and was a score for evaluating ACL-patients. Just like the Lysholm score, it consists of several subscores with a maximum score of 100. Cincinnati Knee Rating System is validated for ACL-patients (Barber-Westin et al. 1999), and has been shown to detect changes over time better than Lysholm score and IKDC (Risberg et al. 1999a).

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

This score was a part of an evaluation package provided by the cell laboratory (Genzyme, Boston, Massachusetts, USA). This is a non validated scoring system that has not been widely used, but it has been used in similar studies (Browne et al. 2005, Micheli et al. 2001). However, the score reports on the same important variables (pain, locking, knee collapse and swelling) as the Cincinnati knee rating system and other well established scoring systems.

SF-36 (Paper II and III)

Short Form-36 (SF-36) is a general health score (Ware, Jr. and Sherbourne 1992) that is widely used in clinical publications and also in orthopaedic research in a variety of conditions. SF-36 contains subscores reflecting physical state, and other subscores reflecting mental state. In many studies of knee conditions it is combined with a knee specific score. It has been compared to Lysholm score in cartilage patients and recommended to be used in combination with a knee specific score (Bartlett et al. 2005a). SF-36 is often used as a “gold standard” to test the external construct validity of other scores. Reference scores for men and women of

different age groups in different populations have been published, including the Norwegian population (Loge and Kaasa 1998).

General comments on knee specific scores

Several scoring systems have been developed for knee conditions. Some have been developed for one specific knee disorder, while others have been developed for knee disorders in general. As mentioned above, several of these scoring systems have been evaluated with regard to validity, reliability and responsiveness. The selection and weighing of the different parameters and questions in the forms are often based on what is important for the investigator or what the investigator believes is important to the patient. In a recent study, patients with different knee disorders rated the questions in the questionnaires of several knee functional scores in regard to how important the questions were to them (Tanner et al. 2007). Of the outcome measures for general knee conditions IKDC and KOOS were rated to be best in reflecting issues that were important to the patients. Based on this, a similar evaluation of IKDC and KOOS was performed in a group of patients who had undergone cartilage surgery, and the authors concluded that IKDC was the best instrument of the two (Hambly and Griva 2008). However, in a letter to the editor (Roos et al. 2009) an incorrect use of the KOOS score in that study was pointed out. In addition, in the Hambly study, responsiveness was not measured, a parameter previously shown to be insufficient for IKDC (Risberg et al. 1999a).

Evaluation of cartilage repair tissue (paper III and IV)

Light microscopy - (paper III and IV)

The following discussion is limited to how specimens in rabbit cartilage studies and biopsies from ACI in humans are evaluated.

Rabbit study (Paper IV)

In an experimental rabbit study, the animal is killed and the entire joint is available for macroscopic 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 the integration to the

surrounding tissue. The quality of the tissue and the integration to the underlying bone can be evaluated for large parts of the defect. However, a three-dimensional defect will for practical reasons also in an animal study only be represented by a limited number of two dimensional sections. Thus, even when the entire defect is available for analysis the measure of quantity and quality of the defect is based on samples of sections from different parts of the defect.

In the present study, we evaluated the tissue in two ways:

1. A qualitative evaluation of several parameters (table 5)

Table 5. Definition of the qualitative score (modified from O'Driscoll)

Hyaline cartilage	
> 60%	2
40-60%	1
< 40%	0
Surface regularity	
Smooth and intact	2
Fissures 25–100% of the thickness	1
Severe disruption, including fibrillation	0
Necrosis:	
Normal cellularity	2
Moderate cell loss	1
Severe cell loss	0
Integration at borders	
Bonded at both sides	2
Bonded at one side, or partially at both sides	1
Not bonded	0
Chondrocyte clustering	
25–100% of the cells	2
25% of the cells	1
No clusters	0

An evaluation system for the repair of cartilage defects in rabbits was developed by O'Driscoll (O'Driscoll et al. 1986). Several authors have been using this score or a modification of it in publications. However, the relevance of such a sum score may be questioned as the importance of each parameter in relation to the others is unknown. In the present study, we therefore chose a more direct approach reporting the score of the same

parameters as in the O'Driscoll score without combining them to a sum score. This approach is also in line with the Histology Endpoint Committee of the International Cartilage Repair Society (ICRS) in their proposed scoring system for biopsies from cartilage repair tissue in humans (Mainil-Varlet et al. 2003).

2. A quantitative evaluation based on point counting

The reproducibility of semiquantitative histological scoring systems has been poor and, thus, the validity is questionable (Hyllested et al. 2002, Mainil-Varlet 2007). We therefore chose to base our conclusions mainly on the results of a quantitative measurement of filling based on point counting (Gundersen et al. 1988). With this method, a grid was superimposed on the micrograph using a computer program (Analysis Pro®, Olympus Soft Imaging Solutions®, Münster, Germany). The grid resulted in approximately 160 test points overlying the micrograph (figure 7). Test points overlaying cartilage, bone or no tissue, respectively, were recorded, and the proportions of cartilage and bone in relation to the total area were calculated. This method turned out to be reproducible with very good agreement between the two observers and between the two time-points.

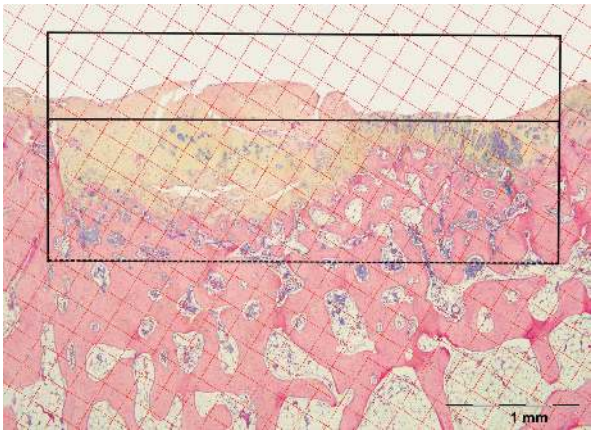

Figure 7. A rectangle of 1 mm height below, and 0.6 mm height above a line between the tidemarks on each side of the defect defined the regions of interest. A grid with 200µm between test lines was superimposed to the micrograph and the amount of tissue inside each frame was quantified by point counting.

Human study (paper III)

Several attempts have been made to obtain a reproducible classification system for biopsies of repair tissue after ACI (Mainil-Varlet et al. 2003).

Table 6. ICRS Visual Histological Assessment Scale (from Mainil-Varlet et al., 2003)

Feature	Score
I. Surface	
Smooth/continuous	3
Discontinuous/irregularities	0
II. Matrix	
Hyaline	3
Mixture: hyaline/fibrocartilage	2
Fibrocartilage	1
Fibrous tissue	0
III. Cell distribution	
Columnar	3
Mixed/columnar-clusters	2
Clusters	1
Individual cells/disorganized	0
IV. Cell population viability	
Predominately Viable	3
Partially viable	1
<10% viable	0
V. Subchondral Bone	
Normal	3
Increased remodeling	2
Bone necrosis/granulation tissue	1
Detached/fracture/callus at base	0
VI. Cartilage mineralization (calcified cartilage)	
Normal	3
Abnormal/inappropriate location	0

This assessment scale (table 6) is a modification of classification systems developed for animal studies where the whole joint is available for analysis. In biopsies from studies in humans, the biopsy is usually only 2-3 mm in diameter. The inter- and intraobserver variabilities of this system have not proven to be satisfactory (Mainil-Varlet 2007). Thus, like in the rabbit study we used the quantitative morphometric measurement method (Gundersen et al. 1988) in a similar way to calculate the percentage of fibrous cartilage in the human ACI biopsies. With this method the investigator defines the character of the tissue for every test point in the grid overlying the specimen (figure 8), in contrast to other studies where the

proportion of fibrous cartilage has been estimated by an overall judgment. Our approach was based on experience from the rabbit study where this method showed very good inter- and intraobserver reproducibility.

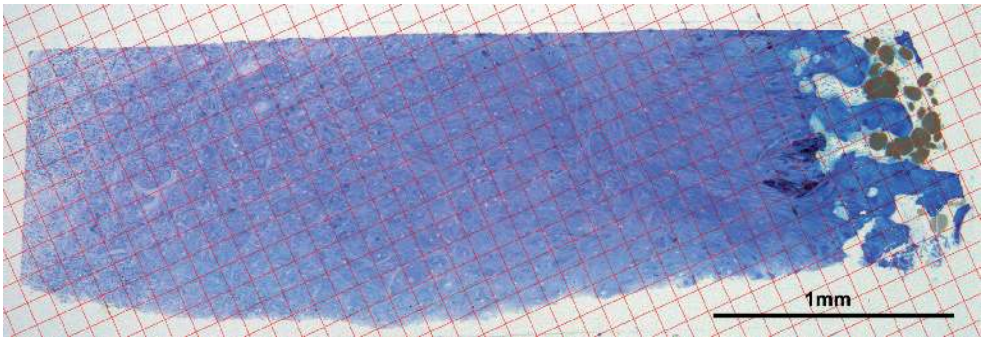

Figure 8. A square grid with 100µm between test lines superimposed to a micrograph to quantify hyaline-like and fibrous cartilage, respectively.

Transmission electron microscopy (paper III)

Transmission electron microscopy (TEM) is a microscopy technique whereby a beam of electrons is transmitted through an ultra thin specimen, interacting with the specimen as they pass through. An image is formed from the interaction of the electrons transmitted through the specimen, which is magnified and focused onto an imaging device, such as a fluorescent film, a layer of photographic film, or a digital camera with an interphase. (http://en.wikipedia.org/wiki/Transmission_electron_microscopy).

With its 1000 times higher resolution TEM gives a more detailed picture of the tissue and additional information compared to light microscopy, with characterization of sections of the biopsies to delineate extracellular matrix organization with particular attention paid on the fibrous elements. The orientation of the collagen fibers important for the functional status of the tissue can be evaluated directly. Moreover, an indirect measure of cell turn over is obtained by investigating the amount of apoptotic bodies, so called matrix vesicles, in the

extracellular matrix. Using TEM, areas with different appearance at light microscopy (hyaline-like versus fibrous cartilage) can be compared at high resolution.

Immunogold technique (paper III)

This is a technique were molecular distributions in the tissue can be studied at high resolution. Molecules (epitopes) are detected by specific antibodies and bound antibodies are detected by protein A conjugated with colloidal gold particles seen as distinct dots in the microscope. In the current thesis the specimens prepared for TEM were subjected to immunogold labeling according to an established protocol (Ramstad et al. 2003) using monoclonal antibodies to human collagen type I (MP Biomedicals, LLC, Solon, Ohio, USA). With this technique, each single collagen fiber will be marked as collagen type I or not. A high proportion of collagen type I fibers demonstrates that the repair tissue is fibrous cartilage.

Evaluation of muscle force (paper III)

Isokinetic quadriceps and hamstrings muscle strength were measured using a Cybex 6000 (Cybex, Division of Lumex, Inc., Ronkonkoma, New York). Before testing, the patients warmed up on a stationary bike for 8 minutes. The test protocol consisted of 5 repetitions at an angular velocity of 60 deg/s (strength), followed by a 1-minute rest period, and then 30 repetitions at 240 deg/s (endurance). The parameter used for analysis was total work expressed as percentage of the contra-lateral leg.

Isokinetic muscle strength measurements have been used previously in evaluation of patients who have undergone ACL reconstruction (Risberg et al. 1999b), and has been established as a reliable method for assessment of muscle performance after ACL reconstruction (Brosky, Jr. et al. 1999). The method has also been reported in the evaluation following knee arthroplasty (Berman et al. 1991). It is reason to believe that quadriceps function is important also for knee function before and after ACI and that measurement of isokinetic muscle strength, both during conservative treatment as well as before and after surgical treatment of cartilage injuries supply valuable information.

Radiological grading (paper I and II)

In paper I grouping of radiographs was carried out by the presence or non-presence of degenerative changes as evaluated by the orthopaedic surgeon. This was based on plain radiographs (weight-bearing or non weight-bearing) and MRI.
In paper II 68 of 84 patients were examined by weight-bearing radiographs and these were evaluated according to Kellgren and Lawrence (Kellgren and Lawrence 1957). The advantage of this system is that it is widely used making comparison to other studies possible. The disadvantage is that it is not completely accurate in the definition of the grades (table 7). An alternative and judged by many to be more precise, is the Albäck classification based solely on mm joint space narrowing (Ahlback 1968).

Table 7. 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

Statistical methods

All statistical analysis in this thesis were performed with the Statistical Package for Social Sciences (SPSS statistical package, Chicago, Illinois, USA version 14 (paper IV) and version 15 (paper II and III),).

Paper I

Paper I reports only descriptive data with no statistics.

Paper II

In paper II the main outcome variable was change in 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. An *a priori* sample size calculation showed that 47 patients would be sufficient to detect a 0.5 unit change in functional level from baseline with a standard deviation of 1.2 units at 80 % power and 5 % significance level, so we had a sufficient number of patients to calculate on changes over time for the whole group, but not sufficient number of patients to compare subgroups. Wilcoxon test was used 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 VAS pain variable as this was regarded to be close to a continuous variable. A linear regression analysis was performed for all outcome variables as the dependent variable with the following independent variables: age at injury, age at operation, sex, body mass index (BMI) at baseline, size of the cartilage injury, localization of the injury, additional ligament/meniscal surgery and cartilage surgery. In such an analysis, the association between each outcome score (dependent variable) and the different independent variables can be calculated. Side to side differences of the Kellgren-Lawrence radiological grading were compared using a Wilcoxon test, as this was also a paired categorical variable.

Paper III

In paper III a Friedman test with a significance level of 0.05 was used to analyze differences between all three time points in the modified 10-point subscales and isokinetic strength. The 10-point scale is an ordinal categorical variable and the number of patients was low. Thus, Friedman non-parametric test for repeated measurements was used. If significant differences were detected, Wilcoxon non-parametric rank test for paired samples was used for a similar comparison between two separate time points. Due to repeated tests, a Bonferroni correction was applied so that p-values < 0.017 ($< 0.05/3$) were regarded as significant in the paired comparisons. In the comparison of isokinetic strength between affected and unaffected side there is a paired situation. Whether such strength measurements can be expected to have a normal distribution is unknown. Therefore, a Wilcoxon non-parametric rank test for paired samples was employed at each separate time point with a significant level of 0.05.

Paper IV

In this study, rabbits were operated with one method in one knee and the control method in the contra-lateral knee. The main parameter was filling of the defect expressed as a proportion of filling. 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. Due to the risk of losing animals during the experiment, a decision to use 12 rabbits was made (one rabbit died, so 11 rabbits completed the study). Based on previous experimental studies (Årøen et al. 2006) a filling difference of more than 25% was considered as a proper level to disregard the null hypothesis of no difference in filling between the two treatments. Each animal served as its own control, and thus a paired student's t-test was used to compare degree of filling of cartilage and bone. A better alternative would probably have been a paired Wilcoxon rank test, as with 11 animals a normal distribution of data could not be expected. However, recalculations with this test did not alter the results.

The mean differences and standard deviation of the differences were used to assess the agreement between the two observers and between measurements at the two time points for each observer (Altman DG 1991).

Wilcoxon rank test for non-parametric paired samples was used for comparison of the semiquantitative evaluation of the repair, as this was an ordinal categorical variable.

Results

Paper I

This study showed that in patients undergoing knee arthroscopy for any indication 66% showed some kind of cartilage pathology. Full thickness lesions (ICRS grade 3-4) were found in 11% of the knees and 6% showed grade 3-4 lesions with an area of 2 cm^2 or more. These findings are in accordance with the results published by Curl and co-workers who in a retrospective study showed a full-thickness lesion in 5% of 31,516 arthroscopies in patients younger than 40 years age (Curl et al. 1997). Other studies from Norway (Hjelle et al. 2002),

and from Poland (Widuchowski et al. 2007) show approximately the same figures. The distribution of cartilage injuries within the different parts of the knee joint in our study is also in accordance with the studies referred above. Cartilage defects are commonly associated with other pathological conditions of the joint such as ACL ruptures and meniscal injuries (Lewandrowski et al. 1997, Murrell et al. 2001, Granan et al. 2008). Our data support previous studies of focal cartilage lesions in patients with anterior cruciate ligament injury, although meniscal injuries were less frequently associated with focal cartilage lesions than in previous studies (Zamber et al. 1989).

As shown in this study, the referred patients with cartilage defects do not constitute a uniform group, while patients in randomized controlled studies have to fulfill strict inclusion criteria. This should be taken into consideration in studies on cartilage repair. The application of a method proven to be effective in a selected patient population may not turn out to be successful used among cartilage patients in general (Engen C et al. 2009). The risk of inappropriate application or overuse of a new technique was pointed out by Mont and co-workers in 1999 (Mont et al. 1999).

Paper II

The study shows that patients with one or more deep cartilage defects in their knee found at arthroscopy 6.2 years previously improve their knee ICRS functional score over time. In a linear regression analysis, we could not detect any association between the type of surgery (including cartilage surgery) and 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 these patients was microfracture; thus, the results may not be generally applicable to all cartilage repair patients. This 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, the majority of cartilage lesions were in patellar locations in groups 1 and 2. Consequently, the groups should not be compared; each should be evaluated separately. The Lysholm score (average 75) and the Cincinnati score (average 73) were low at follow up, reflecting that a cartilage defect has a serious impact on knee function irrespective of treatment.

The improvement in knee scores may reflect a real improvement, due to various therapeutic procedures performed and/or due to a favorable natural history. For example, in group 1, 10 out of 17 patients underwent a procedure; most frequently, debridement of the cartilage lesion. That procedure could have improved knee function by stabilizing the cartilage defect and removing the mechanical symptoms of the knee. However, there are several other 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 orthopaedic surgery (Moseley et al. 2002, Cobb et al. 1959, Kirkley et al. 2008). Finally, another explanation for the improvement in functional score and less pain stated on the VAS-scale may be the reduction in activity level; with less physical activity, the patients may experience less pain.

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 the current study 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 C et al. 2009). Nevertheless, conclusions from RCTs are often generalized to all patients with cartilage injuries.

The current cohort consists of relative young patients (average 32 years at baseline) and with a cartilage injury classified as focal at arthroscopy. Patients with a knee classified as having general osteoarthritis judged by arthroscopy were excluded from the study. Nevertheless, the radiological examination shows significantly more osteoarthritic changes in the affected knees 6.2 years after base line surgery, even in the groups with isolated cartilage injuries (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 (Knutsen et al. 2007) who reported osteoarthritis in more than 30% of the patients five 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 found that a high BMI was associated with a higher Kellgren-Lawrence grade as shown in several other studies (Felson et al. 1997, Spector et al. 1994, Hochberg et al. 1995).

One limitation of this study was that, despite a high follow up rate (84 of 96 living patients; 87%) the number of patients in this study was relatively low; moreover, half of them had additional injuries that caused difficulties in the interpretation of the results. In addition, several patients had been subjected to surgery to the same knee before (42 patients) and/or after (32 patients) baseline arthroscopy. The categorization of the patients into subgroups was intended to give a better description of the material, but the subgroups are too small to be compared statistically, and such a comparison would anyway be of limited value because of several confounding factors. Thus, a regression analysis to correct for confounding factors was performed. Nevertheless, the results should remind surgeons involved in cartilage repair to question if conservative treatment modalities such as active rehabilitation could be equally effective as surgery.

Up to present, no RCTs have included a control group of patients receiving non-surgical or no treatment. In an RCT between mosaic plasty and ACI, where debridement of the lesion was performed at the time of enrolment, 31 % of the patients improved after the initial debridement, and further cartilage surgery was not needed (Dozin et al. 2005). As mentioned above, in a report of 28 patients with a cartilage defect in the knee, 22 patients functioned well 14 years after diagnosis (Messner and Maletius 1996). Together with the results from the current study, this indicates that non-surgical treatment or less invasive surgery may be sufficient to relieve symptoms for many patients, and that a control group randomized to non-surgical treatment should be included in future RCTs.

Paper III

This study confirms that first generation ACI results in the formation of fibrocartilage as evaluated by electron microscopy, and immunohistochemistry. Secondly, the study confirms that despite an improved functional score from baseline, the knee function is seriously affected, as muscle strength is still markedly impaired after 7.4 years.

The clinical results are similar to those presented by others using the same modified 10-point scales of the Cincinnati knee rating system (Browne et al. 2005, Micheli et al. 2001). The Cincinnati score (Bartlett et al. 2005b, Bentley et al. 2003, Krishnan et al. 2006a) and the SF 36 score (Bartlett et al. 2005a) after 7.4 years are in the same range as obtained in other studies.

In the muscle strength measurements, our group of patients demonstrates lower extension and flexion total work (expressed as percentage of contra-lateral leg) compared to ACL reconstructed patients and similar extension total work and lower flexion total work compared to knee arthroplasty patients in these studies (Berman et al. 1991, Risberg et al. 1999b). This indicates that ACI patients suffer marked functional impairment. In a group of healthy female handball players a significant side to side difference (5.5%) was found for flexion total work at 240 deg/s, with no difference in the other measurements (Holm et al. 2004). The side-to-side differences in the current study are more pronounced and can therefore not be explained by normal side-to-side variation. Shelbourne et al (Shelbourne et al. 2003) claim that restoration of muscle strength with emphasis on quadriceps strength may relieve symptoms in patients with a so-called de-conditioned knee, and that failure in regaining strength may contribute to the de-conditioning. The importance of quadriceps strength in knee function has also been shown in another recent study: Reduced preoperative quadriceps strength in ACL reconstructed patients was associated with poorer functional outcome and persistent quadriceps weakness after two years (Eitzen et al. 2009). This may also be the case in the current study, as most patients had suffered from chronic knee pain for years. We do not have detailed data of the training load and intensity over the years after ACI. However, the majority of the patients were followed closely by an experienced physiotherapist during the entire follow up period. At follow up most patients were training at a regular basis indicating that the patients worked to maintain their muscular strength, but obviously this effort was not sufficient to normalize the strength deficit. Unfortunately, we do not have preoperative tests, so we cannot say to what extent this impairment is a result of the cartilage injury itself, of the surgery, or both. However, the majority of the patients experienced long standing symptoms before surgery. Under such circumstances, it might be more difficult to regain normal muscle strength.

The proportions of hyaline like cartilage and fibrocartilage vary between studies. Different definition of hyaline like and fibrous cartilage and different ways of grouping the results make

comparison difficult. Some authors find that the majority of the patients have a hyaline or hyaline like repair tissue (some include mixed hyaline/fibrous repair tissue in this group), (Brittberg et al. 1994, Henderson et al. 2005, Moriya et al. 2007, Peterson et al. 2002) some report that about 50% of the patients developed hyaline or hyaline like repair tissue, (Knutsen et al. 2004, Krishnan et al. 2006b) while others as in the current study, find that the majority show fibrocartilage or fibrous repair tissue (Sharma et al. 2007, Tins et al. 2005). It has been common to use qualitative or semiquantitative histological grading systems to evaluate cartilage biopsies. However, the validity and reproducibility of such scores have been poor (Hyllested et al. 2002). We therefore chose a more direct approach in the current study including a quantitative morphometric measurement to calculate the percentage of fibrous cartilage. This quantitative method could possibly partly explain the high percentage of fibrous cartilage in our study. Different ways to evaluate and classify biopsies may also explain variation in results between studies. There are, of course, also probably real differences between series.

Several biopsy studies after first generation ACI have demonstrated a more hyaline like repair tissue in the deeper layers and a more fibrous tissue with remnants of the periosteum flap in the superficial layers (Brittberg et al. 1994, Henderson et al. 2007, Peterson et al. 2002). In the current study, this was not a consistent finding. Hyaline like tissue could be observed as islands both in the deep and the middle layer and most of the tissue integrating with bone had a fibrous appearance. This may partly be explained by the generally low proportion of hyaline like cartilage in our specimens. The patients in the current study were operated at an early stage of this surgical technique, and the procedure included a cross Atlantic two way transport of cartilage and cells. Today bovine serum for cell culture has been replaced by autologous serum. Shorter transport from laboratory to the hospital is possible, cell culture techniques have been further optimized (Saris et al. 2008) and the implantation of biomaterials seeded with cells have been introduced (Bartlett et al. 2005b, Marcacci et al. 2005, Iwasa et al. 2009, Kon et al. 2008). Clinical biopsy studies on second generation ACI with the use of cells seeded in a scaffold are limited, and clinical studies comparing first generation ACI with the use of periosteal flap to later generations ACI have so far not been conducted. ACI with a collagen cover (ACI-C) was compared to ACI with cells seeded in a collagen matrix (MACI) in an RCT, and showed fibrous tissue in the majority of the patients with no difference between the groups (Bartlett et al. 2005b). In 63 patients treated with arthroscopic implantation of chondrocytes in a hyaluronan scaffold (Hyalograft-C®) biopsies showed 56%

fibrocartilage in biopsies taken before 18 months post implantation, 27% fibrocartilage in biopsies taken later than 18 months, and no fibrocartilage in biopsies from asymptomatic patients with biopsies taken later than 18 months (Brun et al. 2008).

TEM gives a more detailed picture of the tissue and additional information compared to light microscopy. Scanning electron microscopy (SEM), used to evaluate the surface structure, have been used to study biopsies following ACI in clinical studies (Horas et al. 2003, Zheng et al. 2007) and in experimental studies (Munirah et al. 2007). Our focus was to use TEM to characterize sections of the biopsies to delineate extracellular matrix organization with particular attention paid on the fibrous elements including immunogold technique for demonstration of collagen I fibers. No other reports on TEM in similar patients are available for comparison.
Other weaknesses of the study are the limited number of patients and the lack of a control group. However, in contrast to most other studies that have included biopsies, the present study has nearly a complete set of biopsies from a consecutive group of 21 patients. The results from the muscle strength measurements are also useful without a control group due to the paired situation with the contralateral leg as a control.

Paper IV

In this study, there was no difference in the degree of filling when using scaffolds with MSCs compared to scaffolds without cells as judged by morphometry. In the semiquantitative analysis, there was an increased cluster formation in the MSCs treated defects, but no statistical difference in the other parameters.

Cluster formation is a sign of repair in early osteoarthritis (Frenkel and Di Cesare 1999), and in cartilage repair, cluster formation may be interpreted as a positive phenomenon as cell proliferation is central to new tissue formation. Consequently, cluster formation should be considered a positive sign and indicating some additive effect of the MSCs in our study. In addition, there was a trend towards higher scores for all parameters in the cell treated defects, and if combined to a total score (as has been common in similar studies) there is a significant higher sum for the cell treated defects.

The reproducibility of semiquantitative histological scoring systems has been poor and, thus, the validity is questionable (Hyllested et al. 2002). We therefore chose to let our conclusions rest mainly on the results of a quantitative measurement of filling (Gundersen et al. 1988) that turned out to be reproducible with very good agreement between the observers and between the two time-points.

Two previous studies have used hyaluronan scaffold with and without MSCs in rabbits (Radice et al. 2000, Kayakabe et al. 2006). Radice et al (Radice et al. 2000) did not find any difference between the hyaluronan scaffold with or without MSCs after observation periods of 8 and 16 weeks. Kayakabe (Kayakabe et al. 2006) observed a better filling compared to empty defects (with no scaffold) only when fibroblast growth factor-2 (FGF-2) was added to the MSC-loaded hyaluronan scaffold. The current study supports these observations.

Our results are also in accordance with the study by Solchaga et al. (Solchaga et al. 2005) who compared different scaffolds without cells in a similar rabbit model: 3 mm diameter and 1.5 mm deep defects on the medial femoral condyle were created and the scaffolds studied were HYAFF-11, auto cross-linked polysaccharide polymer (ACP) and two different polyester-based scaffolds. The filling of cartilage and bone above and below tidemark was measured in the Solchaga study, and the degree of filling at 20 weeks seems to be in the same order of magnitude as in the current study, although a slightly different calculation method was used.

In an osteochondral lesion, cells from the bone marrow adjacent to the lesion may contribute to the repair and partly outweigh the effect of the added cells. Gao et al (Gao et al. 2002) observed better healing of a 3 mm osteochondral defect in the rabbit with hyaluronan scaffold loaded with mesenchymal progenitor cells compared to empty scaffolds in an experiment where the bony part of the defects first was filled up with calcium phosphate. Hyaluronan scaffold loaded with autologous chondrocytes showed better healing than empty scaffold and empty defects in the rabbit when 6 x 5 mm pure chondral defects were created (Grigolo et al. 2001). Hence, the effect of adding MSCs or chondrocytes may be more important when the access to cells from the bone marrow is limited.

A considerable component of spontaneous healing of the defects could explain the small differences in the current study. A high degree of spontaneous healing is known to occur with

defect diameter up to 3 mm in the rabbit model with less spontaneous healing in larger defects (Shapiro et al. 1993).

Other biomaterials have been studied with or without MSCs in studies of rabbit knees. Some authors report better filling or higher score with cells in a scaffold than without, both for MSCs (Guo et al. 2004, Uematsu et al. 2005) and for chondrocytes (Frenkel et al. 1997, Willers et al. 2005).

Growth factors may promote chondrocyte differentiation of MSCs (Im et al. 2006). We did not supplement the scaffolds and MSCs with growth factors in our study, and this may partly explain the limited effect of the added MSCs. However, in contrast to similar studies the cells were cultured in autologous serum known to contain several growth factors (Tallheden et al. 2005). Previous experiments from our group have also shown that autologous serum induces a more rapid proliferation and a more stable gene expression of MSCs compared to fetal bovine serum (Shahdadfar et al. 2005).

In the present study, MSCs were seeded to the scaffold 48 hours prior to implantation. In the commercial use of Hyaff-11 with chondrocytes, the cells are cultured 14 days in the scaffold before implantation (Marcacci et al. 2005). In experimental studies with MSCs in scaffolds the time from seeding to implantation, when stated, is less than 48 hours (Guo et al. 2004, Uematsu et al. 2005, Liu et al. 2006, Kayakabe et al. 2006). A reason to choose a relatively short interval from seeding to implant is that differentiation of MSCs into chondrocytes is probably facilitated by local factors in the joint and in the cartilage.

MSCs in a hyaluronan scaffold may be a promising treatment approach, but further studies are needed to establish the most suitable scaffold for MSCs, to optimize the handling of the cells before implantation and to increase hyaline cartilage synthesis following implantation of the cells. If the MSCs under such optimized conditions turn out to be superior to chondrocyte implantation in experimental cartilage repair, the procedure should be introduced to clinical practice following well controlled randomized clinical trials.

GENERAL CONCLUSIONS

1. Our study and similar studies have shown that among patients undergoing arthroscopy (all numbers are approximate):

- The relationship between traumatic and gradual onset is 60/40
- 60-70 % have cartilage pathology
- 20% have localized lesions
- 10-12% have ICRS grade 3-4 injuries
- 5-7% have grade 3-4 of 2 cm^2 or more

Of these deepest and largest lesions:

- 50% are the only pathology of that knee
- 50% are localized to the medial femoral condyle
- 5% are osteochondritis dissecans

Patellar dislocation and ACL injuries were most commonly associated with cartilage lesions.

2. Patients with a cartilage defect in their knee detected at arthroscopy can expect to improve their functional score over a 6-7 year time period. This is also possible if the lesion is not treated. However, they cannot expect to regain a normal knee function. Also in a young patient population, radiological changes can be expected at this time point.

3. Standard histology of biopsies after ACI shows variable results. In our study, all biopsies showed predominately fibrous cartilage. Electron microscopy including immunogold histochemistry confirmed these findings, and showed fibrous cartilage also in the "best" appearing areas at light microscopy.

4. Patients with a chronic cartilage lesion, also if treated with autologous chondrocyte implantation, will probably have permanently impaired muscular strength of 10-20% compared to the contra-lateral knee.

5. Autologous mesenchymal stem cells may be an alternative to autologous chondrocytes in cell based repair of cartilage injuries. Several animal studies including our study indicate that it is safe. MSCs can easily be obtained without harming the joint. However, at present many

questions are still unanswered. What is the optimal tissue source for the cells? What is the best scaffold or biomaterial to carry the cells? Which growth factors should be added?

To sum up, cartilage injuries are common and are the cause of major disability in a large number of patients. Many patients are able to cope with their cartilage injury, and may also expect to improve their function over time, but cannot expect to maintain their activity level. However, we will still have a substantial number of patients in need for a better surgical treatment. To date the advanced cell based techniques have not proven to be better than other, more simple surgical or conservative methods.

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