Cartilage Injuries and the Repair Process

Asbjørn Årøen

ACKNOWLEDGEMENTS 6

PAPERS INCLUDED IN THIS THESIS 7

ABBREVIATIONS 7

INTRODUCTION 8

EMBRYOLOGY 8

CARTILAGE ANATOMY 8

Chondrocytes 8

Extracellular matrix 9
Tissue fluid 9
Structural macromolecules of the extracellular matrix 10
Collagen 10
Proteoglycans 10
Non collagenous proteins and glycoproteins 11

Anatomical structure of articular cartilage 11

CARTILAGE PHYSIOLOGY 12

Oxygen-tension in articular cartilage 12

Interspecies variations in articular cartilage structure 12

Structural and functional differences in normal articular cartilage due to gender and age 13

POTENTIAL FOR HEALING IN ARTICULAR CARTILAGE 13

BIOMECHANICAL PROPERTIES OF ARTICULAR CARTILAGE 14

Articular Cartilage as a Mechanical Element 14

Articular Cartilage as a Wear-Resistant Bearing: Lubrication Mechanism 14

Articular Cartilage as a Weight-Bearing Tissue 15
Compressive stiffness under static loading conditions 15
Viscoelastic mechanism and energy dissipation 15

EPIDEMIOLOGY 16

PATHOGENESES OF CARTILAGE LESIONS 17

Microdamage/Blunt Trauma. 17

Chondral fracture. 18

Osteochondral fracture. 18

Osteochondritis dissecans (OCD). 18

Osteonecrosis. 19

EVALUATION OF CARTILAGE INJURIES IN THE KNEE 19

TREATMENT OF A CARTILAGE INJURY OF THE KNEE 20

Non- Surgical Treatment 20

Surgical Treatment 20
Removal of the loose fragment or the injuried cartilage 20
Fixation of the loose fragment 20

Repair of Injured Articular Cartilage 21
Biological perspectives for successful cartilage repair 21
Biomechanical perspectives for successful cartilage repair 21
Cartilage repair techniques 22
Bone marrow-based repair techniques: 22
Subchondral drilling: 23
Microfracture: 23
Osteochondral graft 24
Periosteal graft 24
Cell-based repair 25
Chondrocyte transplantation 25
Mesenchymal stem cell transplantation 26

Postoperative treatment 26

CARTILAGE RESEARCH ISSUES 27

Review on the literature of experimental models 27

Usefulness and drawbacks of experimental models in cartilage repair 28

Why use experimental models in cartilage repair 28
Assessment of the cartilage repair 28
Safety 28
Proof of principle of healing 28

Review of the literature of clinical trials on cartilage repair 29

METHODS FOR EVALUATING CARTILAGE REPAIR 29

Radiology 29

Biomechanical methods 30

Biochemical methods 30

Histology 30

Knee function score 31

THE CURRENT THESIS 31

Background 31

Aims of the thesis 32

MATERIAL AND METHODS 33

Collection of epidemiological data (paper I) 33

Chondrocyte culturing and changes of hydrostatic pressure (paper II) 33

Animals (paper III & IV) 33

Surgery (paper III & IV) 34

Embedding and section for histology (paper III & IV) 34

Morphology (paper III & IV) 34

Morphometry (paper III & IV) 35

Synovial fluid analyses (paper IV) 35

Statistics (paper I, II, III & IV) 35

METHODOLOGY CONSIDERATIONS 36

Data collection methods (paper I) 36

Chondrocyte cultures (paper II) 36

Animals (paper III & IV) 36

Surgery (paper III & IV) 37

Embedding and section for histology (paper III & IV) 37

Morphometry (paper III & IV) 37

Synovial fluid analyses 37

Statistics 38

MAIN RESULTS AND CLINICAL INTERPRETATION OF THE PAPERS INCLUDED IN THE THESIS 38

Paper I 38

Paper II 38

Paper III 38

Paper IV 39

GENERAL DISCUSSION 39

CONCLUSIONS AND CLINICAL IMPLICATIONS OF THE CURRENT THESIS 39

CONCLUSION 41

SUGGESTIONS FOR FUTURE DIRECTIONS 41

TABLES 43

FIGURES 45

REFERENCE LIST 52

PAPERS I-IV

Acknowledgements

The present work was carried out during the years 1997 to 2003. This work was done at different collaborating academic institutions, including: the Institute of Surgical Research, Rikshospitalet, Akershus University Hospital, the Musculoskeletal Research Center, the University of Pittsburgh, USA, and the Oslo Sports Trauma Research Center (OSTRC). The possibility of combining a clinical resident position at Akershus University Hospital and the research at these well-known academic institutions has been instrumental in obtaining the combination of basic experimental research and clinical research in this thesis. The department of surgery at Akershus University Hospital has been especially supportive, both during my stay in Pittsburgh and during the process of fulfilling the experimental work afterwards. The seminars and financial support from the OSTRC improved the project and was one of the keystones in its fulfillment. Financial support in 1997, 2002, and 2003 from the Research Funding at Akershus University Hospital was another keystone in this project.

The practical and intellectual support and assistance from my colleagues Stig Heir and Sverre Løken during this study was invaluable in the achievement of the present work.

Thanks to the laboratory personal who helped us conduct experimental work, and to my colleagues in supporting the clinical paper.

Professor Finn P. Reinholt at the Institute and Department of Pathology, Rikshospitalet, deserves special thanks for letting me use the laboratory facility, and for his extensive experience in advising me in the evaluation of the histological slides and the histomorphometry. His office always had an open door for discussion of cartilage research and his attitude and thoughtful discussion of this issue gave me a new and broader scientific view of cartilage repair research.

My supervisor Professor Lars Engebretsen Department of Orthopedics, Ullevål University Hospital, advised and supported me through all the phases of this work. His extensive clinical experience was most important in focusing on the research goals of what needed to be clarified in the process of trying to solve one of the most challenging issues in orthopedic practice. He also deserves a lot of credit for letting me try new ideas throughout the thesis work, which were of great importance for my scientific education.

I am also indebted to Bente, my beloved wife, for all her love and support and for actually retaining her early attitude of encouragement and certainty throughout the work with this thesis. Thanks to our son Sigurd for bringing a brightening light to our lives every day.

Oslo, December 2004

Asbjørn Årøen

Papers included in this thesis

I. Årøen A, Løken S, Heir S, Alvik E, Ekeland A, Granlund O G, Engebretsen L. Articular Cartilage Lesions in 993 Consecutive Knee Arthroscopies. American Journal of Sports Medicine 2004; (32): 211-215.

II. Suh J-K, Baek GH, Årøen A, Malin CM, Niyibizi C, Evans CH, Westerhaus-Larson A. Intermittent sub-ambient interstitial hydrostatic pressure as a potential mechanical stimulator for chondrocyte metabolism. Osteoarthritis and Cartilage (1999) 7, 71-80

III. Årøen A, Heir S, Løken S, Reinholt F, Engebretsen L. Retention Rate of Periosteal Flap Cover of Articular Cartilage defects in an Experimental Rabbit Model. Acta Orthopaedica Scandinavica (published April 05)

IV. Årøen A, Heir S, Løken S, Reinholt F, Engebretsen L. Healing of Articular Cartilage Defects. An Experimental Study of Vascular and Minimal Vascular Microenvironment. Submitted to Journal of Orthopaedic Research

Abbreviations

OSTRC = Oslo Sports Trauma Research Center
CPM = Continuous Passive Motion
ACL = Anterior Cruciate Ligament
COMP= Cartilage Oligomeric Protein
ECM = Extracellular Matrix
MRI = Magnetic Resonance Imaging
ELISA=Enzyme-Linked Immunosorbent Assay
BMP= Bone Morphogenetic Protein
Gdf5= Growth and differentiation factor
CH21= Extracellular fatty acid binding protein (later renamed as Ex-FABP)
HAP= Hyaloronic Acid Protein
TGF= Transforming Growth Factor
EGF= Epidermal Growth Factor
IGF= Insulin-like Growth Factor
Wnt= Wingless
Å=Ångstrøm unit

Introduction

Malfunction of the knee joint is often associated with cartilage injury (Aroen et al., 2004). Whether or not healing or restoration of lost or wounded portions of articular cartilage with newly formed fully functional cartilage is possible remains one of the unsolved problems in orthopedic practice. Although the answer to this question is still unknown (Messner and Gillquist, 1996) there are reasons to believe that the improved understanding of articular cartilage biology, pathophysiology, and biomechanics will eventually provide an acceptable answer. In this current thesis, issues of importance for a better understanding of this challenging injury of the knee are addressed. The overall aim is that such knowledge will contribute to improved treatment options for the patient.

Embryology

Articular cartilage is a highly specialized tissue that allows for unique functions within the synovial joints. Formation of articular cartilage starts at the third week of development of the human fetus from the mesodermal layer. This forms a series of mesodermal tissue blocks, the somites, on each side of the neural tube, which further develops into the vertebrae. Soon after its formation, each somite becomes differentiated in a ventromedial part, the sclerotome and a dorsolateral part of the dermomyotome. At the end of the fourth week, the sclerotome becomes polymorphous and forms a loosely woven tissue known as mesenchyme. This tissue harbors stem cells that differentiate further into fibroblasts, chondroblasts, and osteoblasts, to create the musculoskeletal system including the human joint. In cartilage, a single cell linage, represented by different subpopulations of chondrocytes, constitutes the only cell type of this tissue. Figure 1 shows the ultrastructural characteristics of this cell.

Joint cartilage in articular surfaces, which line the ends of many bones, and cartilage at some other sites (ex. in the nose, ears and throat) are not replaced by bone and persist as permanent cartilage. It is not clearly understood why chondrocytes in these cartilages do not develop into bone.

During joint formation, cells in the area of the joint space first increase in density and then down-regulate some of their chondrocyte markers. These cells subsequently undergo apoptosis, leading to the formation of a joint cavity. Growth and differentiation factor 5 (Gdf5, a BMP family member) has a central function in joint formation as it has been found that mutations in the genes encoded for this protein both in mouse and human mutations result in a lack of joint formation (Murakami et al., 2004). The formation of the human joint is possibly the most complex process during skeletal development, where several factors are involved and many of them are probably not yet fully characterized. Figure 2 shows a schematic representation of the steps involved in enchondral formation. In Table 1 the currently known signaling molecules in the formation of human joints are listed. Several of these have also been attempted in experimental studies on cartilage repair (Gouttenoire et al., 2004,Li et al., 2005,Stevens et al., 2004).

Cartilage anatomy

Chondrocytes

Articular cartilage consists of chondrocytes and an extracellular matrix with water. Chondrocytes are highly differentiated cells that account for only 5% of total tissue volume. They are quite metabolically active, but show a limited ability to replicate.

Chondrocytes are responsible for maintaining the extracellular matrix through the synthesis and secretion of proteoglycans, collagens, and other matrix proteins. In the view of the limitations of the supplies of nutrients, because of the long distance from the nourishing bloodstream, this is quite remarkable. The chondrocytes have also an, albeit limited, ability to degrade matrix. This is important during the slow metabolic turnover that occurs in the articular cartilage during the daily motion of the joints in order to remove and restore articular cartilage from minor damage and wear. Mature chondrocytes have organelles typical of protein secretory cells with an elaborated rough endoplasmic reticulum and well-developed Golgi complex (fig.1). Large accumulations of glycogen and lipid vesicles are characteristic of mature chondrocytes and reflect their high metabolic activity. Studies on growing cartilage using an injection of radioactive sulfate, which is incorporated into the proteoglycan, reveal that the radioactivity appears first in the cytoplasm of the chondrocytes and subsequently in the intracellular organelles. Synthesis and sulfation of the glycosaminoglycans take place in the Golgi complex (Junquerira et al., 1986). Additionally, the core protein of the proteoglycans synthesized in the rough endoplasmic reticulum combines here with repeated units of glycosaminoglycans to form proteoglycans. Ultrastructural studies of collagen synthesis after ^{3}H-proline administration demonstrate that the radioactivity first appears in the rough endoplasmic reticulum, then in the Golgi complex, and finally in the extracellular matrix (Junquerira et al., 1986).

Cartilage, and in particular the chondrocytes, shrinks during conventional histologic preparation, causing an irregular shape of the chondrocyte and retraction towards the center of the lacuna. In living tissue, the chondrocytes or groups of chondrocytes fill the lacuna completely. Under the light microscope in properly fixed tissue the chondrocyte surface appears smooth, and this has also been confirmed in modern electron microscopy (fig.1). The regulation of the metabolic activity of the chondrocytes has for a long time been an area of intensive research. It is well known that the chondrocyte function depends on proper hormonal balance. The synthesis of glycosaminoglycans is accelerated by growth hormones thyroxine and testosterone, and it is inhibited by cortisone, hydrocortisone, and estradiol (Buckwalter and Mankin, 1997). However, one of the most important stimulatory mechanisms is the biomechanical forces acting on articular cartilage. As shown in paper II of this thesis, the subambient negative pressure has a profound effect on the production of extracellular matrix components (Suh et al., 1999). These effects are probably meditated through the cytoskeleton of the chondrocytes (Benjamin et al., 1994). New technology has revealed that the sensitivity of mechanical stimuli of the chondrocyte is decreased in osteoarthritic cartilage, indicating that the regulation of the normal balance between anabolic and catabolic activities of the chondrocyte has been severely disrupted (Trickey et al., 2004).

Extracellular matrix

The matrix of articular cartilage can be regarded as composite with two main groups of components: the tissue fluid with soluble molecules and the framework of structural macromolecules. The interaction of the tissue fluid with the macromolecular framework gives the tissue its mechanical properties, i.e. stiffness and resilience.

Tissue fluid

Water constitutes as much as 75-80 percent of the wet weight of articular cartilage, and the interaction of water with the matrix macromolecules substantially influences the mechanical properties of the tissue. This fluid contains gases, small proteins, metabolites, and a high concentration of cations to balance the negatively charged proteoglycans. At least some

of the water can move in and out of the tissue. The volume, concentration, and behavior of the water within the tissue depends primarily on its interaction with the structural macromolecules, in particular the large aggregating proteoglycans (aggrecans) that help to maintain the fluid within the matrix and the concentrations of electrolytes in the fluid. The aggrecan monomer includes large numbers of negatively charged sulfate and carboxyl groups that attract positively charged ions such as sodium, and decrease the concentrations of negatively charged ions such as chloride (fig.3). The increase in the total concentration of inorganic ions causes an increase in the osmolarity of the tissue that is creating a Donnan effect. The collagen network is able, under normal conditions, to resist the Donnan osmotic pressure caused by the inorganic ions associated with proteoglycans. However, a sharp cut in the cartilage would disrupt this network and the swelling of articular cartilage observed in response to this injury is a result of the Donnan effect.

Structural macromolecules of the extracellular matrix

The structural macromolecules of the cartilage, collagens, proteoglycans, and non-collagenous proteins contribute 20 to 40% of the wet weight of the tissue. The three classes of macromolecules differ in their concentrations within the tissue and in their contributions to the tissue properties. Collagens contribute about 60% of the dry weight of cartilage; proteoglycans 25%, and non-collagenous proteins and glycoproteins 15 to 20%. Collagens are distributed relatively uniformly throughout the depth of cartilage, except for the collagen rich superficial zone. This collagen fibrillar meshwork provides the articular cartilage its structure. Proteoglycans and noncollagenous proteins organize and stabilize the macromolecular network of the tissue and provide the chondrocytes with anchorage to the network (fig.3).

Collagen

The main structural framework in articular cartilage is collagen type II, which accounts for approximately 90-95% of the total collagen in hyaline cartilage. The tensile strength of collagen fiber is equivalent to steel (Harkness, 1968). The collagen content is highest in the superficial cartilage (up to 90% of dry weight) and it falls sharply with distance from the articular surface (Venn and Maroudas, 1977). Other collagens in hyaline cartilage are of type VI, IX, X, XI, XII, and XIV, though the exact role of all these are still not known yet (Lyyra, 1997). The type II collagen molecule consists of a triple helical structure formed by three polypeptide chains that are covalently cross-linked. The type IX and XI collagens interact with the type II collagen to create a collagen meshwork and thus contribute to the tensile properties of the tissue (Buckwalter and Mankin, 1997). The presence of type X collagen, only of the deep zone close to the calcified cartilage zone of the articular cartilage and the hypertrophic zone of the growth plate, suggest that this collagen has a role in the mineralization of cartilage (Kirsch and Vondermark, 1992).

Proteoglycans

The association of the collagens and proteoglycans are illustrated in the Figure 3. The main proteoglycan of articular cartilage is aggrecan, which fills most of the intrafibrillar space of cartilage matrix, contributing about 90% of the total cartilage matrix proteoglycan mass. Aggrecan consists of large numbers of chondrotin sulfate and keratin sulfate chains attached to a protein core. As illustrated in Figures 3 and 4, these aggrecans non-covalently associate with hyaluronic acid (hyaluron) and link proteins (small non-collagens proteins) to form aggregates. The formation of aggregates helps to anchor the proteoglycans within the matrix,

preventing their displacement during deformation of the tissue and helps to organize and stabilize the relationship between proteoglycans and the collagen meshwork (Hedlund et al., 1999a).

The small non-aggregating proteoglycans (10% of cartilage proteoglycan mass) have shorter protein cores, bind to other macromolecules, and probably influence chondrocyte function. Decorin and fibromodulin binds to type II collagen and probably participates in the maintenance of the structural organization of articular cartilage, having regulatory functions (Hedbom and Heinegard, 1993).

Noncollagenous proteins and glycoproteins

These molecules are poorly understood to date, and only a few aspects of their function are known. These molecules are primarily protein and have a few attached monosaccharides and oligosaccharides. The members of this group consist of cartilage oligomeric protein (COMP), Anchorin CII, link protein, fibronectin, tenascin fibromodulin, decorin, 148-kDa protein (CMP), 58-kDa protein, condrocalcin and CH21 (Heinegard and Oldberg, 1989). Most clinical interest has been focused on cartilage oligomeric protein (COMP), which is concentrated within the territorial matrix of the chondrocytes and only seems to be located in articular cartilage. It has been suggested that this molecule could be a sensitive marker of the degeneration of articular cartilage, as its presence in blood and synovial fluid is associated with breakage of the tissue homeostasis of articular cartilage. However, there have been difficulties in proving the specificity of this marker. A 10-fold increase of this protein is found in synovial fluid in patients who have MRI confirmed bone bruises after acute anterior cruciate ligament tears (Fang et al., 2001).

The link protein is important for stabilization of proteoglycan aggregate, as illustrated in Figure 3. Fibromodulin binds to collagen and influences collagen fibrillogenesis in vitro (Heinegard and Oldberg, 1989). Genetic deficiency of this protein has be shown to be associated with degenerative changes in the knee joint in mice, indicating that fibromodulin might be associated with the risk of osteoarthrosis (Gill et al., 2002). The effect of fibromodulin is hypothesized to be modulation of collagen fibril formation and to prevent mineralization along collagen (Heinegard and Oldberg, 1989). The 58-kDa protein may promote the attachment of the chondrocytes and interact with collagen (Sommarin et al., 1989).

Another known molecule in this group is Anchorin CII, a collagen binding chondrocyte surface protein, which may help to anchor chondrocytes to the collagen fibril of the extracellular matrix. The precise function of the other noncollagenous proteins thus currently relies on further research in this area (Heinegard and Oldberg, 1989); however, it is probable that this information will be critical to improve our current understanding.

Anatomical structure of articular cartilage

Cartilage developed in the human body exists in three different forms: elastic cartilage (ears, respiratory tract), fibrocartilage (intervertbral disks, attachment of certain ligaments to bones and symphyses) and articular hyaline cartilage (human joints and costae perichondrium). There is also evidence that articular cartilage in different anatomical joints have different properties (Eger et al., 2002). The articular cartilage has four structurally distinguishable zones from the surface down to the subchondral bone (fig. 5). These zones vary in cell morphology and matrix composition to meet the functional demands in the tissue. The structure, composition, and mechanical properties of cartilage vary with depth from the articular surface and with site on the joint. Within the cartilage the tissue varies with distance

from the cell (pericellular, territorial, and interterritorial matrix), and the chondrocytes vary with their appearance and synthetic activities (Hedlund et al., 1999c). This phenomenon is referred to as cartilage compartmentalization.

For example, will removal of the superficial layer completely alter the biomechanical stiffness of the articular cartilage (Korhonen et al., 2002). It is not obvious that cells from one zone grown in a culture are capable of forming articular cartilage tissue that includes all four zones used in repair procedures. The zones found in articular cartilage are illustrated in Figure 5 and have the following anatomical characteristics.

I. The superficial tangential zone. The uppermost superficial zone consists of a thin acellular fibril sheet called *lamina splendens*, which creates a low friction joint surface. Under this sheet, flattened, discoid chondrocytes align parallel to the articular surface in a matrix with a relatively low concentration of proteoglycans.
II. The middle zone. A more oval shape of chondrocytes are found in this area and the extracellular matrix contains more proteoglycan, with thicker and more obliquely positioned collagen fibrils, than in the superficial zone.
III. The deep zone. Radial columns of chondrocytes align along collagen fibrils that are perpendicular to the joint surface. The matrix in this zone has the highest concentration of proteoglycans.
IV. The calcified zone. Found just above the subchondral bone plate. In this zone the cells are smaller and contain only a few organelles.

Cartilage physiology

Oxygen-tension in articular cartilage

Another distinguishing feature of the articular cartilage is the low oxygen tension that the chondrocytes are supplied with. The deepest layers of articular cartilage are calcified and impermeable to fluid or gas, and the tissue has no vascular supply. Thus oxygen, glucose, and other nutrients must diffuse into cartilage at its surface from the synovial fluid. As a result of this diffusion mechanism the maximum thickness of articular cartilage is limited. Synovial fluid has a low oxygen tension (~50 mmHg ~ 7 % pO2) compared to that of arterial blood supply (>90 mmHg, >12 % pO2); hence, articular cartilage is normally hypoxic compared to vascularized tissue (Henrotin et al., 2005,Stockwell, 1971). The exact oxygen tension in articular cartilage is unknown, though it is probably dependent of cartilage thickness in the area and the number of chondrocytes. It is likely that the physiological oxygen tension of articular cartilage is lower than 10% at the surface and possibly as low as 1% in the deepest layers.

Articular chondrocytes have a characteristic morphology and metabolism depending upon their position in the cartilage. This may in part be a result of the oxygen tension in relation to the position of the chondrocyte in the articular cartilage

Interspecies variations in articular cartilage structure

Although there are variations, articular cartilage is basically made of similar components and shows similar structural organization in the different joints and between different species. A number of different animals have been used for cartilage research, but an ideal model to investigate articular cartilage healing remains to be established. Interestingly, in a sibling pair study, it was not possible to demonstrate a significant relation between

inherited traits and the prevalence of knee osteoarthritis, but a significant association between cartilage volume and such traits was shown (Zhai et al., 2004).

The impact of genetic interspecies variation on cartilage structure is illustrated in Table 2, and there is considerable variation in many parameters among the different species often used in cartilage research (Hunziker et al., 2002,Hunziker and Quinn, 2003,Jackson et al., 2001,Saied et al., 1997,Stockwell, 1971). Only a limited number of papers provide a systematic analysis of the quantitative parameters in articular cartilage. This deficiency in cartilage repair research limits the general validity when it comes to the possible impact of the experimental results on clinical practice.

The breeding of the animal species can also be associated with weakness in the articular cartilage structure (Ytrehus et al., 2004b), as well as the use of specific medications as use of Quinolones (Ciproxin) (Ball et al., 1999,Norrby, 1991,Wolfson and Hooper, 1991). Figure 6 shows the use of the different species in cartilage repair research until the end of December 2003. From this figure it is evident that we have the most thorough knowledge about the repair process of articular cartilage in experimental rabbits. Many of the findings can probably be extrapolated to human articular cartilage, though it must be kept in mind that the rabbit articular cartilage has twice as many cells as the human articular cartilage, and a thicker subchondral bone. The inverse relationship between cellularity and thickness often obscures the fact that the total number of cells in 1 mm^2 of cartilage lying beneath of articular surface is approximately the same in joints with cartilage of widely differing anatomical locations and also in different species (Stockwell, 1971).

Structural and functional differences in normal articular cartilage due to gender and age

One study (Jones et al., 2000) on children reproted that articular cartilage is thicker in boys than in girls. The same study, and a separate experimental study, stated that this is potentially influenced by physical activity (Newton et al., 1997). The finding of softer cartilage in women has been reported by other authors (Uchio et al., 2002). Furthermore, it has been demonstrated that there is loss of water in chondrocytes progressively as patients grow older. In fetal or immature cartilage the cell volume is much higher than in adults. With increasing age, there is a progressive decrease in cell content and in matrix synthesis, the latter reaching its lowest point when the individual is 20 to 30 years of age (Poole et al., 2001,Stockwell, 1971).

Even in the absence of cartilage disease it has been reported that the articular cartilage is thinner and stiffer in elderly people and that these features are most pronounced in elderly women (Hudelmaier et al., 2001).

Potential for healing in articular cartilage

According to the current dogma, articular cartilage has a very limited ability to heal. This dogma is based on clinical experience and progressive deterioration often observed following an initial injury in osteoarthritis. However, chondrocytes from young individuals may have a potential for healing a tissue injury. This view is supported by the observations of a cartilage regeneration in young experimental animals (Rubak, 1982,Wei et al., 1997) and in young patients with rheumatoid arthritis that where diagnosed with cartilage injuries (Bernstein et al., 1977). These observations indicate that there is a potential for healing in ages below 20 years in humans, but the factors that release or modulate this healing potential are not fully understood. Factors of relevance may be the presence of cell types such as stem cells in the joint tissues, the number of chondrocytes available to take part in the repair

process, or extracellular matrix components factors outside the target tissue that are able to support the reparative process. Thus, as observed in paper II in this thesis, and also by other authors, proper biomechanical stimulation appears to be one important parameter to stimulate the reparative process (Salter et al., 1980,Suh et al., 1999)

Biomechanical properties of articular cartilage

Articular cartilage as a mechanical element

The articular cartilage of diarthrodial joints is involved in several mechanical functions. Through its interaction with synovial fluid, articular cartilage provides an exceptional lubricating mechanism with an extremely low frictional coefficient; approximately one hundred times lower than that of ice on ice (Suh et al., 1997). By its complex structure of composite nature interacting with interstitial fluid, articular cartilage also plays an important role in minimizing the magnitude of the contact stress on the articulating surface, thereby protecting the underlying bone structure (Suh et al., 1997). The remarkable stiffness to compression is achieved by the closed spacing between the negatively charged proteoglycans in the articular cartilage framework, which decelerates the fluid flow in the tissue and reduces the compression forces transmitted to the subchondral bone (fig.3). During normal gait, the knee joint might be subjected to local contact stress as high as 14 MPa. The threshold for cartilage injury is estimated to be 25 MPa (Thambyah et al.). Especially deep flexion of the knee (more than 120 degrees) did produce contact stresses close to the limits of damage of articular cartilage in a cadaver study without additional weight (Thambyah et al.). Given a certain load, the breakage of the structural framework depends on the velocity of the load. The adaptive responses of the articular cartilage may be overloaded either by a single impact load or by repetitive loading. Although several experimental models exist, it has been difficult to find the threshold levels for injury to articular cartilage. However, studies have shown that increasing energy over time causes more damage to the articular cartilage (Jeffrey et al., 1997,Jeffrey et al., 1995).

Articular cartilage as a wear-resistant bearing: lubrication mechanism

Two types of lubrication mechanisms have been identified in the diarthrodial joint (Suh et al., 1997). The first is the fluid-film lubrication mechanism, which occurs in the articulating joint either by a hydrodynamic mechanism (MacConaill, 1932) or by a squeeze film mechanism (Hlavacek and Novak, 1995,Hlavacek, 1995). In this lubrication process, a thin layer of synovial fluid-film, usually thinner than 25 μm, is created due to the high viscosity of synovial fluid during joint articulation. This fluid-film is directly responsible for much of the load bearing that occurs in the diarthrodial joint. The second lubrication mechanism is the boundary lubrication mechanism first suggested by Charnley (Charnley, 1960). He stated that the hyaluronic acid protein (HAP) complex in the synovial fluid has a high affinity for the articulating surface, and thus functions as an important boundary lubricant in the joint. However, it was later found that the lubricant material is not the HAP complex, but actually the adsorbent glycoprotein fraction in the synovial fluid (Radin et al., 1970,Swann et al., 1981).

In both of the lubrication mechanisms described above, the lubrication of a synovial joint is largely dependent upon the viscosity of the joint lubricant, i.e. the synovial fluid. Biochemically, synovial fluid is a dialysate of blood plasma containing varying amounts of glycosaminoglycan and glycoprotein molecules secreted into the joint cavity by the synovial

cells. The rheological properties of this macromolecular fluid are determined by the size, conformation, and concentration of the HAP complex, and degradation often occurs in various states of joint pathology such as rheumatoid arthritis and osteoarthritis (Suh et al., 1997).

Articular cartilage as a weight-bearing tissue

Compressive stiffness under static loading conditions

In articular cartilage, proteoglycans exist in a large aggregated conformation trapped within the collagen meshwork. These molecules play a major role in providing cartilage with a weight-bearing capability under compression at a quasi-static loading rate. Two mechanisms are involved in the ability of proteoglycans to provide articular cartilage with weight-bearing capabilities. First, loaded with a high concentration of fixed negative charges on the glycosaminoglycans, the proteoglycan aggregates create strong intramolecular and intermolecular electrostatic repulsive forces. This constitutes the primary mechanism of bulk compressive stiffness of articular cartilage (Maroudas, 1975,Mow et al., 1984,Suh et al., 1997). Secondly, the fixed negative charges in the proteoglycan aggregate also attract mobile cations such as Na^+ and Ca^{2+} into the intramolecular space. This creates a Donnan osmotic swelling pressure as high as 0.35 MPa, which is about three times higher than normal atmospheric pressure (Maroudas, 1975,Suh et al., 1997). Under a free-swelling environment without external mechanical loading, both the electrostatic repulsive force and the swelling pressure created by the ionically charged proteoglycan molecules are resisted and balanced by the pre-tension developed in the collagen network. This provides the overall compressive stiffness of articular cartilage under quasi-static loading conditions.

Viscoelastic Mechanism and Energy Dissipation

Because of the hydrophilic characteristics of the proteoglycan molecules and the collagen fibrils, a large amount of water is entrapped in the intramolecular and intermolecular space within the cartilage matrix (Maroudas, 1975, Mow et al., 1984,Torzilli and Mow, 1976,Torzilli et al., 1983). Most of this water is mobile and can slowly flow through the dense cartilage matrix when a pressure gradient exists within the tissue (Torzilli et al., 1983).

During walking or running, articular cartilage is subjected to compressive forces that rise to several times body weight within a very short period of time. Under this dynamic loading environment, interstitial fluid entrapped within the cartilage matrix enables the tissue to resist such high compressive force without mechanical failure. When a mechanical force is applied to the tissue matrix at a high rate, it causes an instantaneous increase in hydrostatic pressure, and thus creates a large pressure gradient within the tissue matrix. Due to the small pore size (80 Å in diameter) of the intermolecular space and the low permeability ($\sim 10^{-15}$ m^4/Ns) of the tissue matrix, the interstitial fluid flows at an extremely slow velocity (Kwan et al., 1984). Thus, the instantaneously increased hydrostatic pressure will be sustained within the tissue matrix for an extended period of time. This provides articular cartilage with its primary load resisting mechanism under a rapidly rising load without causing an excessive mechanical deformation of structural molecules (collagen and proteoglycan), thereby protecting the cartilage matrix from mechanical damage.

When the interstitial fluid flows through the dense matrix, the frictional interaction between the fluid and the matrix is created, thus providing a mechanism for energy dissipation (Suh et al., 1997,Suh et al., 1999,Torzilli et al., 1983). This phenomenon, the so-called *flow-*

dependent biphasic viscoelasticity of articular cartilage, provides the additional protection of the tissue matrix from mechanical damage. Furthermore, independent of the interstitial fluid flow, the proteoglycan molecules and the collagen fibers have demonstrated significant viscoelastic characteristics themselves (Suh et al., 1997,Suh and Bai, 1998). Thus, the cartilage matrix constitutes the intrinsic viscoelasticity in shear deformation (Hayes and Bodine, 1978). This second phenomenon is called *flow-independent intrinsic viscoelasticity*, and has been shown to significantly govern the short-term viscoelastic behavior of articular cartilage immediately after a mechanical load is applied to the tissue (Suh et al., 1997). This suggests that the flow-independent intrinsic viscoelasticity provides an important protection mechanism of cartilage matrix from mechanical damage when articular cartilage is subjected to a rapidly fluctuating mechanical load, such as that which occurs while walking or running.

In summary, the combination of the flow-dependent biphasic viscoelasticity and the flow-independent intrinsic viscoelasticity of the tissue matrix results in high compressive dynamic moduli for articular cartilage. This provides the articular cartilage with the primary load bearing mechanism within the physiological loading environment.

Epidemiology

Chondral injuries of the knee are common. There is a lack of knowledge regarding the natural history of cartilage lesions. We do not know when a small lesion results in pain or further progresses into degenerative disease. Currently, the majority of these lesions are not offered surgical treatment with the techniques available today.

Curl and coauthors (1997) reported the discovery of a chondral lesion in 63% of the knees in over 31,000 knee arthroscopic procedures, with an association between meniscal and articular cartilage lesions. Articular cartilage damage has been reported in association with 23% of acute anterior cruciate ligament ruptures, and in 54% of knees with chronic ACL laxity or instability (Indelicato and Bittar, 1985). One recent study observed that 19% of ACL ruptures showed associated chondral lesions and that there was a increased odds ratio concerning chondral lesions with the time period since initial injury (Tandogan et al., 2004). Many of the chondral lesions in ACL-ruptured knees occur in combination with meniscal injuries, as only 4-5% show to have full thickness cartilage lesions with both menisci intact (Shelbourne et al., 2003). The mean area of the lesion in the study of Shelbourne et al. is 1.7 cm^2. The study also reported that at the five year follow-up the course following the chondral lesions was favorable. However, several possible biases are obvious in the cited study. Firstly, the ACL-reconstruction may induce several stimuli to healing of the cartilage injury. Secondly, the study is a short-term follow-up study, and only 60% of the patients were included at follow-up. In addition, the demands of the knee function after the ACL-reconstruction were low among the patients, as the majority of them were not actively participating in sports activities. Recently, attempts have been made to classify such injuries prospectively in knees subjected to an arthroscopic procedure, and these studies document that chondral injuries are common (Aroen et al., 2004,Hjelle et al., 2002). Associations to ACL-injuries (18%) are common as well as to meniscus injury (13%) (Aroen et al., 2004). An area of cartilage injury of more than 1 cm^2 was found in 4-5 % in one study (Hjelle et al., 2002), and in another study 6% of the patients showed an area of injury of more than 2 cm^2 (Aroen et al., 2004).

In a study of the outcome after chondral injuries in association with meniscal lesions, only injuries of more than 2 cm^2 progressed to radiographic changes indicative of osteoarthrosis (Ciardullo et al., 1999). Another study described a 14 year follow-up on 28 young athletes with isolated traumatic chondral injuries or osteochondritis dissecans, where three patients received Pridie drilling (Messner and Maletius, 1996). At follow-up, radiographic signs of

arthrosis were demonstrated in the injured knee in 16 patients, with a higher incidence of degenerative changes for the patients over 30 years of age. Although the study did not offer a full description of the natural course, it is probably the best long-term follow-up study of patients with chondral lesions subjected to minimal or no treatment.

Other authors have looked at the natural history of osteochondritis dissecans lesions after removal of the fragment, and reported a high incidence of arthrosis at long-term follow-up (Linden, 1977).

However, epidemiological data is still sparse on presence of articular cartilage lesions. Only a long-term study aimed at evaluating the natural history of the smaller cartilage lesions in comparison to larger ones, and the effect of treated associated lesions, will provide some of the desired facts related to this injury. As long as such data are unavailable, a cost-benefit analysis of the effect of cartilage treatment is difficult, although one study suggests that early intervention in serious cases might be cost-effective (Lindahl et al., 2001)

PATHOGENESES OF CARTILAGE LESIONS

For some of the conditions causing cartilage injury, e.g. osteochondritis dissecans and the articular cartilage lesions as a sequel of knee traumas, the exact pathogenesis is not known. The depth of a cartilage injury is also related to the cause of the lesion and may imply different prognosis of the natural healing without treatment. It may be useful to divide cartilage injuries into five different types, both in clinical situations and in experimental models, as type of injury may be crucial for the choice of treatment and the interpretation of results. In addition, the outcome may also be influenced by such variables as age, obesity, activity level, and limb misalignment

Microdamage/Blunt Trauma

The lack of innervation, both of neurogenic and of vascular elements in articular cartilage, may cause an initial asymptomatic course of these injuries. Experimentally, it is suggested that injuries of a certain size may lead to osteoarthrosis over time. Both a single severe impact and repetitive blunt loading may cause microfractures of the matrix. One of the initial responses to impact is loss of chondrocytes in the region of trauma, which is one of the major signs of the start of a degenerative process. In a rabbit model of chondral injury, D'Lima et al demonstrated that up to 34% of chondrocytes in the injured area undergo apoptosis (i.e., programmed cell death) in contrast to 1% basal rate of apoptosis (D'Lima et al., 2001). Blunt cartilage injury is also associated with important metabolic alterations in the matrix, including degeneration of collagen and loss of proteoglycans. Experimental use of caspase inhibitors, which targets the enzymes that cause apoptosis, reduced both cell death and loss of glycosaminoglycans from the injuried cartilage (Lo and Kim, 2004). From this evidence it seems that these inhibitors may play a role after traumatic injury to the joint surface and as such decrease the potential for late osteoarthritic changes. However, the diagnosis of such injuries are not easy and it is not verified that, for instance, bone bruises seen on MRI, with no alterations to the cartilage surface, result in the same degenerative changes as produced experimentally. Even though the bone bruises seem to resolve in a MRI evaluation at a one year follow-up (Miller et al., 1998), there is evidence that the most serious bone bruises diagnosed are associated with cartilage thinning adjacent to the site of the initial osteochondral lesion at a six year follow-up (Faber et al., 1999).

Chondral fracture

By definition a chondral fracture is confined to the articular cartilage and does not penetrate to the subchondral bone. Accordingly, the vascular system in adult individuals is not involved and no reparative inflammatory response is initiated. The limited ability of mature chondrocytes to migrate, proliferate and initiate a repair response is well-known (Hunziker, 2002). Although some repair cells may be observed at the bottom of the defect, and probably represent chondrogenic progenitor cells originating from the synovial membrane, these cells are not able to make any significant amount of repair tissue (Hunziker and Rosenberg, 1996). This is different from other mesenchymal tissues such as fat, muscle, and bone, where stem cells are recruited to repair and recover tissue integrity. In articular cartilage there are no obvious stem cells that can enhance the reparative response. As with blunt trauma, chondral fracture rapidly results in chondrocyte apoptosis in the region of injury. Using an in vitro cartilage explant model, Tew et al (2000) showed that experimental wounding of cartilage leads to loss of cells through both necrosis and apoptosis. However, many of the surviving cells in the region of the injury subsequently undergo a proliferate response in an attempt to repair the tissue (Ytrehus et al., 2004). Although synthesis of type II collagen and matrix macromolecules is increased in surviving chondrocytes (which proliferate and form clusters in the periphery of the injuried zone), the partial thickness lesions are not repaired (Hunziker, 2002,Tew et al., 2000). Interestingly, defects made with a sharp scalpel have been shown to induce a more restricted cell death compared to blunt injuries of articular cartilage (Redman et al., 2004).

Osteochondral fracture

These injuries are most often seen in association with patella dislocations (Matelic et al., 1995,Stanitski et al., 1993), although such a lesion may occur as a result of ankle trauma as well (Clark et al., 1995). Hemarthrosis or a swollen knee after a trauma in a child or an adolescent has been shown to be associated with a high frequency of osteochondral fractures (Matelic et al., 1995,Stanitski et al., 1993). Although very similar to the chondral fractures, these injuries theoretically do have a better potential for healing because some of the subchondral bone are attached to the osteochondral fragment, and as such represents a potential for bony healing at the base of the defect.

Osteochondritis dissecans (OCD)

The pathogenesis and treatment of these lesions are still unclear. Loosening of the osteochondral fragment is caused by events taking place in the subchondral bone, and often a zone of sclerosis of the bone located in the bottom of the defect is noted. Although it is possible to grow chondrocytes from these osteochondral fragments and they often macroscopically have normal structure, there are histological changes in the cartilage morphology (Guthrie et al., 1992). This phenomena is illustrated in the histological slide from one patient who got this loose fragment removed (figure 7). Studies from veterinary medicine suggest that factors like rapid growth, heredity, anatomical conditions, biomechanical stress locations, and dietary factors may be important in the pathogenesis. Veterinary studies have nicely demonstrated that heredity and too early regression or interruption of the blood vessels in the developing articular cartilage in pigs, are factors predisposing for this condition (Ytrehus et al., 2004,Ytrehus et al., 2004). Whether these results can be extrapolated to humans is still not known. However, conservative treatment of

children and adolescents is more often successful, whereas some kind of surgical treatment (removal of the sclerotic bone and fixation of the fragment or cartilage repair procedure) is often needed in the adult (Cahill et al., 1989,Schenck, Jr. and Goodnight, 1996). The majority of these adult patients present with a locking or locked joint in contrast to the younger patients where joint pain is the dominating symptom.

Osteonecrosis

Spontaneous osteonecrosis of the knee is a superficial subchondral lesion classically seen in the medial femoral condyle. It has been suggested that osteonecrosis is a result of a stress fracture in the subchondral bone (Yamamoto and Bullough, 2000). In this kind of lesion the supportive function of the subchondral bone to the articular cartilage disappears and the consequence may turn to be dramatic for the function of the articular cartilage and the joint function. Fortunately, this kind of lesion is rather unusual and mostly occurs in older age groups where a prosthetic arthroplasty is an alternative. However, knee arthroplasty is only found to be successful in 71% of this patient group (Mont et al., 2000). Osteonecrosis occurs also in younger age groups, but often as a complication to steroid use. The use of a fresh osteochondral allograft in such cases has been reported in a series of 18 patients, where the procedure resulted in pain relief, improved function, and high degree of patient satisfaction (Bugbee W-ICRS 2004).

EVALUATION OF CARTILAGE INJURIES IN THE KNEE

Several cartilage injury classification systems have been proposed, but a general problem is that they have not been validated concerning the reproducibility of data between different orthopedic surgeons. Illustrative is the thorough review on the clinical reports of cartilage treatment by (Jakobsen et al., 2005), where it is documented that in 64 included studies, 20 different scores have been used. The International Cartilage Repair Society (ICRS) evaluation package has been redesigned from the first version in 1998 and is now intended to be used as International Knee Documentation System (IKCD) (Brittberg and Winalski, 2003). Two important parameters systematically incorporated into this evaluation package are the description of the depth of the cartilage lesion and the anatomical location. According to this system, the depth of the cartilage defect is ranked on a 4-graded scale. Grade 1 and 2 represent partial cartilage defects discriminated by their depth in the cartilage. ICRS grade 3 can be a full-thickness injury in which the defect in the articular cartilage extends down to the subchondral bone (through the calcified cartilage), while grade 4 means penetration of the subchondral bone. The latest version of this evaluation package further divides these four major groups into different subgroups (Brittberg and Winalski, 2003). Only two studies have actually used a version of this evaluation package for classification of cartilage defects (Aroen et al., 2004,Hjelle et al., 2002). In the studies presented in Figure 8 several different scores have been used. The Lysholm score, used as one of the parameters in three of the studies (Horas et al., 2003,Knutsen et al., 2004,Peterson et al., 2000), was later judged suitable in evaluating chondral injuries (Kocher et al., 2004).

Reliable imaging techniques for non-invasive evaluation of a cartilage injury and the results of the cartilage repair procedures have not yet been established. With new techniques it might be possible to recognize early changes in order to help the orthopaedic surgeon to intervene with an appropriate treatment at an earlier stage (Burstein et al 2003). However, the application of such methods are still in the future, and, at present, a combination of conventional arthroscopy and standing x-rays still provide valuable information in classifying cartilage injuries.

Treatment of a cartilage injury of the knee

Non- Surgical Treatment

This is an alternative of treatment in cases where an osteochondritis dissecans is not unstable in a young patient diagnosed on x-ray or MRI as a result of examination of knee pain. Avoidance of sports activities and weight bearing for six weeks can often induce complete resolution of the symptoms (Hughes et al., 2003). However, we do not know whether or not such a procedure helps to heal a cartilage injury related to a bone bruise. Another situation when non-surgical treatment often is chosen is when the cartilage defect is an associated finding to the meniscal or ACL injury where any of the latter represents the main problem. The natural history of a cartilage lesion in relation to ACL and/or meniscal injuries has not been documented in detail.

Surgical treatment

Removal of the loose fragment or the injuried cartilage

In these cases, the arthroscopic procedure is performed as a result of symptoms related to the loose fragment. The literature is limited regarding the results subsequent to this treatment, even though there is reason to believe that the procedure is not so uncommon in clinical practice. The consequences of this treatment are dependent on location and the size of the fragment. If the fragment is thin or consists of multiple fragments it cannot be fixated. Today, most of these patients would be subjected to some kind of cartilage repair procedure in order to restore the articular cartilage surface. However, no evidence of serious complications was seen by Kennedy et al.1966 following removal of the fragment. More recent literature concludes that removal of the loose fragment located in the patellofemoral joint would improve the mechanical symptoms, although the procedure has been associated with persistent patellofemoral crepitus and discomfort (Peters and McLean, 2000). The patellofemoral joint is the compartment least subjected to axial compression, so it is generally believed that the consequences would be even worse in the medial and lateral femoral tibial compartment. Review papers on this issue recommend fixation or cartilage repair procedures for these lesions (Schenk, Jr. and Goodnight, 1996). If the articular cartilage lesion is less than 2 to 3 cm^2 and has good peripheral cartilage support at the edges it may take several years before degenerative arthritis develops (Brittberg et al., 1994,Shelbourne et al., 2003).

Fixation of the loose fragment

Fixation of loose fragments has been advocated by many authors to allow healing to occur. This is often technically demanding arthroscopically. Use of metal pins or screws are often associated with the need of later removal of the hardware in a second operation (Nakagawa et al., 2004a,Kivisto et al., 2002,Zarzycki, 2001). Biodegradable meniscus arrows, pins, and screws have also been introduced, although reports on synovitis and further chondral injuries as a result of these fixation devices have been published (Friederichs et al., 2001,Marandola and Prietto, 1993,Nakagawa et al., 2004,Tegnander et al., 1994,Wouters et al., 2004). Mosaic arthroplasty has been reported as an alternative to stabilize the fragment, and such a procedure may induce stimulation of the subchondral bone in order to obtain healing (Yoshizumi et al., 2002).

Repair of Injured Articular Cartilage

Biological Perspectives for Successful Cartilage Repair

During embryological development, mesenchymal cells undergo specific, yet diverse differentiation to constitute different mesenchymal soft tissues. Originating with the formation of the mesoderm in the third week, cells migrate to form a loose network of connective tissue known as mesenchyme. The development of articular cartilage represents one avenue of this cellular lineage. This implies that successful repair of cartilage defects will be based upon an understanding of the specific factors that influence the recruitment and differentiation of mesenchymal cells.

The mature chondrocytes produce the unique extracellular matrix (ECM) of articular cartilage *in vivo*. The composition of the ECM is critical to the functional properties of repaired cartilage, and consequently to the long-term success of cartilage repair. This includes the site-specific distribution of type II collagen, proteoglycans, and the proteins as well as the tissue fluid (Haapala et al., 2000,Lyyra et al., 1999,Lyyra et al., 1999,Peterson et al., 2002,Torzilli et al., 1983) .

The synthesis of these molecules by chondrocytes is responsive to various physical and pharmacological factors. A cyclic compressive load has a stimulatory effect on the biosynthetic activity of articular cartilage *in vitro* (Davisson et al., 2002). Intermittent positive hydrostatic pressure and shear has been shown to stimulate the synthesis of glycosaminoglycans, aggrecan, and type II collagen in chondrocyte cultures (Waldman et al., 2003b,Davisson et al., 2002). Similar stimulation of proteoglycan synthesis has also been induced by intermittent negative pressure (Suh et al., 1999). Finally, administration of non-steroidal anti-inflammatory agents (NSAIDS) inhibits the secretion of proteoglycans in both cellular and cartilage explant cultures (Collier and Ghosh, 1991). This inhibition seems to be reversible when these agents are removed from the culture media.

Several hormones and factors released in response to hormonal stimulation are important for the regulation of metabolic activities of chondrocyte. Growth hormones, calcitonin, androgens, and growth factors such as insulin-like growth factor I (IGF-1), transforming growth factor beta (TGF-β), bone morphogenetic protein-2 (BMP-2), and epidermal growth factor (EGF) stimulate the chondrocyte proliferation and the synthesis of type II collagen and proteoglycan (Suh et al., 1997). TGF-β1 also stimulates chondrogenesis in periosteal explants and down-regulates interleukin-1 receptors on chondrocytes (Harvey et al., 1991). It has been demonstrated that growth hormone alone has no metabolic effect, but produces a synergistic effect with insulin-like growth factor I to stimulate chondrocyte extracellular matrix synthesis (Smith et al., 1989). While the specific cellular mechanisms and signal pathways have not yet been fully described for articular chondrocytes, there is evidence that the cytoskeleton is involved in chondrocyte metabolism (Trickey et al., 2004). A more complete understanding of the cellular mechanisms of articular cartilage is critical to the development of new methods to improve the functional qualities of repaired cartilage.

Biomechanical perspectives for successful cartilage repair

Articular cartilage is an avascular tissue that is subjected to a harsh mechanical loading environment. The fate of repaired cartilage tissue depends on its ability to meet the biomechanical demands that enables healthy tissue to function within this environment.

One of the most important biomechanical criteria for successful cartilage repair is reconstitution of the repair tissue with biomechanical properties that are comparable to the

surrounding normal cartilage. The difficulty of achieving the same biomechanical properties in the repair tissue has been evident from *in vivo* studies (Peterson et al., 2002,Laasanen et al., 2003). In general, the biomechanical properties of repair tissues are inferior and affected with variations within the repair, and there is poor integration with the neighboring cartilage and an increase of subchondral bone density and thickness (Lyyra et al., 1999b,Peterson et al., 2002,Russlies et al., 2003,Russlies et al., 2004). Consequently, shear stresses are increased along the interface between the repair and surrounding normal tissues (Wayne et al., 1991a,Wayne et al., 1991b), and leads to failure at the interface of the repair tissue and the surrounding tissues. An *in vitro* study demonstrated that structural integration at this interface can be improved by stimulating cellular metabolism (Reindel et al., 1995).

Other important biomechanical criteria for successful cartilage repair include appropriate reconstruction of both the subchondral bone and the superficial tangential zone. The reconstruction of appropriate subchondral bone, along with an appropriate tissue thickness, is critical to the maintenance of a proper mechanical stress environment in the repaired tissue (Qiu et al., 2003). The reconstruction of the proper superficial tangential zone with a densely packed collagen meshwork will provide the cartilage surface with a wear-resistant capacity. An appropriate superficial tangential layer also provides a balancing mechanism against the proteoglycan-induced pressure and the mechanically-induced hydrostatic pressure. This balance is critical to the maintenance of the proper structural morphology of the repaired tissue (Suh et al., 1997).

Cartilage repair techniques

Bone marrow-based repair techniques

These techniques involve a disruption of subchondral bone in an attempt to induce fibrin clot formation and to initiate primitive stem cell migration from the bone marrow into the cartilage defect site. It is expected that the fibrin clot formation stimulates the primitive stem cells to initiate a repair response and to differentiate into chondrocytes under the biological and biomechanical conditions experienced in the joint. Three main techniques have been introduced in the literature based on the concept of bone marrow-based repair techniques: abrasion, subchondral drilling, and microfracture. These techniques differ according to the method of exposure of the subchondral bone and the aggressiveness of the surgical intervention.

Abrasion arthroplasty

This treatment emphasizes the removal of the damaged cartilage to the normal tissue edge in such a way that repair tissue formed in the fibrin clot can make a proper connection with normal cartilage tissue. At second-look arthroscopy and biopsy, Johnson et al showed that the cartilage defect was filled with fibrocartilage, and that the reparative fibrocartilage maintained its integrity with the host hyaline cartilage for up to six years (Johnson, 2001).

Although fibrocartilage often appears to offer the patient significant pain relief (Bert and Maschka, 1989,Steadman et al., 2003), this tissue lacks several key structural components to carry out the mechanical functions as a wear-resistant bearing as well as a weight-bearing surface. As shown in Figure 9, the fibrocartilage has a significantly decreased amount of proteoglycan molecules, as compared to the normal hyaline cartilage. As a result, the fibrocartilage does not produce a proper compressive stiffness against applied mechanical load, and thus is subjected to an excessive deformation under physiological loading. This in turn causes a mechanical rupture of the repaired tissue matrix and eventually leads to a

recurrence of degeneration and fissure of the repaired cartilage. Furthermore, in this repair technique, the abraded subchondral bone rarely tends to recover its intact structural morphology. This incomplete reconstruction of subchondral bone can cause an aberrant mechanical stress environment in the repaired tissue matrix, thus furthering the degeneration of the tissue. One study with five years follow-up revealed that 25% of the patients have been converted to total knee replacement after abrasion arthroplasty as the initial procedure to stop or hold the degenerative changes observed at the initial arthroscopy (Bert and Maschka, 1989).

Subchondral drilling

Subchondral drilling involves drilling pins through the subchondral bone (Pridie, 1959). This technique is expected to preserve some portion of subchondral bone structure, and thus help restore a proper mechanical environment in the regenerating tissue. Like abrasion arthroplasty, drilling through subchondral bone induces vascular infiltration, fibrin clot formation, and a subsequent inflammatory response, thus facilitating the repair. Using an adult rabbit model, Mitchell et al demonstrated that hyaline-like tissue initially filled the defect, but that the reparative tissue lost its hyaline appearance after eight months, and at one year it resembled dense collagenous tissue with apparent surface fibrillation (Mitchell and Shepard, 1976). Retrospective studies on this method indicate that it provides some symptom relief and enhances the intrinsic healing capacity of the joint (Gudas et al., 2002,Messner and Maletius, 1996).

Microfracture

This method was first proposed by Steadman and is designed to be less invasive than drilling (Steadman et al., 1998). In this procedure, an awl is used to create multiple microfractures (3-4 mm apart) in the subchondral bone arthroscopically. The microfracture of subchondral bone disrupts intraosseous blood vessels and leads to fibrin clot formation, release of growth factors, and introduction of mesenchymal cells into the cartilage defect. Together, these can act as a biological stimulus for differentiation and proliferation of chondral progenitor cells and enhances the repair process. Additionally, the rough surface created around the microfracture site is expected to provide better attachment of the fibrin clot. It is also believed that the awl creates less heat necrosis than the drilling procedure, thus improving tissue healing potential (Steadman et al., 2001). Follow-up studies of this procedure with postoperative continuous passive motion (CPM) have demonstrated a significant improvement at second-look arthroscopy (Blevins et al., 1998). The initial animal study only showed a temporary increase of thickness of the subchondral bone through 59% filling of the defect in comparison to 39% in the controls (Frisbie et al., 1999). Another animal study, which is part of this thesis (paper IV), shows that this surgical method creates a 50% filling of the defect, but also has an apparently permanent increase of the subchondral bone plate, which can lead to further degeneration of the joint.

The efficacy of the three above-mentioned techniques is controversial and questionable. These methods are best considered as a treatment option that has little likelihood of harming and might have chance of helping the patient, especially if it is combined with a systematic rehabilitation program. However, the reports indicating that these methods can induce some degenerative changes imply that they should only be used when a

cartilage injury is the most likely reason for the knee problem and the patient is motivated to go through a full rehabilitation program.

Osteochondral grafts

Since first introduced by Lexer in 1908, the efficacy of osteochondral allograft transplants has been studied extensively by many investigators (Aroen et al., 1998). In 1976, Mankin et al reported the results with a series of large cryopreserved osteochondral allografts. While the results of transplanted cryopreserved cartilage in their series were considered good, serious complications occurred when approximately 75% of the replaced joints exhibited subchondral collapse and/or fractures of the allograft that led to cartilage destruction. Ample evidence indicates that cryopreservation irreversibly damages the cartilage and its biomechanical properties (Bujia et al., 1995,Kubo et al., 2001). Some other investigators claim that properly frozen tissue may maintain both the viability of chondrocytes and extracellular matrix integrity (Jomha et al., 2004).

The immune response to allografts has been a matter of much interest (Phipatanakul et al., 2004,Rodrigo et al., 1989,Stevenson, 1987). One study (Kawabe and Yoshinao, 1991) demonstrated lymphocyte accumulation in the area of fresh allografts, indicating a cellular immune response. This response is apparently less pronounced in frozen allografts (Stevenson, 1987). In a 20 year follow-up study of osteochondral allografts, graft survival was 63%, indicating that graft failure due to rejection was not a significant problem (Shasha et al., 2002). In a human fresh allograft study (Czitrom et al., 1990), the survival rate of viable chondrocytes was over 60% in the grafts examined at 24 months.

Conceptually, autograft is a more suitable source of graft material and results in no immune response. However, it is limited by size constraints and co-morbidity. Hangody et al (1997) introduced the possibility of arthroscopic mosaic arthroplasty, in which autologous osteochondral plugs are harvested from non-weight-bearing regions and transplanted to weight-bearing regions. This technique has been widely used recently; however, the inferior results reported by Bentley et al (2003) decreased the enthusiasm for the use of this technique. Other authors seem to have no problem with the use of this technique (Barber and Chow, 2001,Hangody and Fules, 2003). Experimental studies have also pointed out some problems related to this treatment in regards to the impaction of the plugs leading to chondrocyte death with this technique (Nabavi-Tabrizi et al., 2002). Two similar experimental studies show that the stiffness difference related to different cartilage thickness and the lack of donor/recipient articular healing using plugs might be a problem for the long term state of the joint (Lane et al., 2004,Lane et al., 2001).

Perichondral grafts have not been used much in the last few years; however, one clinical follow-up study on this autograft demonstrates reasonable long-term results (Bouwmeester et al., 2002).

Periosteal graft

Several authors have used the periosteum in order to attempt to repair the articular cartilage defect (Alfredson et al., 1999,Alfredson and Lorentzon, 1999,Siebold et al., 2003). The observation by Salter et al., that periosteum placed in the joint cavity would differentiate into cartilage-like tissue made the approach conceivable. However experimental and humans studies indicate that the chondrogenic potential of periosteum is much less with increasing age. Experimentally, the chondrogenic potential of a periosteal graft is found to be dependent upon the cambium layer, and this later is strongly reduced at an age of 12 months in experimental rabbits (O'Driscoll et al., 2001). Similarly, the chondrogenic potential of

harvested periosteum from human ribs is very low after the age of 22 years in humans (Nakahara et al., 1991). However, the periosteum transplantation technique has been used clinically in combination with CPM, and a decrease in symptoms has been reported in one study (Alfredson and Lorentzon, 1999).

Cell-based repair

Chondrocyte transplantation

The finding that chondrocytes replicate when they are enzymatically isolated from their matrix (Green, 1971) made it conceivable to use cultured chondrocytes as a means to repair cartilage defects (Brittberg et al., 1996,Grande et al., 1989,Peterson et al., 2000,Peterson et al., 2002,Peterson et al., 2003). With this technique, autologous chondrocytes are harvested from articular cartilage arthroscopically. Chondrocytes are released from the harvested cartilage slices using an enzymatic digestion technique, expanded in culture to 5×10^6 cells, and then transplanted into the cartilage defect exposed by an arthrotomy a few weeks later. The use of a periosteal flap technique to cover the transplanted chondrocytes initially yielded encouraging results in animal studies (Brittberg et al., 1996,Grande et al., 1989), and has been reported to induce healing of deep cartilage defects in humans (Brittberg et al., 1994).The initial study reported excellent healing at the femoral articular surface in 14 of 16 patients at a two year follow-up, but less favorable results with patellar lesions (Brittberg et al., 1994). Although the long-term results are reported to be promising, one of three randomized studies published questioned the superiority of this surgical technique (Knutsen et al., 2004). The two other randomized studies on this technique have been questioned regarding their quality and the information provided is therefore limited (Bentley et al., 2003,Horas et al., 2003,LaPrade, 2003,Smith et al., 2003).

The half-life of the periosteal coverage over the defect is short, at least in experimental studies (Driesang and Hunziker, 2000 and paper III of the current thesis). Doubts with the use of periosteum to cover the cartilage defect have also been raised because it seems to increase subchondral bone density (Russlies et al., 2004). Other authors have found the stimulation from the periosteum to be important in differentiating the transplanted chondrocytes (Brittberg et al., 2005).

Other biological vehicles for chondrocyte transplantation are currently being evaluated. Thus, fibrin glue (van Susante et al., 1999) and collagen scaffolds (van Susante et al., 2001,Nehrer et al., 1997) have been launched. Although promising results have been reported *in vitro*, the *in vivo* results have not been satisfactory (van Susante et al., 1999). Hyaluronate supports chondrocyte function, but apparently does not persist *in vivo* for sufficient time to support cell attachment (Suh et al., 1997). Use of a synthetic biodegradable polymer such as polylactic acid (PLA) has been reported to lead to joint resurfacing in animal studies (Chu et al., 1995) , but the long-term results are unknown. Different new scaffolds have been introduced. The case reports have been promising (Marlovits et al., 2004,Solchaga et al., 2000) although comparison to controls is still lacking both experimentally and, in particular, clinically. Currently there seems to be evidence that the cell density seeded in the scaffold should be more than 20 million cells/mL to induce formation of cartilage (Alford and Cole, 2005,Puelacher et al., 1994). LeBaron and Athanasiou (2000) noted that polylactic-polyglycolide scaffolds seeded with a density of less than 10 million cells/mL resulted in the formation of very little cartilage.

Mesenchymal stem cell transplantation

The activation and proliferation of mesenchymal stem cells from bone marrow do theoretically have the potential to rebuild the normal cartilage structure. However, it has also been reported that their numbers decline and their potential to proliferate and differentiate deteriorate as a function of age (Quarto et al., 1995,Huibregtse et al., 2000). Other authors have observed that the repair potential of stem cells remains unchanged even at adult ages (Dressler et al, in press.). Mesenchymal stem cell-based repair of full thickness cartilage lesions has been quite successful experimentally, although in that study the age of the experimental rabbits was four months (Wakitani et al., 1994). Later, a similar approach was applied in clinical practice on osteoarthritic knees, though no significant improvement on symptoms was registered (Wakitani et al., 2002). Overall, the results obtained by using stem cells have been similar to what has been seen both in combination with growth factors and use of chondrocytes (Hunziker, 2002). Better characterization of mesenchymal stem cells and cell matrix systems, which facilitate the chondrogenic differentiation, may increase the repair potential of these cells. Interestingly, one recent study reports a scaffold that appears to have the potential to direct the mesenchymal stem cells towards chondrogenic differentiation (Chen et al., 2004).

Postoperative treatment

Immobilization of the joint induces degenerative changes in articular cartilage, while moderate running exercise causes an increase in both the thickness and the metabolism of articular cartilage (Kiviranta et al., 1994,Newton et al., 1997). Numerous *in vitro* experiments also demonstrate that cyclic mechanical load may stimulate the biosynthetic activities of articular cartilage, as described previously (Aroen et al., 1998). These findings suggest that joint motion has the potential to facilitate the healing process of repaired cartilage.

Several animal studies have demonstrated the beneficial effect of CPM in the repair of full thickness defects (Salter et al., 1975). It has been suggested that CPM should be used for at least six to eight hours per day to effectively stimulate the cartilage repair process (Salter et al., 1975,Shimizu et al., 1987). Currently, there seems to be broad agreement among scientists that CPM increases the healing potential of cartilage defects with a variety of repair techniques (Alfredson and Lorentzon, 1999,Aroen et al., 1998,Brittberg et al., 1994,Knutsen et al., 2004,Marlovits et al., 2004). Theoretically, however, this early motion may create a large shear strain at the interface between repair tissue and adjacent host cartilage, and thus prevent permanent interfacial bonding (Aroen et al., 1998,Bentley et al., 2003,Suh et al., 1997,Lane et al., 2004,Whiteside et al., 2003).

Most clinical trials include non-weight bearing active motion as instructed by a physiotherapist starting between two and three days after surgery (Brittberg et al., 1994). In order to avoid excessive loading of the immature repair tissue, the patients are often instructed to undergo a toe-touch weight-bearing exercise for at least eight weeks. Most clinical papers, however, recommend partial weight bearing for at least six weeks (Brittberg et al., 1994,Knutsen et al., 2004,Marlovits et al., 2004). Only in one clinical paper full weight bearing was allowed immediately after surgery (Bentley et al., 2003). Exercise programs, such as bicycling, swimming, and water running have been recommend in order to prevent muscle atrophy (Aroen et al., 1998).

The cartilage defect patients have a tendency to develop quadriceps weakness postoperatively, and this has to be specifically addressed in order to reduce the symptoms the same way as in unicompartmental arthritis (Marlovits et al., 2004). Patient education programs and supervised exercise secure steady movement and relief of the articular cartilage

in the knee to increase the proteoglycan production as shown in chondrocyte cultures (Suh et al., 1999).

CARTILAGE RESEARCH ISSUES

Review on the literature of experimental models

Several authors have described the natural history of cartilage defects in animal models. Younger animals are described to have a much greater potential of healing compared to adult experimental animals (Wei et al., 1997). A full thickness cartilage lesion has a healing response, while a partial cartilage thickness lesion shows little or no self-repair activity in adult animals (Hunziker, 2002). One experimental study using a pig as an animal model demonstrates that full thickness cartilage lesions or osteochondral defects all show signs of bony ingrowth in the cartilage defect unless some kind of structural barrier is introduced to prevent this (Hunziker et al., 2001). Similar observations have also been made in a sheep model (Russlies et al., 2004). Furthermore, as illustrated in Table 3, the healing response of the lesions seems to be greater in studies of natural history than what is observed in control groups in experimental studies of osteochondral defects (Lietman et al., 2002,Sellers et al., 1997). Unfortunately, most authors tend to use their own or a modified histological scoring system to grade the results. Simple and straight forward parameters such as percent filling of the defect are not available from the data presented (ex. table 3). Thus, out of several experimental studies in rabbits reviewed, only four reported filling of the control osteochondral defect (Adachi et al., 2002,Amiel et al., 1988,Hunziker et al., 2001,Katayama et al., 2004,Kawamura et al., 1998,Kreder et al., 1994,Messner, 1994,Messner et al., 1993b,Sellers et al., 1997,Wei et al., 1997,Lietman et al., 2002). Furthermore it should be noted that a considerable variation exists between "good healers" and "bad healers" within the same group of animals. Experiments with small groups are particularly vulnerable to this kind of bias (Solchaga et al., 1999). The inter-individual variation in the capacity to heal a full thickness cartilage injury is also depended on the age of the animal (Solchaga et al., 2001,Wei et al., 1997) and a genetic capacity to heal a lesion. Two possible designs to overcome this last problem can be to either increase the number of experimental animals or to use a model (as in paper III and IV in this thesis), where bilateral surgery is performed on adult animals in order to minimize variation. Another problem is the design of the cartilage lesion in the experimental animal, where several studies involve a lesion that extends far below the subchondral bone plate and as such represents studies on bone repair rather than cartilage repair (Hunziker, 2002).

In contrast to smaller lesions created in more favorable locations in the animal joint, such as in trochlea, larger osteochondral defects have an incomplete healing response when they are located in the weight bearing area (Jackson et al., 2001). It seems also that older lesions (ten weeks) have less potential for repair than fresh ones (Saris et al., 2003). Not much is known about the importance of location and the significance of full thickness cartilage lesions. One experimental study indicates that the location has no impact on the risk of developing osteoarthrosis (Heir et al., 2004). Lesions of more than 1 cm^2 in human knee joints are associated with increased rim stress forces and such lesions may thus be candidates for surgical repair (Guettler et al., 2004).

Different animal models are described; however, today the rabbit model has provided most of the experimental data on the methods that are used clinically as chondrocyte transplantation, hyaluron polymers, CPM, periosteum transplantation, and also the recent use of perichondrium (Amiel et al., 1988,Brittberg et al., 1996,Grigolo et al., 2001,Poussa et al., 1981,Salter et al., 1975,Solchaga et al., 2000). A horse model has been used to validate the

use of microfracture and mosaic arthroplasty techniques (Frisbie et al., 1999,Hangody and Fules, 2003). A dog model has also been introduced in mosaic arthroplasty, and this model shows the same advantage as the horse model in that a rehabilitation program can be performed (Hangody et al., 1997). Although studies on rats, sheep, goats, and pigs have not provided data for the methods in clinical use, there are well-performed experimental studies concerning cartilage repair and function that may have considerable impact on further treatment modalities

Usefulness and drawbacks of experimental models in cartilage repair

First, the usefulness of experimental models in cartilage repair is widely debated, partly due to the interspecies variations in the cartilage structures as outlined above and as illustrated in Table 2. Second, the loading pattern in an animal that uses four legs to move is clearly different compared with the human way of movement when walking or running. Moreover, there are differences in bone morphology, especially regarding the subchondral bone structure. Third, the ability to control the rehabilitation of the experimental animals is another challenging issue in use of animal models.

Why use experimental models in cartilage repair ?

Assessment of the cartilage repair

In animal models the whole joint or condyle can be evaluated thoroughly by histology in contrast to the clinical studies where only a small biopsy can be retrieved. As all other parameters can be kept constant, except the treatment of the chosen type of defect, the repair tissue obtained can be more directly linked to the treatment.

Safety

The promising future techniques would include scaffolds, in combination with cultured or modified cells, probably in combination with growth factors. In order to avoid safety problems linked to these methods, it is important to do a true evaluation on an animal model to lower the risk before this is included in the clinical practice. The use of differentiation and growth factors also implies the possibility of growth regulation disturbances of repair tissue formed.

Proof of principle of healing

No methods today have shown that they are able to rebuild the cartilage structure after an injury, neither in animals nor in humans. Despite the differences of the articular cartilage in the different species, the general anatomical structure is quite similar (Stockwell, 1971). If in an experimental study it would be possible to recreate the general structure of normal adult articular cartilage, the approach would definitely have a great potential to work also in humans. It currently seems that we are some steps away from this proof of healing principle study, and it seems reasonable to take these steps in an animal model.

In conclusion, despite their drawbacks, animal models have been of great value in the assessment of benefits and potential problems in existing cartilage repair procedures

Review of the literature of clinical trials on cartilage repair

The natural history of full thickness cartilage lesions of the knee in humans is currently unknown. Obviously this makes the evaluation of clinical trials difficult. The confusing results from the experimental models are often overlooked due to the fact that the clinical studies, or series of cases, show symptom relief in most of the existing methods (fig. 8).

Probably the best natural history study to date is a 14 year follow-up study on 28 young athletes with isolated cartilage defects of more than 1.3 cm^2 (graded as Outerbridge grade III or IV) (Messner and Maletius, 1996). Ten patients received no treatment, three patients received Pridie drilling, and the rest underwent shaving or removal of small free bodies. At follow-up, 22 patients had a Lysholm score of more than 84 points, and 21 patients had been able to resume previous sports activities. Radiographic examination revealed signs of arthrosis in the injured knee of 16 patients, with a higher incidence of degenerative changes in patients over 30 years of age. Even though this study gives some indication on the natural course, it is important to remember that most of the lesions were Outerbrigde grade 3 (comparable to ICRS grade 2-3). When it comes to papers evaluating the results after surgical cartilage repair, the symptom relief is most pronounced in studies reporting small series of cases and less in comparative studies between different treatment options, or when larger numbers of patients are involved (fig. 8).

Until January 1, 2005, 3,978 patients subjected to surgical repair procedures have been reported upon (Jakobsen et al., 2005), but only four clinical studies exist that compare the cartilage repair procedure conducted against another treatment procedure (Bentley et al., 2003,Bouwmeester et al., 2002,Horas et al., 2003,Knutsen et al., 2004). The study by Jakobsen et al (2005) also found that in the 3,978 patients reported, some patients had been reported twice. The best methodological study to date is the one by Knutsen et al (2004), which evaluates microfracture against autologous chondrocyte implantation. After a two year period, no major differences between the groups were detected. In two other studies, mosaic arthroplasty was compared with autologous chondrocyte implantation. One of the studies concluded that autologous chondrocyte implantation is superior (Bentley et al., 2003), while the other study favored mosaicarthroplasty (Horas et al., 2003). Later, however, both of these studies were criticized due to serious methodological flaws that rendered them not very useful in evaluating the possible superiority of one technique over the other (LaPrade, 2003,Smith et al., 2003). A retrospective study with a small number of patients (n=26) comparing subchondral drilling and autogenous perichondral grafting, concluded that patients in both groups showed symptom relief, but no significant difference between the two groups was detected ten years later (Bouwmeester et al., 2002). The previous mentioned systematic review on the published clinical trials, including 61 studies with 3,987 patients treated surgically (Jakobsen et al., 2005), concluded that the methodological quality of the studies was generally so low that no recommendations could be given on which cartilage repair procedure to choose.

Methods for evaluating cartilage repair

Radiology

Conventional radiography is useful for evaluating osteochondritis dissecans fragment incorporation, but does not visualize changes in the articular cartilage structure before degenerative changes occur. A notch or tunnel posteroanterior radiographic view is considered the best way to visualize a osteochondritis dissecans lesion (Milgram, 1978).

However, all clinical trials should include a standard weight-bearing X-ray that demonstrates mechanical axes and assesses for established degenerative changes (Ahlback, 1968).

Although MRI has been used as an evaluation method for the presence of repair tissue in the defect (Marlovits et al., 2004) the usefulness of MRI for evaluating cartilage defects is questionable (Burstein and Gray, 2003). There are several methodological issues that must be solved before MRI can be used to assess the quality of the repair cartilage. These include volume averaging of the cartilage and other tissues at the interface, the variable orientation of the cartilage relative to the magnetic field (because of joint curvature or differences in positioning), and the need to ensure full contrast agent penetration. Additionally, there is no general acceptance on a standardized magnetic resonance imaging classification system to use to evaluate cartilage lesions (Brittberg and Winalski, 2003).

Biomechanical methods

Different probes have been proposed and used to evaluate the biomechanical properties of articular cartilage. Artscan ® is probably the one that has been used most (Lyyra, 1997,Lyyra et al., 1999b,Lyyra et al., 1999a). The method is, however, linked with several practical problems since it needs one extra person in the operating theater and it is easy to induce errors during the measurements. In addition, no reference material exists that could be used to sort out the normal variation in biomechanical parameters at different sites in the knee joint related to the age of the patient. Even though biomechanical parameters appear to be relevant, they have not been used much in clinical studies on cartilage defects (Peterson et al., 2002,Vasara et al., 2005).

Biochemical methods

Articular cartilage degeneration reflected by the appearance of tissue components in the joint can be assessed by taking samples of the synovial fluid and analyze these for levels of markers indicating loss of cartilage components. Such markers include COMP (cartilage oligomeric protein) or keratin sulphate and various proteoglycans (Carlson et al., 2002,Heinegard and Oldberg, 1989,Messner et al., 1993b,Messner et al., 1993a). The level of interleukin 6 in the synovial fluid is also linked to a degenerative processes of the articular cartilage (Kaneko et al., 2000). These types of parameters represent indirect measurements of the loss of components or the ongoing processes in the articular cartilage. A more direct measurement would be to analyze the different components of the articular cartilage in a biopsy by e.g. Western Blot or, even more precisely by mass spectrometry, to quantify the collagen type II content and other important extracellular matrix components such as aggrecan and glycoproteins.

Histology

The information extracted from the histology sections is dependant on sampling and handling (fixation, embedding section, staining, and microscopy) variables of the biopsies. The first step is to obtain an adequate fixation of the tissue to prevent later changes of the tissue harvested and prepare for clear and good staining of the tissue. Loss of proteoglycans and cell shrinkage are the most common artifacts observed in cartilage subjected to suboptimal fixation (Engfeldt et al., 1994). The most used fixatives in preparing tissue for light microscopy include formalin, formaldehyde, paraformaldehyde, and ethanol-formaldehyde, as they allow adequate results for an all over examination of the tissue and provide good procedures in the subsequent steps of the preparation sequence. The preparation

of the specimens needs to be performed in a standardized manner. Staining is needed to allow distinction between the different components of the tissue. Hematoxylin and eosin is one of the most commonly used staining combinations. It works well with cartilage by staining the cell nucleus blue and the collagen pink and shows general tissue morphology clearly. Sirius Red F3BA is used for selective detection of collagens and the stain varies between yellow and orange red. The use of polarized light has been reported to help distinguish between collagen types I, II, and III (Nielsen 1998). Furthermore, immunohistochemistry has been introduced to detect the presence of different collagen types. However, the reproducible quantification of changes in distribution of the collagen in the tissue by light microscopy is difficult with such techniques.

To evaluate the extracellular matrix other staining techniques have claimed to be useful. These include Safranin O, which often has been used for an estimation of the proteoglycan level or an indication of GAG depletion (Rosenberg et al). However, the use of this method to quantify the proteoglycan content of cartilage is questionable (Hyllested et al., 2002). Another stain for the extracellular matrix is toluidin blue, which may be used in paraffine and resin embedded sections. In older studies the stoichiometric staining characteristics of toulidine blue in detection of cartilage GAG has been compared to those of Safranin O, and reported to be inferior (Rosenberg 1971 & Poole 1970). Another possible staining for the evaluation of proteoglycans is alcian blue 8GX, but this is considered to be very troublesome in use (Hyllested et al., 2002). In conclusion, histochemistry is well suited for overview staining and as a measure of large scale variations in tissue structure, but the method is at best semi quantitative.

Knee function score

A mixture of different function scores exists and many of them are used in the clinical trials previously reviewed. A recent review noted 27 different scores have been used in follow-up studies on cartilage defects (Jakobsen et al., 2005). The KOOS-score has been evaluated to be a useful tool for estimation of symptoms of degenerative changes and the Lysholm score has also been evaluated according to its usefulness in cartilage injuries (Brittberg and Winalski, 2003,Kocher et al., 2004,Roos et al., 1998). Concerning other scores, including the ICRS-score, the documentation of their usefulness is not known (Brittberg and Winalski, 2003).

The current thesis

Background

Previous studies on treatment of focal cartilage injuries do not provide convincing results in respect to the ideal rehabilitation or the restoration of functional articular cartilage, neither experimentally or clinically. Clearly, as outlined above, a lack of knowledge concerning the number of patients that could benefit from this surgery, how regulation of matrix production of the chondrocytes occurs, and which factors are primary in the cartilage defect exist. Only through increased knowledge regarding these issues will a progress concerning the best treatment options for the individual patient be made. Only a better understanding of the influence of different biological factors of the articular cartilage defect may provide such answers. Most experimental studies do try to solve the issue of the repair of cartilage injuries, ignoring the fact that we do not know the effect of breakage of the subchondral bone plate in the defect. As long as we do not know which number of patients

will benefit from cartilage surgery or the natural history of these defects it hard to evaluate the clinical trials of this issue.

The ideal rehabilitation would definitely stimulate the repair cells in the defect to produce a significant amount of extracellular matrix. As long as we do not know the stimuli needed to obtain this, the ideal rehabilitation program would just be based on personal experience with this, as illustrated by the different rehabilitation programs used in the clinical studies published (Alfredson and Lorentzon, 1999,Bentley et al., 2003,Knutsen et al., 2004). As evidenced from this review, there has been a need for a systematical review of these factors since the introduction of the more biological cartilage repair with autologous chondrocyte transplantation (Brittberg et al., 1994,Grande et al., 1989). The concept of understanding some of the current problems in cartilage surgery provides the background for the current thesis.

Aims of the thesis

The primary aim of this thesis was to increase knowledge relevant to the treatment of focal cartilage defects and to investigate the primary processes in the cartilage defect of the knee. The studies were performed under the belief that only a better understanding of the incidence characteristics of these lesions and the biology in the defect can help us to improve the current treatment alternatives.

A particular aim was to systematically take advantage of one of the most used experimental models in order to challenge some of the axioms or unproven statements in cartilage research.

Specifically, the following statements in the literature were tested:

- **Epidemiology**
  - Chondral or osteochondral injuries are common musculoskeletal sequela of acute or chronic trauma. (Peterson et al., 2000)
  - Damage to articular cartilage is a common problem and it is associated with 16% of knee injuries (O'Driscoll, 1998).
- **Rehabilitation**
  - Full weight bearing 24 hours postoperatively would act as stimulus of the articular cartilage (Bentley et al., 2003)
- **Treatment**
  - Sutured periosteum graft would immediately detach from the cartilage defect if free motion was allowed (Driesang and Hunziker, 2000)
  - Penetration of the subchondral bone plate to access the bone marrow would only result in a transient increased thickness of the subchondral bone plate (Frisbie et al., 1999)
  - Penetration of the subchondral bone allows mesenchymal stem cells to reach the articular cartilage defect and produce a healing response. (Grana 2000,Gill, 2000)
  - Periosteum, in combination with penetration of the subchondral bone plate, is able to restore a functional repair tissue in a cartilage defect (Alfredson and Lorentzon, 1999,Carranza-Bencano et al., 2000,Siebold et al., 2003).

Four sub studies were performed to address the following questions:

- **How large is the problem associated with cartilage defects of the knee?** –A clinically demographic description study of the incidence of cartilage lesions of the knee and the associated knee injuries of knees subjected to knee arthroscopy.
- **How does subambient hydrostatic pressure affect chondrocyte metabolism?** –One experimental chondrocyte culture study was performed to investigate the effect of hydrostatic pressure changes on chondrocyte-cultures in order to look at the beneficial effects of rehabilitation protocols used after cell-therapies in cartilage repair.
- **Do sutured periosteal flaps fall off immediately, or do periosteum take part in the repair process of articular cartilage?** –One experimental animal study was designed to assess the survival of periosteal flaps used in cell-therapies in cartilage repair using restricted motion or free motion of the knee joint
- **Do blood elements and the access to bone marrow elements in the articular cartilage defect induce a reparative response that can provide filling of the defect?** –One experimental animal study was performed to elucidate the difference of access to bone marrow elements, not in the defect, when the defect was covered by a rim sutured periosteal flap to create a biological chamber in the defect.

Material and Methods

Collection of epidemiological data (paper I)

Study I was performed as a systematical registration of all knee arthroscopy performed at three hospitals in the Oslo area, all of which had a considerable amount of arthroscopic surgery. Currently available ICRS registration forms (1997) were used, containing both a patient part related to symptoms and quality of life and an assessment form marked by the orthopedic surgeon, including location, depth, and area of the cartilage defect. During the six months of the data collection a total of 1,005 knee arthroscopies were performed at the three hospitals, and 993 of these were included in the study.

Chondrocyte culturing and changes of hydrostatic pressure (paper II)

Bovine metatarsophalengeal joints were dissected and the chondrocytes harvested to culture in chondrocyte cultures. A custom designed sub-ambient pressure generator was used to apply periods of intermittent sub-ambient hydrostatic pressure to bovine chondrocyte monolayer cultures. After this pressuration, the chondrocyte cultures were analyzed for the response concerning biosynthesis of proteoglycan and type II collagen, using Northern Blot analyses for aggrecan and type II collagen. Scintillation analyses of radioactive sulfate and proline were performed to measure the effect on synthesis of proteoglycan and collagenous protein molecules

Animals (paper III &IV)

In papers III and IV New Zealand rabbits were used. A rabbit model was chosen due to the large numbers of previous reports, as illustrated in Figure 6. In paper III we mostly used black New Zealand rabbits. The availability of such rabbits at the proper age was, however, limited, and we changed to white New Zealand rabbits during paper IV. There is no data available indicating any relevant difference between white and black New Zealand rabbits regarding the issues under study. The 18 animals used in paper III were also included in paper IV, but were then analyzed for a different hypothesis.

The rabbits used in the current two studies were subjected to repair surgery at ages 24 weeks (6 months) and had a mean weight of 3.6 kilograms, which means that these rabbits were considered adults.

Surgery (paper III&IV)

A defect (φ = 4 mm) was created in the patella of both knees in the 34 experimental rabbits. The area selected for the defect was at the center of each patella and chosen for two reasons: first, the patella has the thickest articular cartilage in the rabbit joint; second, the patella had been used in the pre-experimental work with the autologous chondrocyte implantation technique. A defect of φ = 4 mm in the patella accounts for 30 % of the total articular cartilage area of the rabbit patella.

The surgery was performed under anesthesia composed of a mixture of Hypnorm® and Dormicum® with dosages adjusted according to the weight of the animal. The lower limbs were shaved, cleaned with Hibiscrub® 5%, and covered with a sterile draping using Adhesive spray® and Klinedrape® at each surgical procedure. Before each surgical intervention 2 ml of NaCl 0.9 % was injected in the joint and after the joint had been subjected to 50 repeated full range of motions one aliquot of this washout sample of minimum 0.75 ml of the synovial fluid were retrieved from the joint for analysis of proteoglycan levels in the synovial fluid.

In addition, 1.5 ml of local anesthesia (Marcain®) with epinephrine was injected locally at the wound edges at the start of surgery. An antibiotic (Vibramycin®) was given orally as prophylaxis pre- and post-operatively for five days, together with Temgesic® subcutaneously as pain relief. Medial parapatellar incisions were made in the skin and a capsular incision performed just medial to the patella, taking care to leave a small cuff of fascia attached to the patella to allow for a strong capsular closure. The patella was dislocated laterally and inverted. A biopsy punch (φ = 4 mm) was used to create the cartilage lesion. Three experienced surgeons, specially trained in the procedure of harvesting periosteum and performing cartilage repair procedures in rabbits, performed all of the surgery. Dental instruments and a stereo microscope were used to secure removal of all the cartilage in the defect down to the tidemark. Care was taken to avoid any damage of the subchondral bone plate. The wound was closed with 4-0 white Dexon ® (Sherwood Davis & Geck) with abrupted sutures in the fascia and non-abrupted intracutaneous suture, and finally sealed with Op-Site ® (Smith & Nephew) spray. Two weeks later the defects were repaired during rearthrotomy.

Embedding and section for histology (paper III&IV)

The patella specimens were fixed immediately after sacrifice in 4% paraformaldehyde buffered by phosphate at pH = 7.42 for 48 hours, further decalcified in 7% EDTA with 0,5% paraformaldehyde in a 0,1 M phosphate buffer. After decalcification the patella specimens were sectioned in half through the center of the defect. From each of these two halves five sections of 4-5 um were made and the four sections that gave the best overview over the defect from each sample were subjected to the further measurement.

Morphology (paper III&IV)

Both macroscopic and histological evaluations were done by the first author (A.Å). In both experimental studies signs of macroscopic degeneration of the articular cartilage were looked for and noted if present. Further in study III the attachment of the periosteal flap was noted, and if loosening had occurred the remnants of this were looked for through the joint recesses.

Morphometry (paper III&IV)

In study IV, measurements of several parameters, including cartilage thickness in the defect and at the edges, binding of the repair tissue to the subchondral bone plate, thickness of subchondral bone, and the filling of the defect were carried out by the first author (A.Å). Filling of the defect and binding of repair tissue was performed by point counting according to the stereological guidelines described by Romppanen and Collan in 1983. Printouts of each of the four best sections of the defect from each sample were used, and the median value of these four measurements was used for further statistical analysis. Thickness measurements in paper III and IV of periosteal flap, cartilage, subchondral bone, and repair tissue was performed with a semiautomatic interactive image analysis system (Analyses Soft Imaging System, Münster, Germany). The median value of five measurements in each section was used for further analyses.

Synovial fluid analyses (paper IV)

This was only performed in paper IV as an indicator of tissue degeneration involving loss of proteoglycans to the synovial fluid. In the long-term observation group of 36 weeks, and in the sham-operated knees of the control-sham group, a washout sample of the synovial fluid was aspirated before each surgical procedure as described above. A sample of minimum 0.75 ml was then aspirated from the joint and the surgical procedure or sacrifice was performed according to the time point in the experimental protocol. Synovial fluid was analyzed for proteoglycan concentration using standard ELISA techniques (Messner et al., 1993a).

Statistics (papers I, II, III, & IV)

Study I contains a demographic description of the incidence of cartilage injuries in the knee and associated lesions. Since the primary aim was to answer the frequency of these lesions, no further statistical analyses were used in this paper. In papers II and III, ANOVA analyses were performed to determine the statistical significance between various groups. This statistical analysis is a standard method to analyze the differences between groups in experimental studies. A standard t-test was used in papers III and IV according to whether the setting was paired. Most settings were paired since bilateral surgery had been performed.

Pre-experimental analysis, using a power of 0.80 and a significance level of 0.05 to detect sample size, indicated a need of nine experimental animals in the experimental group. This was based on previous experimental studies that a filling percent difference of more than 25% was considered as a proper level. The highest standard deviation observed in the experimental group in respect of filling of the defect was 0.24.

Study III contains 18 experimental animals and study IV contains 16 experimental animals in the long observation group. The long observation period in the studies, the time consuming experimental surgery, and the risk of losing experimental animals were the reasons for the decision to use more than nine experimental rabbits in these two studies.

Methodology considerations

Data collection methods (paper I)

Our ability to adequately grade the articular cartilage lesions arthroscopically has been questioned by several authors. In this study we use the ICRS-classification system, which both describes the location and the square area of the lesion. However, the magnifying feature of the arthroscope could easily lead to an overestimation of the lesion (Oakley et al., 2002,Oakley et al., 2003). The amount of experience of the orthopedic surgeons has been considered important for the ability to classify the area and depth of the cartilage injury, but in a study by Cameron et al (2003) this notion could not be verified.

Other authors have reported overestimation and underestimation of the size of the cartilage lesion area classified arthroscopically of about 50% regarding the lesion area (Schafer et al., 2003). An ordinary arthroscopic probe was used in the current study (Aroen et al., 2004) is considered to give a reasonable estimate of the area of the cartilage injury of knee (Cameron et al., 2003). It has been documented that MRI is not useful for classifying the area of these lesions (Irie et al., 2000).

Chondrocyte cultures (paper II)

In study II, chondrocyte cultures from bovines were used to study the effects of mechanical stimulation of cartilage. The use of cell cultures to estimate the effects of mechanical stimuli of chondrocytes could be in question due to the differentiation that occurred during culturing. However, a previous study has demonstrated that these chondrocytes are able to regain their phenotype (Lemare et al., 1998) and the effects demonstrated in the study (an increase of proteoglycan production and the presence of collagen type II biosynthesis) are further evidence of this model that can be used to estimate chondrocyte metabolism. The use of chondrocyte cultures in several other studies investigating the chondrocyte metabolism (Waldman et al., 2003a,Jin et al., 2000,Carver and Heath, 1999) demonstrates that scientific acceptance of this model due to difficulties to estimate these effects in “in vivo” studies.

The cell passages, though, have to be kept low like in the current studies because if more than five numbers of passages are performed, the chondrocyte phenotype will be reduced in the cell cultures (Schulze-Tanzil et al., 2002).

Animals (papers III & IV)

An important issue is the closure of the epiphyseal growth plates using animals in cartilage research. It is well known that young or adolescent rabbits are associated with a much better repair result than adult rabbits. It is known that rabbits reach skeletal maturity, which includes closed epihysial growth plates, at the age of six months and weights at just below four kilograms (O'Driscoll et al., 2001). Some authors claim that this occurs with weights of more than 3.2 kg (Messner et al., 1993b).

Another well-known issue in evaluation of the experimental results in cartilage repair is the interindividual variance in the healing response of the experimental animals. This was handled by the use of bilateral surgery so that this variance did not influence the comparison of the experimental groups.

Surgery (papers III & IV)

After the pilot experiments we decided that a surgical microscope was essential in order to create a way of handling and suturing the periosteal flap to these cartilage lesions with diameter of 4 mm. It was also important for the complete removal of the cartilage in the defect; the experimental setup using an operating microscope is probably one of the advantages of studies III and IV in comparison to other similar studies. Minimal vascular microenvironment was defined as a chondral lesion down to the subchondral bone. The cover of the defect with a periosteal graft would further reduce the influence of blood elements released into the joint immediately after surgery. This biological chamber constituted the minimal vascular microenvironment, and four drill holes at the base of the defect supplied bone marrow elements from the base of the defect into this chamber.

Embedding and section for histology (papers III & IV)

The preparation of the histological slides of cartilage-bone interface specimens is a well known challenge for scientists involved in this area, as outlined in the *Handbook of Histology Methods for Bone and Cartilage* by Yuehuei & Martin. The hardness of the subchondral bone of rabbit patella demands decalcification of these specimens for long periods of time. Still, the difference between cartilage tissue and bone makes the section difficult and often results in cracks or errors in slides. This was the reason for using the method of the four best sections of each specimen and calculating the median values of these four to obtain the best overview of the factors influencing the repair process in the defect. Histology from the pilot experiments demonstrated bone marrow in the center of patella occupying about one half of the section, and that the penetration of the subchondral bone plate was sufficient to obtain passage to the bone marrow elements.

Morphometry (paper III&IV)

The published histological scoring systems are mostly related to osteoarthrosis and nearly normal cartilage (Hyllested et al., 2002). In our studies, this grading was considered to be too subjective in classification of the repair tissue obtained. Recent literature also recommends a more straightforward measurement of tissue quality parameters and clinical parameters related to experimental knee joints in order to make a comparison and evaluation of the different experimental papers. A sophisticated scoring system may obscure the findings, and they are, after all, only capable of distinguishing between normal and severe degenerative changes (Hyllested et al., 2002).

Synovial fluid analyses

The synovial fluid analyses were performed as estimates of the release of proteoglycans from cartilage indicative of degenerative changes. Other authors have also used this method (Messner et al., 1993a). However, it is found in other studies that this method, using a washout sample of the joint, is subjected to a considerable variation in the measured values (Dahlberg et al.,1994, Kuhne et al.,1998). As no major changes indicative of osteoarthrosis were observed, it is not surprising that this analysis did not detect any significant changes of the concentration of the proteoglycans. Another issue is that variations

of the amount of synovial fluid in the joint would have minor effects on the washout sample retrieved from the joint.

Statistics

The bilateral surgery reduces the impact of interindividual variance seen both in clinical and in experimental cases. A paired situation where each animal serves as its own control will make comparison of differences created by the surgical differences much easier, as the interindividual variance is avoided. Differences between the surgeons' operating techniques were also reduced as all surgeons did the same number of operations with the same technique. When different animals were compared, the unpaired student T-tests were used, which also is a standard statistical test.

Main results and clinical interpretation of the papers included in the thesis

Paper I

This demographic study that describes the number of patients that would be candidates for cartilage repair surgery provided an available procedure for restoration of full articular cartilage tissue existed. It also shows that anterior cruciate ligament injuries are frequently associated with focal cartilage lesions in 18% of fresh injuries and in 29% of old anterior cruciate ligament ruptures. Knees diagnosed with meniscus injuries were accompanied by cartilage injury in 13% of the cases in this study. Patella subluxation was associated with 57% of cartilage injuries as well. In conclusion, 11% of all knees subjected to a knee arthroscopy show a localized cartilage lesion that may be suitable for cartilage repair surgery, and half of these knees display an area of cartilage injury of more than 2 cm^2.

Paper II

This experimental study used cell-cultures of bovine chondrocytes, which were subjected to intermittent subambient interstitial hydrostatic pressure by a custom-designed subambient pressure generator. The cell cultures subjected to this intermittent subambient hydrostatic pressure demonstrated increased production of proteoglycan (40%) and non-collagenous protein (17%) during the pressurization period. An increase in mRNA for aggrecan was also observed. The production of collagen type II was not affected by the pressurization period.

The clinical implication of this study was improved production of the proteoglycans needed to rebuild the extracellular matrix observed in chondrocyte cultures, and hopefully the same effect might also have been achieved by a properly designed rehabilitation program in patients with injured and repaired articular cartilage

Paper III

This experimental study analyzes the contribution of the periosteal graft to cover a cartilage defect. Even though the periosteal grafts were sutured as well as glued to the edges of the defect it detached and was displaced after the first week. At sacrifice the graft was found attached to the synovial membrane in several cases. The introduction of drill holes to the bone marrow in the bottom of defects did not affect the retention rate. Free rehabilitation of the experimental rabbits had little impact on the retention rate.

Paper IV

One of the major differences in cartilage repair techniques concerns the microenvironment created in the defect, which can be either vascular or non-vascular according to the operative method chosen. Previous to the current study only cell-culture studies have been conducted to verify the importance of this. In this paper a systematical evaluation of this difference was performed using the same experimental model as in paper III. Although the defects with drill holes in their subchondral bone plates did show a significantly better filling of defects, the repairs created were incomplete in both situations with less than 50% of defects filled. Furthermore, degenerative changes, including erosions and reduced height of the cartilage, were observed at the edges of the defect in both experimental groups. Also interesting was the finding that penetration of the subchondral bone plate was associated with an apparent permanently increased thickness of the subchondral bone, which may have further increased the degeneration of the repair cartilage.

General discussion

The treatment of cartilage injury in patients is difficult, and real therapeutic progress may help high numbers of patients, as illustrated in the current thesis. As outlined previously, several statements or axioms exist in treatment of the cartilage defect of the knee. Currently, the practice of combining cartilage repair with osteotomy has become widely adapted (ICRS-meeting, 2004) and as such, makes the evaluation of the efficiency of these procedures even more difficult. In the current thesis, as in the majority of studies with animal models, the primary outcome variable was histology, whereas in the clinical practice the parameter is pain relief. In addition, the activity level and conditions of loading may differ from animal models to humans. The challenge in this field is illustrated by the observation that neither clinical nor experimental series have provided evidence that normal hyaline articular cartilage can be restored. It is therefore likely that the sometimes confusing results reported in studies on repair of the cartilage defects are due to the studies being founded on shortcomings in the knowledge about injury and injury mechanisms, as well as an incomplete understanding of the crucial factors involved in the repair process. The results from the current thesis added to our understanding of some of the aspects of current surgical procedures and rehabilitation techniques. Independent experimental and prospective clinical trials with cutting-edge techniques should be performed to found the basis of evidence-based procedures with optimal outcomes.

Conclusions and clinical implications of the current thesis

- A considerable number (11%) of all knees subjected to a knee arthroscopy have a full thickness cartilage lesion, and about half of these lesions have a size that may indicate the need for a cartilage repair procedure (paper I).
- Cartilage injury often occurs in association with ACL-injuries (18%) and meniscus injuries (13%) (paper I).
- The proteoglycan secretion of the chondrocytes increased in an experimental study on chondrocyte cultures, where variation in the air pressure was applied (paper II). This might have important clinical implication in the rehabilitation of cartilage injuried patients and in the preparation of cultured chondrocytes matrices also.

- A periosteal flap cover does not seem to stay in place for more than one week in rabbits. Detachment of the flap does not cause formation of a free body. (Paper III).
- The failure of the cartilage repair process causes degenerative changes both in the defect and in the adjacent cartilage, increasing the risk for later development of arthrosis (paper IV).
- Access to bone marrow elements in a cartilage defect covered with a periosteal flap does not induce a sufficient reparative response to fill the defect, and furthermore does not increase differentiation of the repair tissue towards hyaline cartilage (paper IV).
- The use of a technique where the subchondral bone plate is penetrated causes an apparently permanent increase of the subchondral bone plate thickness, which might contribute to the development of degenerative changes at a later stage. In this way the initial repair procedure may reduce the possibility of obtaining complete tissue healing with later cell transplantation procedures if symptom relief is not obtained (paper IV).

Verification of the current statements in the literature that were tested:

- **Epidemiology**
    - Chondral or osteochondral injuries are common musculoskeletal sequele of acute or chronic trauma (Peterson et al., 2000).
    - Damage to articular cartilage is a common problem and was associated with 16% of the knee injuries (O'Driscoll, 1998).
    - **Result concerning these two statements: Partly verified: Articular cartilage damage is common but is often associated with other knee pathology as meniscus injury and anterior cruciate ligament injuries. Although 66% of the knees subjected to a knee arthroscopy contain cartilage pathology, only 4% did receive a cartilage treatment. However, 11% of all knees contain a full thickness lesion that might be eligible for cartilage treatment.**

- **Rehabilitation**
    - Full weight bearing 24 hours postoperatively would act as stimulus of the articular cartilage (Bentley et al., 2003).
    - **Result: Not verified: The current chondrocyte culture study demonstrates that exercises that reduce the hydrostatic pressure in articular cartilage would stimulate the production of proteoglycans, which is a main component of the articular cartilage**

- **Treatment**
    - Sutured periosteum graft would immediately detach from the cartilage defect if free motion is allowed (Driesang and Hunziker, 2000).
    - **Result: Not verified: Used in the most common experimental model, which is a rabbit, will in 80% of the cases still be placed in the defect after one week, but detach after two weeks.**
    - Penetration of the subchondral bone plate to access the bone marrow would only result in a transient increased thickness of the subchondral bone plate (Frisbie et al., 1999).

- **Result: Not verified: Penetration of the subchondral bone plate will permanently increase the thickness of the subchondral bone plate and additional degenerative changes in the adjacent cartilage occurs as a result of an incomplete repair.**
- Penetrations of the subchondral bone allow mesenchymal stem cells to reach the articular cartilage defect and produce a healing response. (Grana 2000,Gill, 2000b)
- **Result: Partly verified: A healing response occurs, but only 50% of the defect is filled and this repair tissue is fibrous tissue.**
- Periosteum, in combination with penetration of the subchondral bone plate, is able to restore a functional repair tissue in a cartilage defect. (Alfredson and Lorentzon, 1999,Carranza-Bencano et al., 2000,Siebold et al., 2003).
- **Result: Not verified: This method, based on the results in experimental studies III and IV, does not have the ability to restore functional articular cartilage**.

Conclusion

According to the four studies included in the current thesis, and the review of the experimental and clinical studies published, it seems clear that are no procedures available that are able to restore normal articular cartilage. However, the currently applied approaches induce a reparative response that provides some relief of the symptoms and indicates that the restoration of normal cartilage may be possible. There is, however, an apparently long way to go. Below are listed some issues that should be explored in order to obtain this normal articular cartilage repair.

Suggestions for future directions

Issue I: What is the natural history of cartilage injury of the knee?

Studies

- Natural history study of cartilage defects of the knee and the long-term prognosis.
- The natural history of combination ACL-rupture and cartilage injury.

Methodology

- Follow-up studies on the patients identified with cartilage injury in study I of this thesis, with radiographic and clinical examination after five years and further tests after ten years.
- Evaluation of the biomechanical values in the area of MRI detected bone bruises, compared to normal areas, in combination with ACL-injuries and ELISA-analyses of the proteoglycan levels in the synovial fluid in relation to the later progression of degenerative changes.

Issue II: What is the effect of a systematic rehabilitation program?

Studies

- The ideal rehabilitation program for cartilage injuries with and without surgical stimulation of resurfacing.

Methodology

- Patients with a cartilage defect of the knee, with symptoms indicating a surgical cartilage repair procedure, should preoperatively be followed with a systematic rehabilitation program to assess if a significant reduction in symptoms can be observed using standardized scoring systems.

Issue III: Is there a need for a stronger fixation of the cell grafts applied to increase the repair potential of the currently used procedures?

Studies
- Experimental studies on methods to increase the attachment of the repair tissue in the cartilage defect.
- Age-related changes in the cambium layer in the human tibiae.

Methodology
- Experimental studies on other methods to fix and increase the adhesion of the periosteum in the defect.
- Histology examination of the presence of the cambium layer in relation to the age in humans, which could be harvested from patients undergoing ACL-reconstruction.

Issue IV: What are the consequences of the degenerative changes observed in the association of a cartilage defect as in study IV, including increased subchondral thickness and chondrocyte loss and erosion at the edges caused by the cartilage injury?

Studies
- A clinical study to quantify the increase in the subchondral bone thickness in patients treated with the microfracture procedure.
- An experimental study to explore if complete filling of the cartilage defect would reverse the degenerative changes and chondrocyte loss observed at the adjacent cartilage.

Methodology
- MRI measurement of the subchondral bone thickness in the defect and the adjacent cartilage in patients treated with the microfracture procedure.
- One biopsy from the defect and the neighboring cartilage harvested under the operation in order to measured the subchondral bone plate thickness.
- An experimental animal study using the same methodology of histology measurement of subchondral bone thickness and cartilage thickness as in this thesis, though assuring complete filling of the defect at repair using either biological tissue or non-biological tissue replacement in the defect, to evaluate if the observed degenerative changes can be reversed or prevented.

Tables

Table 1. Different signaling molecules/growth factors/cytokines of special interest in the formation of the human joint in relation to their specific action to differentiate the proliferating chondrocyte to create a normal human joint. Adapted from "Inborn errors of development" by Epstein et al. Oxford University Press 2004.

Signaling Molecule	Function
FGF 18	Inhibits chondrocyte proliferation, inhibits Ihh expression, promotes osteogensis
Ihh	Promotes chondrocyte proliferation, specifies the location of bone collar formation, induce PTHrP expression in periarticular chondrocytes
PTHrP	Inhibits hypertrophic differentiation of chondrocytes
BMP2	Function unclear, but are expressed in developing joint
BMP6	Function unclear, but are expressed in developing joint
GDF5	Specifies presumptive joint positions, segmentation of mesenchymal condensation
GDF6	Function unclear, but are expressed in developing joint
TGFβ	Inhibits hypertrophic differentiation of chondrocytes, maintains articular cartilage
Wnt4	Accelerates maturation of chondrocytes, accelerate onset of bone collar formation
Wnt14	Induce GDF5 expression

Table 2. Some parameters related to the knee articular cartilage in species often used in cartilage research. The information is collected from the following studies: (Hunziker et al., 2002,Hunziker, 2002,Jackson et al., 2001,Lane et al., 2004,Mcllwraith et al., 2004,Murray et al., 2001,Stockwell, 1971,Brismar et al., 2003,Cake et al., 2004).

Species	Cartilage (mm)	Subchondral bone (mm)	Number of cells (mm^3)	Expected lifetime	Cartilage diseases
Humans	2,0-3,33	0,190	10,000	80 Years	10 %
Rats	0,266-0,43		265,000		Seldom
Rabbits	0,4-1,0	0,5-0,625	22,000	4 Years	Seldom
Dogs	0,21-0,67		44,000	10 Years	Sometimes
Goats	1,1-1,4			8 Years	
Sheep	0,84±0,3	0,4-0,8	53,000	8 years	
Pigs	0,4-0,5		18,000	12 years	9-49%
Monkeys					
Bovine	1,7± 0,1		20,000	15-20 years	Often
Horses	2	2,8-4,2		20 years	Often

Table 3. Results from rabbit studies concerning the healing response in the knee joint obtained in natural history studies and experimental studies. Studies employing osteochondral experimental defects are included since this is the most commonly used experimental model. However, only four studies reported the three parameters of interest (observation time, weight/age and filling of the control defect).

Authors	N_0	Filling of defect	Observation time	Weight or age
(Sellers et al., 1997)	10	Less than 25%	24 weeks	8 months
(Kreder et al., 1994)	7	30%	6 weeks	3,2 kg
(Lietman et al., 2002)	8	85%	18 weeks	3,2 kg
(Qiu et al., 2003)	7	100%	16 weeks	5 months

Figures

Figure 1. Electron micrograph of cartilage chondrocyte from rat epiphysis illustrating the well developed machinery for synthesis of proteins for secretion.

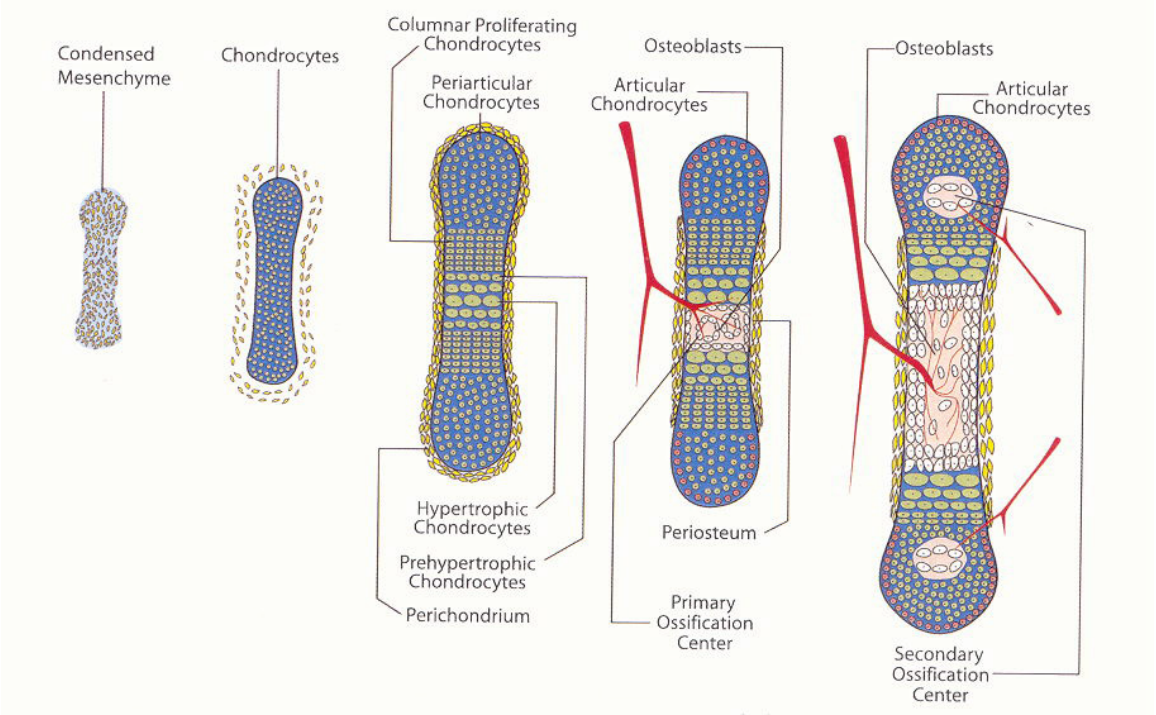
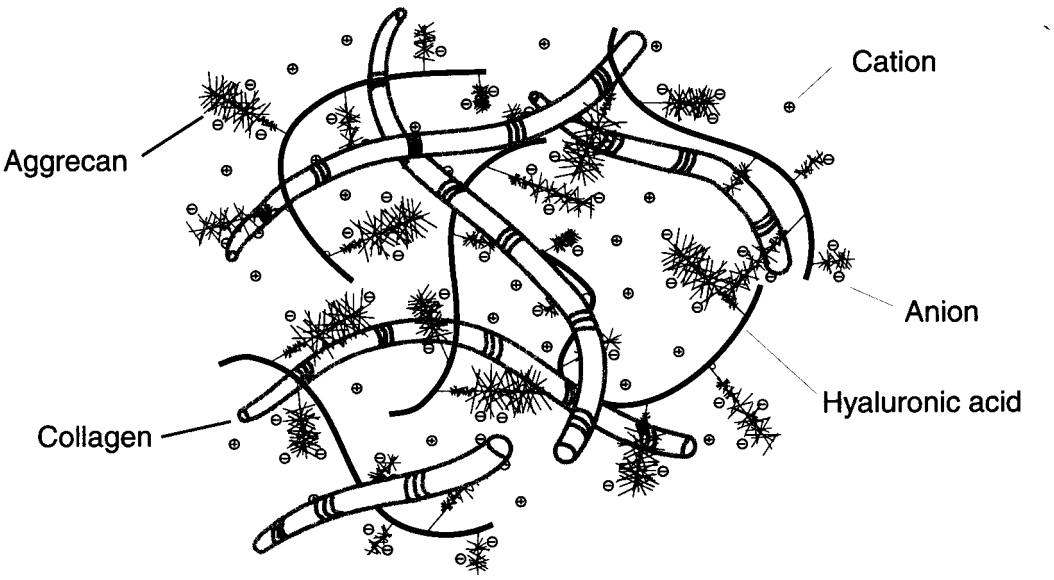


Figure 2. A schematic drawing illustrating the differentiation development from embryological condensed mesenchyme to a human weight-bearing bone. Adapted from "Inborn errors of development" by Epstein et al. Oxford University Press 2004.



The anions on the proteoglycans of aggrecan attract cations. This causes the ion concentration within the tissue to be greater than that in the surrounding fluid, causing an osmotic pressure difference. Fluid is thus imbibed into cartilage, placing the collagen fibrils in tension.

Figure 3. An illustration of the collagen and aggrecan organization in articular cartilage from the *Handbook of Histology Methods for Bone and Cartilage* by Yuehuei & Martin, Human Press 2003,USA.

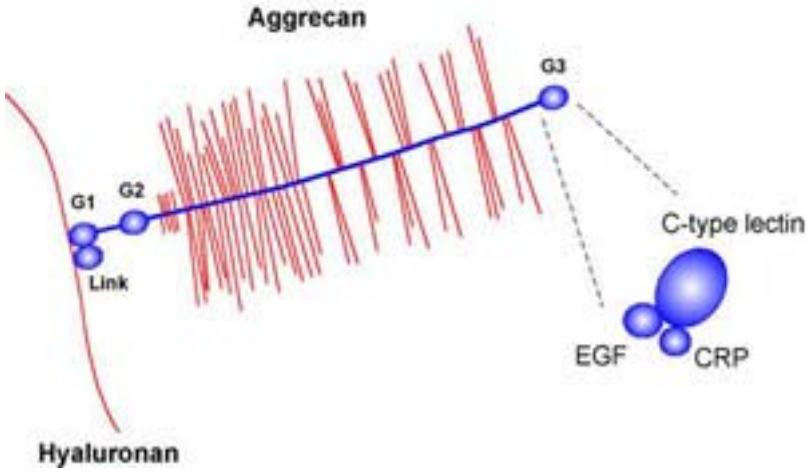


Figure 4. Illustration of the proteoglycan aggregates in articular cartilage. The aggregates consist of central hyaluron filaments and multiple attached aggrecan monomers. Aggregates form older individuals have shorter hyaluron filaments and fewer monomers attached per unit length (Buckwalter and Mankin, 1997). Recent years have revealed more exact knowledge of protein core domains of G1 (hyaluronan binding region), G2, keratan sulfate rich region, chondroitin sulfate rich domain and its C-terminal G3, C-type lectin domain. Identified functional properties of the domains include collagen binding (KS-rich domain), binding of matrilin 1, tight interactions with fibulins and tenascin (G3 domain) and interactions with the hyaluronan binding domain (G1 domain)(Hedlund et al., 1999b,Heinegard and Oldberg, 1989).

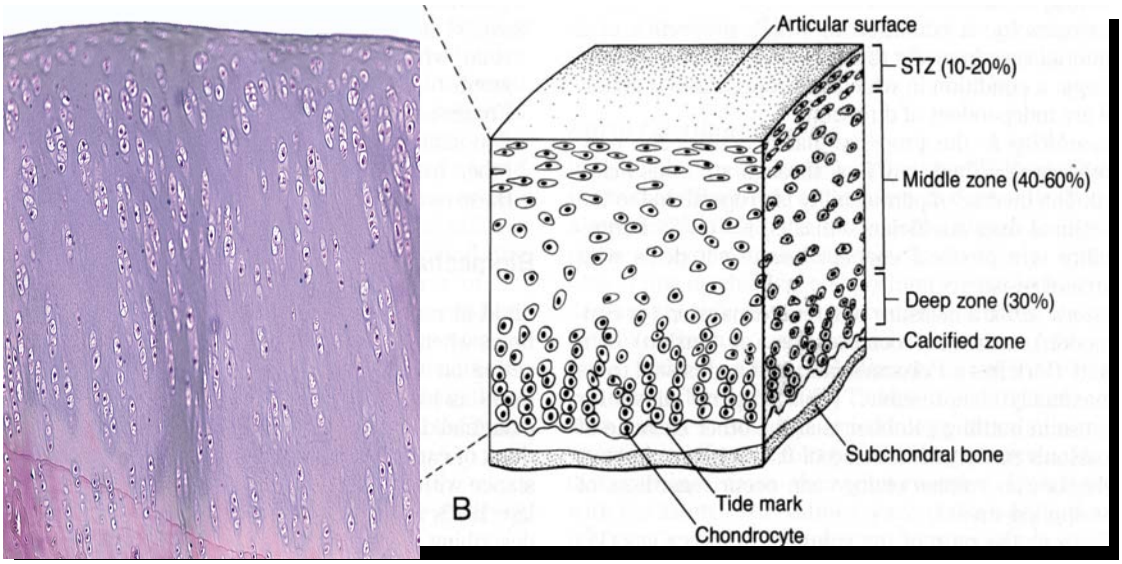


Figure 5. Micrograph and cartoon illustrating the four zones of articular cartilage.

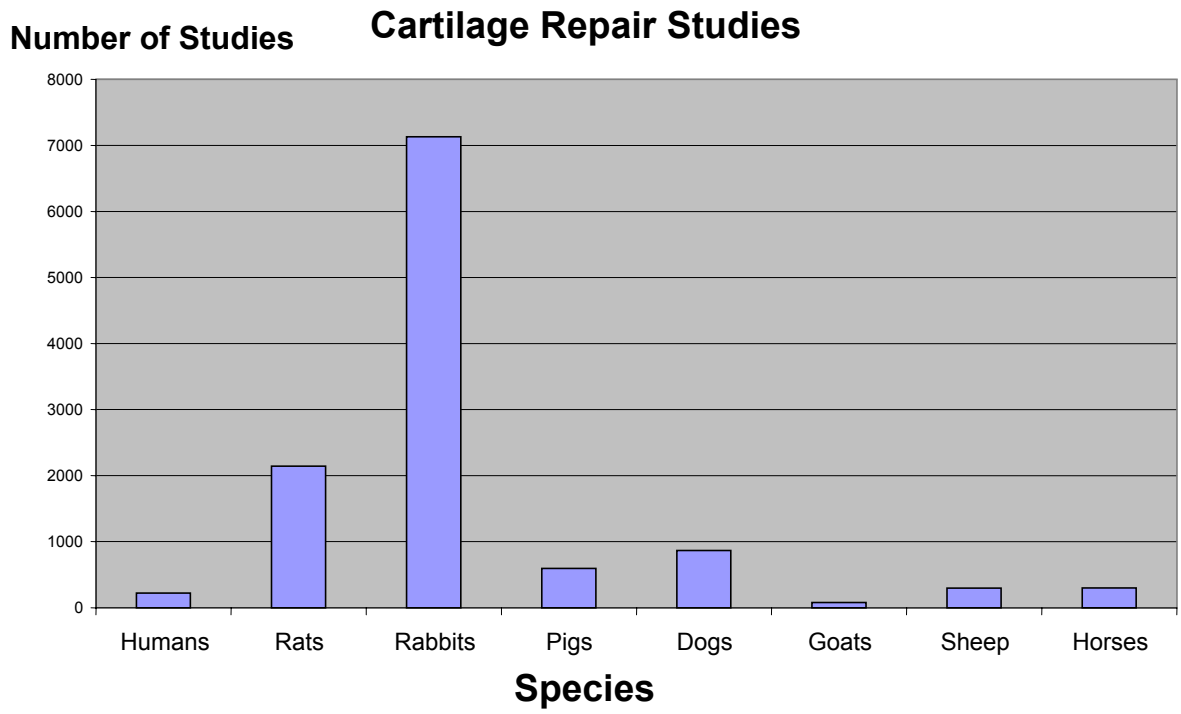


Figure 6. Number of cartilage repair studies in the different species available in the Medline search of December 2003.

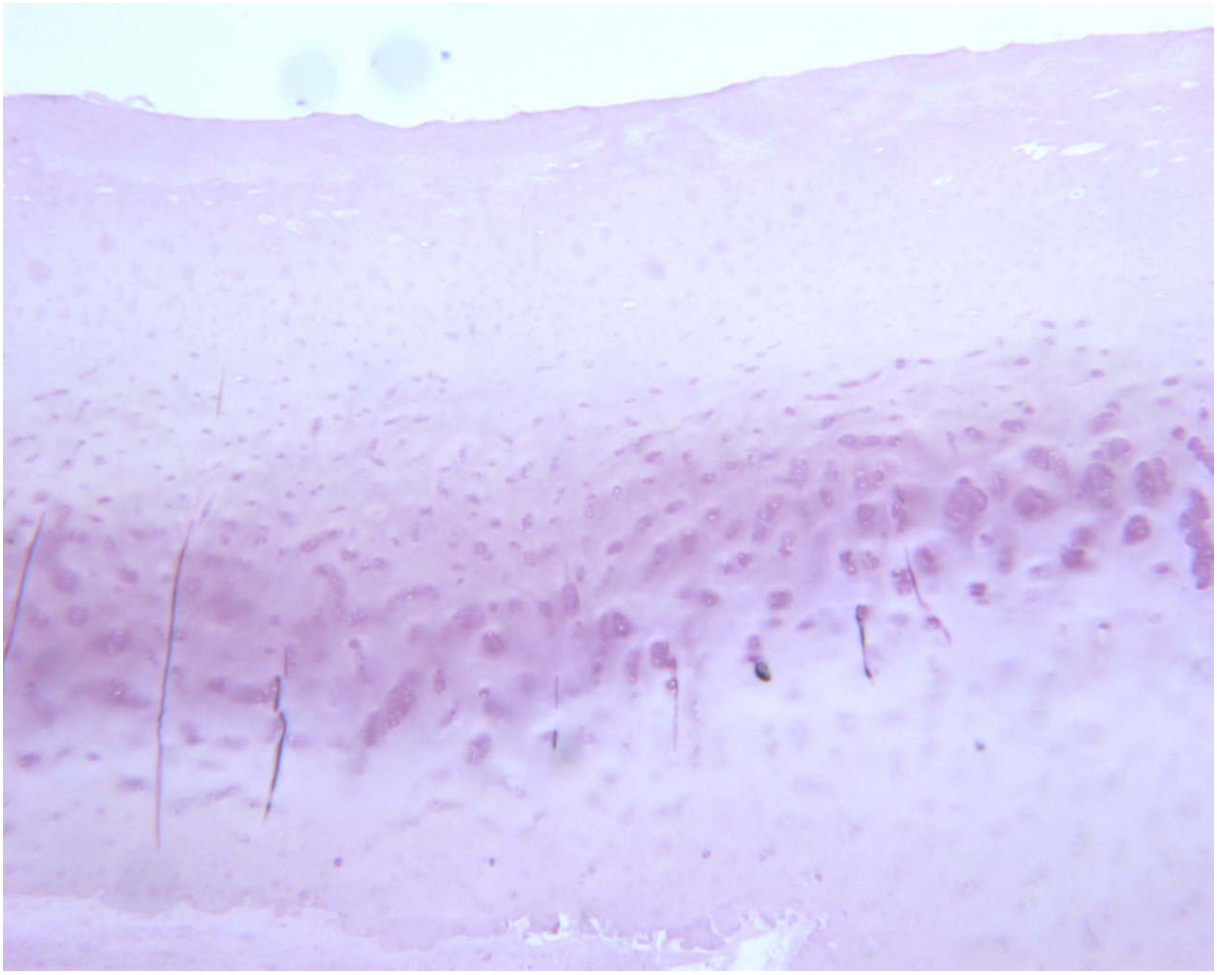
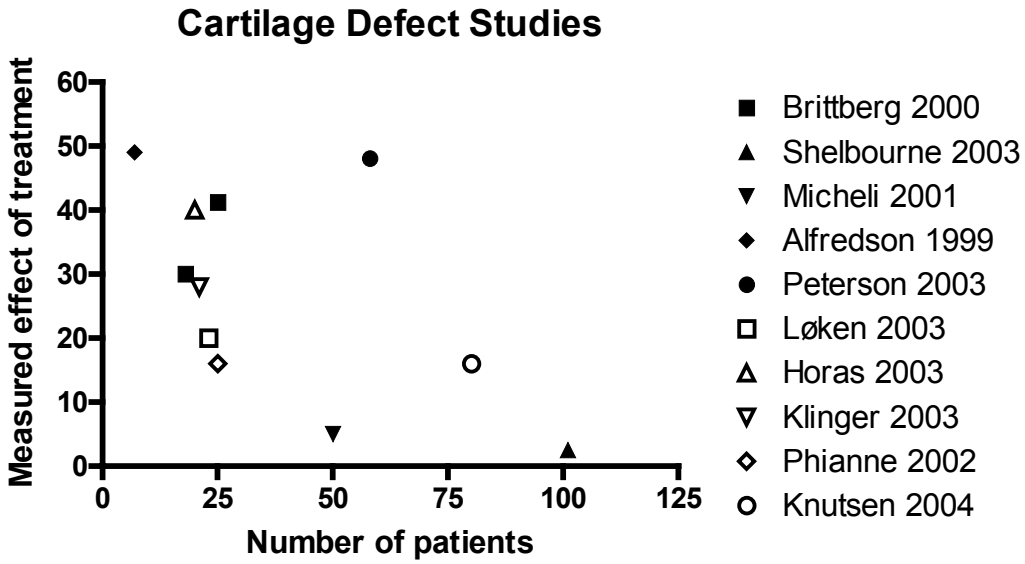


Figure 7. Histology from a loose fragment removed from the knee joint in a 38 year old women with osteochondritis dissecans. This fragment, which has been unstable for several months/years, is covered by fibrocartilage in superficial parts where large portions of the chondrocytes are necrotic. This illustrates the fact that even when it may look macroscopically normal, these fragments may already have ultrastructural changes that make a successful fixation and incorporation of a loose fragment difficult (Guthrie et al., 1992).



.

Figure 8. An overview of the effect in percent improvement on the knee score used in the actual study related to number of patients in clinical studies

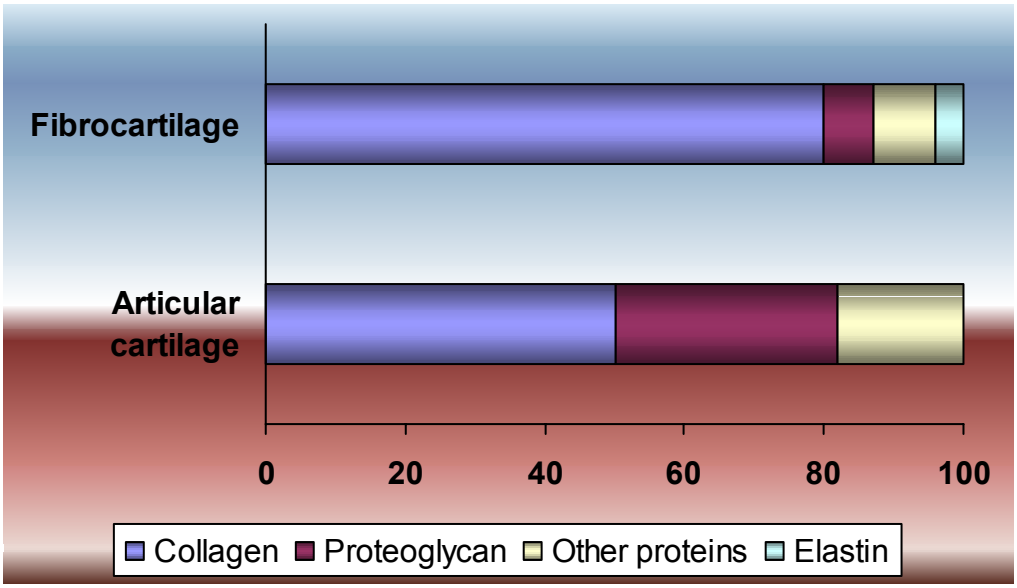


Figure 9. Illustration of the main components of fibrocartilage, which is the end result of most cartilage repair procedures and normal articular cartilage. The data are collected from a previous publication by Suh et al (1997)

Reference List

Adachi N, Sato K, Usas A, Fu F H, Ochi M, Han C W, Niyibizi C, Huard J. Muscle derived, cell based ex vivo gene therapy for treatment of full thickness articular cartilage defects. J Rheumatol 2002; (29): 1920-1930.

Ahlback S. Osteoarthrosis of the knee. A radiographic investigation. Acta Radiologica Diagn. Suppl. 277, 7-72. 1968.

Alford J W, Cole B J. Cartilage restoration, part 1 - Basic science, historical perspective, patient evaluation, and treatment options. Am J Sports Med 2005; (33): 295-306.

Alfredson H, Lorentzon R. Superior results with continuous passive motion compared to active motion after periosteal transplantation - A retrospective study of human patella cartilage defect treatment. Knee Surg Sports Traumatol Arthrosc 1999; (7): 232-238.

Alfredson H, Thorsen K, Lorentzon R. Treatment of tear of the anterior cruciate ligament combined with localized deep cartilage defects in the knee with ligament reconstruction and autologous periosteum transplantation. Knee Surg Sports Traumatol Arthrosc 1999; (7): 69-74.

Amiel D, Coutts R D, Harwood F L, Ishizue K K, Kleiner J B. The chondrogenesis of rib perichondrial grafts for repair of full thickness articular cartilage defects in a rabbit model: a one year postoperative assessment. Connect Tissue Res 1988; (18): 27-39.

Aroen A, Jones D G, Fu F H. Arthroscopic diagnosis and treatment of cartilage injuries. Sports Med Arthrosc Rev 1998; (6): 31-40.

Aroen A, Loken S, Heir S, Alvik E, Ekeland A, Granlund O G, Engebretsen L. Articular Cartilage Lesions in 993 Consecutive Knee Arthroscopies. Am J Sports Med 2004; (32): 211-215.

Ball P, Mandell L, Niki Y, Tillotson G. Comparative tolerability of the newer fluoroquinolone antibacterials. Drug Safety 1999; (21): 407-421.

Barber F A, Chow J C Y. Arthroscopic osteochondral transplantation: Histologic results. Arthroscopy 2001; (17): 832-835.

Benjamin M, Archer C W, Ralphs J R. Cytoskeleton of Cartilage Cells. Microsc Res Tech 1994; (28): 372-377.

Bentley G, Biant L C, Carrington R W J, Akmal M, Goldberg A, Williams A M, Skinner J A, Pringle J. A prospective, randomized comparison of autologous chondrocyte implantation versus mosaicplasty for osteochondral defects in the knee. J Bone Joint Surg 2003; (85B): 223-230.

Bernstein B, Forrester D, Singsen B, King K K, Kornreich H, Hanson V. Hip-Joint Restoration in Juvenile Rheumatoid-Arthritis. Arthritis Rheum 1977; (20): 1099-1104.

Bert J M, Maschka K. The arthroscopic treatment of unicompartmental gonarthrosis: a five-year follow-up study of abrasion arthroplasty plus arthroscopic debridement and arthroscopic debridement alone. Arthroscopy 1989; (5): 25-32.

Blevins F T, Steadman J R, Rodrigo J, Silliman J. Treatment of articular cartilage defects in athletes: An analysis of functional outcome and lesion appearance. Orthopedics 1998; (21): 761-768.

Bouwmeester P S J M, Kuijer R, Homminga G N, Bulstra S K, Geesink R G T. A retrospective analysis of two independent prospective cartilage repair studies: autogenous perichondrial grafting versus subchondral drilling 10 years post-surgery. J Orthop Res 2002; (20): 267-273.

Brismar B H, Lei W, Hjerpe A, Svensson O. The effect of body mass and physical activity on the development of guinea pig osteoarthrosis. Acta Orthop Scand 2003; (74): 442-448.

Brittberg M, Lindahl A, Nilsson A, Ohlsson C, Isaksson O, Peterson L. Treatment of Deep Cartilage Defects in the Knee with Autologous Chondrocyte Transplantation. N Engl J Med 1994; (331): 889-895.

Brittberg M, Nilsson A, Lindahl A, Ohlsson C, Peterson L. Rabbit articular cartilage defects treated with autologous cultured chondrocytes. Clin Orthop Related Res 1996; 270-283.

Brittberg M, Sjogren-Jansson E, Thornemo M, Faber B, Tarkowski A, Peterson L, Lindahl A. Clonal growth of human articular cartilage and the functional role of the periosteum in chondrogenesis. Osteoarthritis Cart 2005; (13): 146-153.

Brittberg M, Winalski C S. Evaluation of cartilage injuries and repair. J Bone Joint Surg 2003; (85A): 58-69.

Buckwalter J A, Mankin H J. Articular cartilage .1. Tissue design and chondrocyte-matrix interactions. J Bone Joint Surg 1997; (79A): 600-611.

Bujia J, Kremer D, Sudhoff H, Viviente E, Sprekelsen C, Wilmes E. Determination of viability of cryopreserved cartilage grafts. Eur Arch Otorhinolaryngol 1995; (252): 30-34.

Burstein D, Gray M. New MRI techniques for imaging cartilage. J Bone Joint Surg Am 2003; (85-A Suppl 2): 70-77.

Cahill B R, Phillips M R, Navarro R. The results of conservative management of juvenile osteochondritis dissecans using joint scintigraphy. A prospective study. Am J Sports Med 1989; (17): 601-605.

Cake M A, Read R A, Appleyard R C, Hwa S Y, Ghosh P. The nitric oxide donor glyceryl trinitrate increases subchondral bone sclerosis and cartilage degeneration following ovine meniscectomy. Osteoarthritis Cart 2004; (12): 974-981.

Cameron M L, Briggs K, Steadman J R. Reproducibility and reliability of the outerbridge classification for grading chondral lesions of the knee arthroscopically. Am J Sports Med 2003; (31): 83-86.

Carlson C S, Guilak F, Vail T P, Gardin J F, Kraus V B. Synovial fluid biomarker levels predicts articular cartilage damage following complete medial meniscectomy in the canine knee. J Orthop Res 2002; (20): 92-100.

Carranza-Bencano A, Garcia-Paino L, Padron J R A, Dominguez A C. Neochondrogenesis in repair of full-thickness articular cartilage defects using free autogenous periosteal grafts in the rabbit. A follow-up in six months. Osteoarthritis Cart 2000; (8): 351-358.

Carver S E, Heath C A. Influence of intermittent pressure, fluid flow, and mixing on the regenerative properties of articular chondrocytes. Biotechnol Bioeng 1999; (65): 274-281.

Charnley J. The Lubrication of Animal Joints in Relation to Surgical Reconstruction by Arthroplasty. Ann Rheum Dis 1960; (19): 10-19.

Chen G P, Liu D C, Tadokoro M, Hirochika R, Ohgushi H, Tanaka J, Tateishi T. Chondrogenic differentiation of human mesenchymal stem cells cultured in a cobweb-like biodegradable scaffold. Biochem Biophys Res Commun 2004; (322): 50-55.

Chu C R, Coutts R D, Yoshioka M, Harwood F L, Monosov A Z, Amiel D. Articular cartilage repair using allogeneic perichondrocyte-seeded biodegradable porous polylactic acid (PLA): a tissue-engineering study. J Biomed Mater Res 1995; (29): 1147-1154.

Ciardullo A, Aglietti I, Giron F, Biddau F. Natural history of femoral chondral defects in patients with meniscectomy. J Sports Traumatol Rel Res 1999; (21): 187-195.

Clark T W I, Janzen D L, Ho K, Grunfeld A, Connell D G. Detection of Radiographically Occult Ankle Fractures Following Acute Trauma - Positive Predictive Value of An Ankle Effusion. Am J Roentg 1995; (164): 1185-1189.

Collier S, Ghosh P. Comparison of the effects of non-steroidal anti-inflammatory drugs (NSAIDs) on proteoglycan synthesis by articular cartilage explant and chondrocyte monolayer cultures. Biochem Pharmacol 1991; (41): 1375-1384.

Curl W W, Krome J, Gordon E S, Rushing J, Smith B P, Poehling G G. Cartilage injuries: A review of 31,516 knee arthroscopies. Arthroscopy 1997; (13): 456-460.

Czitrom A A, Keating S, Gross A E. The viability of articular cartilage in fresh osteochondral allografts after clinical transplantation. J Bone Joint Surg 1990; (72A): 574-581.

D'Lima D D, Hashimoto S, Chen P C, Lotz M K, Colwell C W. Cartilage injury induces chondrocyte apoptosis. J Bone Joint Surg 2001; (83A): 19-21.

Dahlberg L, Roos H, Saxne T, Heinegard D, Lark MW, Hoerrner LA, Lohmander LS: Cartilage Metabolism in the Injured and Uninjured Knee of the Same Patient. Ann Rheum Dis 53:823-827, 1994

Davisson T, Kunig S, Chen A, Sah R, Ratcliffe A. Static and dynamic compression modulate matrix metabolism in tissue engineered cartilage. J Orthop Res 2002; (20): 842-848.

Dressler M R, Butler D L, Boivin G P. Effects of age on the repair ability of mesenchymal stem cells in rabbit tendon. J Orthop Res (In Press, Corrected Proof).

Driesang I M K, Hunziker E B. Delamination rates of tissue flaps used in articular cartilage repair. J Orthop Res 2000; (18): 909-911.

Eger W, Schumacher B L, Mollenhauer J, Kuettner K E, Cole A A. Human knee and ankle cartilage explants: catabolic differences. J Orthop Res 2002; (20): 526-534.

Engfeldt B, Reinholt F P, Hultenby K, Widholm S M, Muller M. Ultrastructure of Hypertrophic Cartilage - Histochemical Procedures Compared with High-Pressure Freezing and Freeze-Substitution. Calcif Tissue Int 1994; (55): 274-280.

Faber K J, Dill J R, Amendola A, Thain L, Spouge A, Fowler P J. Occult osteochondral lesions after anterior cruciate ligament rupture. Six-year magnetic resonance imaging follow-up study. Am J Sports Med 1999; (27): 489-494.

Fang C, Johnson D, Leslie M P, Carlson C S, Robbins M, Di Cesare P E. Tissue distribution and measurement of cartilage oligomeric matrix protein in patients with magnetic resonance imaging-detected bone bruises after acute anterior cruciate ligament tears. J Orthop Res 2001; (19): 634-641.

Friederichs M G, Greis P E, Burks R T. Pitfalls associated with fixation of osteochondritis dissecans fragments using bioabsorbable screws. Arthroscopy 2001; (17): 542-545.

Frisbie D D, Trotter G W, Powers B E, Rodkey W G, Steadman J R, Howard R D, Park R D, McIlwraith C W. Arthroscopic subchondral bone plate microfracture technique augments healing of large chondral defects in the radial carpal bone and medial femoral condyle of horses. Vet Surg 1999; (28): 242-255.

Gill M R, Oldberg A, Reinholt F P. Fibromodulin-null murine knee joints display increased incidences of osteoarthritis and alterations in tissue biochemistry. Osteoarthritis Cart 2002; (10): 751-757.

Gill T J. The role of the microfracture technique in the treatment of full-thickness chondral injuries. Op Tech Sports Med 2000; (8): 138-140.

Gouttenoire M, Valcourt U, Ronziere M C, Aubert-Foucher E, Mallein-Gerin F, Herbage D. Modulation of collagen synthesis in normal and osteoarthritic cartilage. Biorheology 2004; (41): 535-542.

Grande D A, Pitman M I, Peterson L, Menche D, Klein M. The Repair of Experimentally Produced Defects in Rabbit Articular-Cartilage by Autologous Chondrocyte Transplantation. J Orthop Res 1989; (7): 208-218.

Grana W A. Healing of articular cartilage- A review. Am J Knee Surg. 2000 (13): 29-31.

Green W T. Behavior of articular chondrocytes in cell culture. Clin Orthop 1971; 248-260.

Grigolo B, Roseti L, Fiorini M, Fini M, Giavaresi G, Aldini N N, Giardino R, Facchini A. Transplantation of chondrocytes seeded on a hyaluronan derivative (hyaff-11) into cartilage defects in rabbits. Biomaterials 2001; (22): 2417-2424.

Gudas R, Simonaityte R, Riauba L, Pocius G, Kalesinskas R. Treatment of joint surface pathology by Pridie drilling. Medicina (Kaunas) 2002; (38): 720-729.

Guettler J H, Demetropoulos C K, Yang K H, Jurist K A. Osteochondral Defects in the Human Knee: Influence of Defect Size on Cartilage Rim Stress and Load Redistribution to Surrounding Cartilage. Am J Sports Med 2004; (32): 1451-1458.

Guthrie S, Plummer J M, Vaughan L C. Aetiopathogenesis of canine elbow osteochondrosis: a study of loose fragments removed at arthrotomy. Res Vet Sci 1992; (52): 284-291.

Haapala J, Arokoski J, Pirttimaki J, Lyyra T, Jurvelin J, Tammi M, Helminen H J, Kiviranta I. Incomplete restoration of immobilization induced softening of young beagle knee articular cartilage after 50-week remobilization. Int J Sports Med 2000; (21): 76-81.

Hangody L, Kish G, Karpati Z, Udvarhely I, Szigeti I, Bely M. Autogenous osteochondral graft technique for replacing knee cartilage defects in dogs. Orthopedics [5], 175-181. 1997.

Hangody L, Fules P. Autologous Osteochondral Mosaicplasty for the Treatment of Full-Thickness Defects of Weight-Bearing Joints: Ten Years of Experimental and Clinical Experience. J Bone Joint Surg 2003; (85A): 25-32.

Harkness R D. Mechanical properties of collagenous tissues. In: Biology of collagen. London: Raven Press, 1968.

Harvey A K, Hrubey P S, Chandrasekhar S. Transforming growth factor-beta inhibition of interleukin-1 activity involves down-regulation of interleukin-1 receptors on chondrocytes. Exp Cell Res 1991; (195): 376-385.

Hayes W C, Bodine A J. Flow-independent viscoelastic properties of articular cartilage matrix. J Biomech 1978; (11): 407-419.

Hedbom E, Heinegard D. Binding of Fibromodulin and Decorin to Separate Sites on Fibrillar Collagens. J Biol Chem 1993; (268): 27307-27312.

Hedlund H, Hedbom E, Heineg r D, Mengarelli-Widholm S, Reinholt F P, Svensson O. Association of the aggrecan keratan sulfate-rich region with collagen in bovine articular cartilage. J Biol Chem 1999; (274): 5777-5781.

Heinegard D, Oldberg A. Structure and Biology of Cartilage and Bone-Matrix Noncollagenous Macromolecules. Faseb Journal 1989; (3): 2042-2051.

Heir S, Aroen A, Loken S, Sulheim S, Reinholt FP, Engebretsen L. The intra-articular location of a partial thickness cartilage defect does not influence the risk of osteoarthrosis. Poster 556, 67. 2004. ICRS.

Henrotin Y., Kurz B. and T. Aigner Oxygen and reactive oxygen species in cartilage degradation: friends or foes? Osteoarthritis Cart 2005 (13): 643-654

Hjelle K, Solheim E, Strand T, Muri R, Brittberg M. Articular cartilage defects in 1,000 knee arthroscopies. Arthroscopy 2002; (18): 730-734.

Hlavacek M. The Role of Synovial-Fluid Filtration by Cartilage in Lubrication of Synovial Joints .4. Squeeze-Film Lubrication - the Central Film Thickness for Normal and Inflammatory Synovial-Fluids for Axial Symmetry under High Loading Conditions. J Biomech 1995; (28): 1199-1205.

Hlavacek M, Novak J. The Role of Synovial-Fluid Filtration by Cartilage in Lubrication of Synovial Joints .3. Squeeze-Film Lubrication - Axial Symmetry under Low Loading Conditions. J Biomech 1995; (28): 1193-1198.

Horas U, Pelinkovic D, Herr G, Aigner T, Schnettler R. Autologous chondrocyte implantation and osteochondral cylinder transplantation in cartilage repair of the knee joint - A prospective, comparative trial. J Bone Joint Surg 2003; (85A): 185-192.

Hudelmaier M, Glaser C, Hohe J, Englmeier K H, Reiser M, Putz R, Eckstein F. Age-related changes in the morphology and deformational behavior of knee joint cartilage. Arthritis Rheum 2001; (44): 2556-.

Hughes J A, Cook J V, Churchill M A, Warren M E. Juvenile osteochondritis dissecans: a 5-year review of the natural history using clinical and MRI evaluation. Pediatr Radiol 2003; (33): 410-417.

Huibregtse B A, Johnstone B, Goldberg V M, Caplan A I. Effect of age and sampling site on the chondro-osteogenic potential of rabbit marrow-derived mesenchymal progenitor cells. J Ortho Res 2000; (18): 18-24.

Hunziker E B. Articular cartilage repair: basic science and clinical progress. A review of the current status and prospects. Osteoarthritis Cart 2002; (10): 432-463.

Hunziker E B, Driesang I M K, Saager C. Structural barrier principle for growth factor-based articular cartilage repair. Clin Orthop Rel Res 2001;S182-S189.

Hunziker E B, Quinn T M. Surgical removal of articular cartilage leads to loss of chondrocytes from cartilage bordering the wound edge. J Bone Joint Surg 2003; (85A): 85-92.

Hunziker E B, Quinn T M, Hauselmann H J. Quantitative structural organization of normal adult human articular cartilage. Osteoarthritis Cart 2002; (10): 564-572.

Hunziker E B, Rosenberg L C. Repair of partial-thickness defects in articular cartilage: cell recruitment from the synovial membrane. J Bone Joint Surg 1996; (78A): 721-733.

Hyllested J L, Veje K, Ostergaard K. Histochemical studies of the extra cellular matrix of human articular cartilage - a review. Osteoarthritis Cart 2002; (10): 333-343.

Indelicato P A, Bittar E S. A Perspective of lesions associated with ACL insufficiency of the Knee - A review of 100 Cases. Clin Orthop Rel Res 1985;77-80.

Irie K, Yamada T, Inoue K. A comparison of magnetic resonance imaging and arthroscopic evaluation of chondral lesions of the knee. Orthopedics 2000; (23): 561-564.

Jackson D W, Lalor P A, Aberman H M, Simon T M. Spontaneous repair of full-thickness defects of articular cartilage in a goat model - A preliminary study. J Bone Joint Surg 2001; (83A): 53-64.

Jakobsen R, Engebretsen L, Slauterbeck JR. An analysis of the quality of cartilage repair studies. J Bone Joint Surg Am, submitted November 17th 2004. 2005.

Jeffrey J E, Gregory D W, Aspden R M. Matrix Damage and Chondrocyte Viability Following a Single Impact Load on Articular-Cartilage. Arch Biochem Biophys 1995; (322): 87-96.

Jeffrey J E, Thomson L A, Aspden R M. Matrix loss and synthesis following a single impact load on articular cartilage in vitro. Biochimica et Biophysica Acta (BBA) - General Subjects 1997; (1334): 223-232.

Jin G, Sah R L, Li Y S, Lotz M, Shyy J Y J, Chien S. Biomechanical regulation of matrix metalloproteinase-9 in cultured chondrocytes. J Orthop Res 2000; (18): 899-908.

Johnson L L. Arthroscopic abrasion arthroplasty: a review. Clin Orthop 2001; S306-S317.

Jomha N M, Anoop P C, McGann L E. Intramatrix events during cryopreservation of porcine articular cartilage using rapid cooling. J Orthop Res 2004; (22): 152-157.

Jones G, Glisson M, Hynes K, Cicuttini F. Sex and site differences in cartilage development - A possible explanation for variations in knee osteoarthritis in later life. Arthritis Rheum 2000; (43): 2543-2549.

Junquerira L C, Carneiro, Long J. Basic histology. Lange Medical Publications, Los Altos, California 1986.

Kaneko S, Satoh T, Chiba J, Ju C Y, Inoue K, Kagawa J. Interleukin-6 and interleukin-8 levels in serum and synovial fluid of patients with osteoarthritis. Cytokines Cellular & Molecular Therapy 2000; (6): 71-79.

Katayama R, Wakitani S, Tsumaki N, Morita Y, Matsushita I, Gejo R, Kimura T. Repair of articular cartilage defects in rabbits using CDMP1 gene-transfected autologous mesenchymal cells derived from bone marrow. Rheumatology 2004; (43): 980-985.

Kawabe N, Yoshinao M. The repair of full-thickness articular cartilage defects. Immune responses to reparative tissue formed by allogeneic growth plate chondrocyte implants. Clin Orthop 1991; 279-293.

Kawamura S, Wakitani S, Kimura T, Maeda A, Caplan A I, Shino K, Ochi T. Articular cartilage repair - Rabbit experiments with a collagen gel-biomatrix and chondrocytes cultured in it. Acta Orthop Scand 1998; (69): 56-62.

Kirsch T, Vondermark K. Remodeling of Collagen Type-I, Type-Ii and Type-X and Calcification of Human Fetal Cartilage. Bone and Mineral 1992; (18): 107-117.

Kiviranta I, Tammi M, Jurvelin J, Arokoski J, Saamanen A M, Helminen H J. Articular-Cartilage Thickness and Glycosaminoglycan Distribution in the Young Canine Knee-Joint After Remobilization of the Immobilized Limb. J Orthop Res 1994; (12): 161-167.

Kivisto R, Pasanen L, Leppilahti J, Jalovaara P. Arthroscopic repair of osteochondritis dissecans of the femoral condyles with metal staple fixation: a report of 28 cases. Knee Surg Sports Traumatol Arthrosc 2002; (10): 305-309.

Knutsen G, Engebretsen L, Ludvigsen T C, Drogset J O, Grontvedt T, Solheim E, Strand T, Roberts S, Isaksen V, Johansen C. Autologous chondrocyte implantation compared with microfracture in the knee - A randomized trial. J Bone Joint Surg 2004; (86A): 455-464.

Kocher M S, Steadman J R, Briggs K K, Sterett W I, Hawkins R J. Reliability, validity, and responsiveness of the Lysholm knee scale for various chondral disorders of the knee. J Bone Joint Surg 2004; (86A): 1139-1145.

Korhonen R K, Wong M, Arokoski J, Lindgren R, Helminen H J, Hunziker E B, Jurvelin J S. Importance of the superficial tissue layer for the indentation stiffness of articular cartilage. Med Eng Phys 2002; (24): 99-108.

Kreder H J, Moran M, Keeley F W, Salter R B. Biologic Resurfacing of A Major Joint Defect with Cryopreserved Allogeneic Periosteum Under the Influence of Continuous Passive Motion in A Rabbit Model. Clin Ortho Rel Res 1994; 288-296.

Kubo T, Arai Y, Namie K, Takahashi K, Hojo T, Inoue S, Ueshima K, Shiga T, Yutani Y, Hirasawa Y. Time-sequential changes in biomechanical and morphological properties of articular cartilage in cryopreserved osteochondral allografting. J Orthop Sci 2001; (6): 276-281.

Kuhne SAM, Neidhart M, Everson MP, Hantzschel H, Fine PR, Gay S, Hauselmann HJ, Gay RE: Persistent high serum levels of cartilage oligomeric matrix protein in a subgroup of patients with traumatic knee injury. Rheum Int 18:21-25, 1998

Kwan M K, Lai W M, Mow V C. Fundamentals of fluid transport through cartilage in compression. Ann Biomed Eng 1984; (12): 537-558.

Laasanen M S, Toyras J, Vasara A I, Hyttinen M M, Saarakkala S, Hirvonen J, Jurvelin J S, Kiviranta I. Mechano-acoustic diagnosis of cartilage degeneration and repair. J Bone Joint Surg 2003; (85A): 78-84.

Lane J G, Massie J B, Ball S T, Amiel M E, Chen A C, Bae W C, Sah R L, Amiel D. Follow-up of osteochondral plug transfers in a goat model - A 6-month study. Am J Sports Med 2004; (32): 1440-1450.

Lane J G, Tontz J, Ball S T, Massie J B, Chen A C, Bae W C, Amiel M E, Sah R L, Amiel D. A morphologic, biochemical, and biomechanical assessment of short-term effects of osteochondral autograft plug transfer in an animal model. Arthroscopy: 2001; (17): 856-862.

LaPrade R F. Autologous Chondrocyte Implantation Was Superior to Mosaicplasty for Repair of Articular Cartilage Defects in the Knee at One Year. J Bone Joint Surg 2003; (85A): 2259.

LeBaron R G, Athanasiou K A. Ex vivo synthesis of articular cartilage. Biomaterials 2000; (21): 2575-2587.

Lemare F, Steimberg N, Le Griel C, Demignot S, Adolphe M. Dedifferentiated chondrocytes cultured in alginate beads: Restoration of the differentiated phenotype and of the metabolic responses to interleukin-1 beta. J Cell Physiol 1998; (176): 303-313.

Lexer E. Die verwendungen der freien knochenplastik nebst versuchen uber Gelenkverstefung and Gelenktransplantation. Arch F Klin Chir 1908; 939.

Li W J, Tuli R, Okafor C, Derfoul A, Danielson K G, Hall D J, Tuan R S. A three-dimensional nanofibrous scaffold for cartilage tissue engineering using human mesenchymal stem cells. Biomaterials 2005; (26): 599-609.

Lietman S A, Miyamoto S, Brown P R, Inoue N, Reddi A H. The temporal sequence of spontaneous repair of osteochondral defects in the knees of rabbits is dependent on the geometry of the defect. J Bone Joint Surg 2002; (84B): 600-606.

Lindahl A, Brittberg M, Peterson L. Health economics benefits following autologous chondrocyte transplantation for patients with focal chondral lesions of the knee. Knee Surg Sports Traumatol Arthrosc 2001; (9): 358-363.

Linden B. Osteochondritis dissecans of the femoral condyles: a long-term follow-up study. J Bone Joint Surg 1977; (59A): 769-776.

Lo M Y, Kim H T. Chondrocyte apoptosis induced by collagen degradation: inhibition by caspase inhibitors and IGF-1. J Orthop Res 2004; (22): 140-144.

Løken S, Ludvigsen TC, Knopp A, Engebretsen L. Autolog chondrocytimplantasjon- 5 års resultater fra Ullevål Universitetssykehus. 181. 2003. Oslo, Norsk Kirurgisk Forening. Vasli, L. 23-10-2003.

Lyyra T. Development, validation and clinical application of indention technique for arthroscopic measurement of cartilage stiffness. Thesis 1997.

Lyyra T, Arokoski J P, Oksala N, Vihko A, Hyttinen M, Jurvelin J S, Kiviranta I. Experimental validation of arthroscopic cartilage stiffness measurement using enzymatically degraded cartilage samples. Phys Med Biol 1999a; (44): 525-535.

Lyyra T, Kiviranta I, Vaatainen U, Helminen H J, Jurvelin J S. In vivo characterization of indentation stiffness of articular cartilage in the normal human knee. J Biomed Mater Res 1999b; (48): 482-487.

MacConaill M A. The function of intra-articular fibrocartilages with special reference to the knee and inferior radio-ulnar joints. J Anat 1932; 210-227.

Mankin H J, Fogelson F S, Thrasher A Z, Jaffer F. Massive resection and allograft transplantation in the treatment of malignant bone tumors. N Engl J Med 1976; (294): 1247-1255.

Marandola M S, Prietto C A. Arthroscopic Herbert screw fixation of patellar osteochondritis dissecans. Arthroscopy 1993; (9): 214-216.

Marlovits S, Striessnig G, Kutscha-Lissberg F, Resinger C, Aldrian S M, Vecsei V, Trattnig S. Early postoperative adherence of matrix-induced autologous chondrocyte implantation for the treatment of full-thickness cartilage defects of the femoral condyle. Knee Surg Sports Traumatol Arthrosc 2004.

Maroudas A. Biophysical chemistry of cartilaginous tissues with special reference to solute and fluid transport. Biorheology 1975; (12): 233-248.

Matelic T M, Aronsson D D, Boyd D W, Lamont R L. Acute Hemarthrosis of the Knee in Children. Am J Sports Med 1995; (23): 668-671.

Mcllwraith CW, Frisbie DD, Cross MW. The quest for a better model? A comparative study of articular cartilage thickness on femoral trochlea and femoral condyle of species used in preclinical studies compared to cartilage thickness in the human knee. ICRS. poster 668, 84. 2004.

Messner K. Durability of Artificial Implants for Repair of Osteochondral Defects of the Medial Femoral Condyle in Rabbits. Biomaterials 1994; (15): 657-664.

Messner K, Gillquist J. Cartilage repair - A critical review. Acta Orthop Scand 1996; (67): 523-529.

Messner K, Gillquist J, Bjornsson S, Lohmander L S. Proteoglycan Fragments in Rabbit Joint Fluid Correlated to Arthrosis Stage. Acta Orthop Scand 1993a; (64): 312-316.

Messner K, Lohmander L S, Gillquist J. Neocartilage After Artificial Cartilage Repair in the Rabbit - Histology and Proteoglycan Fragments in Joint Fluid. J Biomed Mater Res 1993b; (27): 949-954.

Messner K, Maletius W. The long-term prognosis for severe damage to weight-bearing cartilage in the knee - A 14-year clinical and radiographic follow-up in 28 young athletes. Acta Orthop Scand 1996; (67): 165-168.

Milgram J W. Radiological and pathological manifestations of osteochondritis dissecans of the distal femur. A study of 50 cases. Radiology 1978; (126): 305-311.

Miller M D, Osborne J R, Gordon W T, Hinkin D T, Brinker M R. The Natural History of Bone Bruises: A Prospective Study of Magnetic Resonance Imaging-Detected Trabecular Microfractures in Patients with Isolated Medial Collateral Ligament Injuries. Am J Sports Med 1998; (26): 15-19.

Mitchell N, Shepard N. The resurfacing of adult rabbit articular cartilage by multiple perforations through the subchondral bone. J Bone Joint Surg1976; (58A): 230-233.

Mont M A, Baumgarten K M, RifaI A, Bluemke D A, Jones L C, Hungerford D S. Atraumatic Osteonecrosis of the Knee. J Bone Joint Surg 2000; (82A): 1279.

Mow V C, Holmes M H, Lai W M. Fluid transport and mechanical properties of articular cartilage: a review. J Biomech 1984; (17): 377-394.

Murakami S, Akiyama H, Crombrugghe B. Inborn errors of development. The molecular basis of clinical disorders and morphogenesis. Oxford University Press, 2004.

Murray R C, Vedi S, Birch H L, Lakhani K H, Goodship A E. Subchondral bone thickness, hardness and remodeling are influenced by short-term exercise in a site-specific manner. J Orthop Res 2001; (19): 1035-1042.

Nabavi-Tabrizi A, Turnbull A, Dao Q, Appleyard R. Chondrocyte damage following osteochondral grafting using metal and plastic punches: comparative study in an animal model. J Orthop Surg (Hong Kong) 2002; (10): 170-172.

Nakagawa T, Kurosawa H, Ikeda H, Nozawa M, Kawakami A. Internal fixation for osteochondritis dissecans of the knee. Knee Surg Sports Traumatol Arthrosc 2004.

Nakahara H, Goldberg V M, Caplan A I. Culture-Expanded Human Periosteal-Derived Cells Exhibit Osteochondral Potential In vivo. J Ortho Res 1991; (9): 465-476.

Nehrer S, Breinan H A, Ramappa A, Shortkroff S, Young G, Minas T, Sledge C B, Yannas I V, Spector M. Canine chondrocytes seeded in type I and type II collagen implants investigated in vitro. J Biomed Mat Res 1997; (38): 95-104.

Newton P M, Mow V C, Gardner T R, Buckwalter J A, Albright J P. The effect of lifelong exercise on canine articular cartilage. Am J Sports Med 1997; (25): 282-287.

Norrby S R. Side-Effects of Quinolones - Comparisons Between Quinolones and Other Antibiotics. Eur J Clin Microbio Inf Dis 1991; (10): 378-383.

O'Driscoll S W. The healing and regeneration of articular cartilage. J Bone Joint Surg 1998; (80A): 1795-1812.

O'Driscoll S W M, Saris D B F, Ito Y, Fitzimmons J S. The chondrogenic potential of periosteum decreases with age. J Orthop Res 2001; (19): 95-103.

Oakley S P, Portek I, Szomor Z, Turnbull A, Murrell G A C, Kirkham B W, Lassere M N. Poor accuracy and interobserver reliability of knee arthroscopy measurements are improved by the use of variable angle elongated probes. Ann Rheum Dis 2002; (61): 540-543.

Oakley S P, Portek I, Szomor Z, Turnbull A, Murrell G A C, Kirkham B W, Lassere M N. Accuracy and reliability of arthroscopic estimates of cartilage lesion size in a plastic knee simulation model. Arthroscopy 2003; (19): 282-289.

Peters T A, McLean I D. Osteochondritis Dissecans of the Patellofemoral Joint. Am J Sports Med 2000; (28): 63-67.

Peterson L, Brittberg M, Kiviranta I, Akerlund E L, Lindahl A. Autologous chondrocyte transplantation - Biomechanics and long-term durability. Am J Sports Med 2002; (30): 2-12.

Peterson L, Minas T, Brittberg M, Lindahl A. Treatment of osteochondritis dissecans of the knee with autologous chondrocyte transplantation - Results at two to ten years. J Bone Joint Surg 2003; (85A): 17-24.

Peterson L, Minas T, Brittberg M, Nilsson A, Sjogren-Jansson E, Lindahl A. Two- to 9-year outcome after autologous chondrocyte transplantation of the knee. Clin Orthop 2000; 212-234.

Phipatanakul W P, VandeVord P J, Teitge R A, Wooley P H. Immune response in patients receiving fresh osteochondral allografts. Am J Orthop 2004; (33): 345-348.

Poole A R, Kojima T, Yasuda T, Mwale F, Kobayashi M, Laverty S. Composition and structure of articular cartilage - A template for tissue repair. Clin Orthop 2001;S26-S33.

Poussa M, Rubak J, Ritsila V. Cartilage Formation by Periosteal Grafts. Acta Orthop Scand 1981; (52): 594.

Pridie K. A method of resurfacing osteoarthritic knee joints. J Bone Joint Surg 1959; (41 B): 618-619.

Puelacher W C, Kim S W, Vacanti J P, Schloo B, Mooney D, Vacanti C A. Tissue-Engineered Growth of Cartilage - the Effect of Varying the Concentration of Chondrocytes Seeded Onto Synthetic-Polymer Matrices. Int J Oral and Maxillofac Surg 1994; (23): 49-53.

Qiu Y S, Shahgaldi B F, Revell W J, Heatley F W. Observations of subchondral plate advancement during osteochondral repair: a histomorphometric and mechanical study in the rabbit femoral condyle. Osteoarthritis Cart 2003; (11): 810-820.

Quarto R, Thomas D, Liang C T. Bone Progenitor-Cell Deficits and the Age-Associated Decline in Bone Repair Capacity. Calcif Tissue Int 1995; (56): 123-129.

Radin E L, Swann D A, Weisser P A. Separation of a hyaluronate-free lubricating fraction from synovial fluid. Nature 1970; (228): 377-378.

Redman S N, Dowthwaite G P, Thomson B M, Archer C W. The cellular responses of articular cartilage to sharp and blunt trauma. Osteoarthritis Cart 2004; (12): 106-116.

Reindel E S, Ayroso A M, Chen A C, Chun D M, Schinagl R M, Sah R L. Integrative repair of articular cartilage in vitro: adhesive strength of the interface region. J Orthop Res 1995; (13): 751-760.

Rodrigo J J, Schnaser A M, Reynolds H M, Jr., Biggart J M, III, Leathers M W, Chism S E, Thorson E, Grotz T, Yang Q M. Inhibition of the immune response to experimental fresh osteoarticular allografts. Clin Orthop 1989;235-253.

Romppanen T, Collan Y. Pratical guidelines for a morphometric study. Acta Stereol 1983;274-297.

Roos E M, Roos H P, Ekdahl C, Lohmander L S. Knee injury and Osteoarthritis Outcome Score (KOOS)--validation of a Swedish version. Scand J Med Sci Sports 1998; (8): 439-448.

Rubak J M. Reconstruction of Articular-Cartilage Defects with Free Periosteal Grafts - An Experimental-Study. Acta Orthop Scand 1982; (53): 175-180.

Russlies M, Behrens P, Ehlers E M, Brohl C, Vindigni C, Spector M, Kurz B. Periosteum stimulates subchondral bone densification in autologous chondrocyte transplantation in a sheep model. Cell Tissue Res 2004.

Russlies M, Ruther P, Koller W, Stomberg P, Behrens P. Biomechanical properties of cartilage repair tissue after different cartilage repair procedures in sheep. Z Orthop Ihre Grenzgeb 2003; (141): 465-471.

Saied A, Cherin E, Gaucher H, Laugier P, Gillet P, Floquet J, Netter P, Berger G. Assessment of articular cartilage and subchondral bone: Subtle and progressive changes in experimental osteoarthritis using 50 MHz echography in vitro. J Bone Min Res 1997; (12): 1378-1386.

Salter R B, Simmonds D F, Malcolm B W, Rumble E J, Macmichael D. Effects of Continuous Passive Motion on Healing of Articular-Cartilage Defects - Experimental Investigation in Rabbits. J Bone Joint Surg 1975; (57A): 571-572.

Salter R B, Simmonds D F, Malcolm B W, Rumble E J, Macmichael D, Clements N D. The Biological Effect of Continuous Passive Motion on the Healing of Full-Thickness Defects in Articular-Cartilage - An Experimental Investigation in the Rabbit. J Bone Joint Surg 1980; (62A): 1232-1251.

Saris D B, Dhert W J, Verbout A J. Joint homeostasis. The discrepancy between old and fresh defects in cartilage repair. J Bone Joint Surg 2003; (85 B): 1067-1076.

Schafer D, Boss A, Hintermann B. Accuracy of arthroscopic assessment of anterior ankle cartilage lesions. Foot Ankle Int 2003; (24): 317-320.

Schenk R C, Jr., Goodnight J M. Current Concept Review - Osteochondritis Dissecans. J Bone Joint Surg 1996; (78 A): 439-456.

Schulze-Tanzil G, de Souza P, Castrejon H V, John T, Merker H J, Scheid A, Shakibaei M. Redifferentiation of dedifferentiated human chondrocytes in high-density cultures. Cell Tissue Res 2002; (308): 371-379.

Sellers R S, Peluso D, Morris E A. The effect of recombinant human bone morphogenetic protein-2 (rhBMP-2) on the healing of full-thickness defects of articular cartilage. J Bone Joint Surg 1997; (79A): 1452-1463.

Shasha N, Aubin P P, Cheah H K, Davis A M, Agnidis Z, Gross A E. Long-term clinical experience with fresh osteochondral allografts for articular knee defects in high demand patients. Cell Tissue Bank 2002; (3): 175-182.

Shelbourne K D, Jari S, Gray T. Outcome of untreated traumatic articular cartilage defects of the knee - A natural history study. J Bone Joint Surg 2003; (85A): 8-16.

Shimizu T, Videman T, Shimazaki K, Mooney V. Experimental-Study on the Repair of Full Thickness Articular-Cartilage Defects - Effects of Varying Periods of Continuous Passive Motion, Cage Activity, and Immobilization. J Orthop Res 1987; (5): 187-197.

Siebold R, Lichtenberg S, Habermeyer P. Combination of microfracture and periostal-flap for the treatment of focal full thickness articular cartilage lesions of the shoulder: a prospective study. Knee Surg Sports Traumatol Arthrosc 2003; (11): 183-189.

Smith G D, Richardson J B, Brittberg M, Erggelet C, Verdonk R, Knutsen G, Ashton B A, Ashton I K, Harrison P E, Horas U, Pelinkovic D, Aigner T, Schnettler R. Autologous Chondrocyte Implantation and Osteochondral Cylinder Transplantation in Cartilage Repair of the Knee Joint * U. Horas, D. Pelinkovic, T. Aigner, and R. Schnettler reply. J Bone Joint Surg 2003; (85A): 2487-2488.

Smith R L, Palathumpat M V, Ku C W, Hintz R L. Growth hormone stimulates insulin-like growth factor I actions on adult articular chondrocytes. J Orthop Res 1989; (7): 198-207.

Solchaga L A, Goldberg V M, Caplan A I. Cartilage regeneration using principles of tissue engineering. Clin Orthop Rel Res 2001;S161-S170.

Solchaga L A, Johnstone B, Yoo J U, Goldberg V M, Caplan A I. High variability in rabbit bone marrow-derived mesenchymal cell preparations. Cell Transplant 1999; (8): 511-519.

Solchaga L A, Yoo J U, Lundberg M, Dennis J E, Huibregtse B A, Goldberg V M, Caplan A I. Hyaluronan-based polymers in the treatment of osteochondral defects. J Orthop Res 2000; (18): 773-780.

Sommarin Y, Larsson T, Heinegard D. Chondrocyte Matrix Interactions - Attachment to Proteins Isolated from Cartilage. Experimental Cell Research 1989; (184): 181-192.

Stanitski C L, Harvell J C, Fu F. Observations on Acute Knee Hemarthrosis in Children and Adolescents. Journal of Pediatric Orthopaedics 1993; (13): 506-510.

Steadman J R, Briggs K K, Rodrigo J J, Kocher M S, Gill T J, Rodkey W G. Outcomes of microfracture for traumatic chondral defects of the knee: Average 11-year follow-up. Arthroscopy 2003; (19): 477-484.

Steadman J R, Rodkey W G, Briggs K K, Rodrigo J J. The microfracture procedure: Rationale, technique, and clinical observations for treatment of articular cartilage defects. J Sports Traumatol Rel Res 1998; (20): 61-70.

Steadman J R, Rodkey W G, Rodrigo J J. Microfracture: Surgical technique and rehabilitation to treat chondral defects. Clin Orthop 2001;S362-S369.

Stevens M M, Marini R P, Martin I, Langer R, Shastri V P. FGF-2 enhances TGF-beta 1-induced periosteal chondrogenesis. J Orthop Res 2004; (22): 1114-1119.

Stevenson S. The immune response to osteochondral allografts in dogs. J Bone Joint Surg Am 1987; (69): 573-582.

Stockwell R A. The interrelationship of cell density and cartilage thickness in mammalian articular cartilage. J Anat 1971; (109): 411-421.

Suh J K, Årøen A, Muzzonigro T S, Disilvestro M, Fu F H. Injury and repair of articular cartilage: Related scientific issues. Oper Tech Orthop 1997; (7): 270-278.

Suh J K, Baek G H, Aroen A, Malin C M, Niyibizi C, Evans C H, Westerhausen-Larson A. Intermittent sub-ambient interstitial hydrostatic pressure as a potential mechanical stimulator for chondrocyte metabolism. Osteoarthritis Cart 1999; (7): 71-80.

Suh J K, Bai S. Finite element formulation of biphasic poroviscoelastic model for articular cartilage. J Biomech Eng 1998; (120): 195-201.

Swann D A, Hendren R B, Radin E L, Sotman S L, Duda E A. The lubricating activity of synovial fluid glycoproteins. Arthritis Rheum 1981; (24): 22-30.

Tandogan R N, Taser O, Kayaalp A, Taskiran E, Pinar H, Alparslan B, Alturfan A. Analysis of meniscal and chondral lesions accompanying anterior cruciate ligament tears: relationship with age, time from injury, and level of sport. Knee Surg Sports Traumatol Arthrosc 2004; (12): 262-266.

Tegnander A, Engebretsen L, Bergh K, Eide E, Holen K J, Iversen O J. Activation of the complement system and adverse effects of biodegradable pins of polylactic acid (Biofix) in osteochondritis dissecans. Acta Orthop Scand 1994; (65): 472-475.

Tew S R, Kwan A P, Hann A, Thomson B M, Archer C W. The reactions of articular cartilage to experimental wounding: role of apoptosis. Arthritis Rheum 2000; (43): 215-225.

Thambyah A, Goh J C H, De S D. Contact stresses in the knee joint in deep flexion. Med Eng Phys (In Press, Corrected Proof).

Torzilli P A, Dethmers D A, Rose D E, Schryuer H F. Movement of interstitial water through loaded articular cartilage. J Biomech 1983; (16): 169-179.

Torzilli P A, Mow V C. On the fundamental fluid transport mechanisms through normal and pathological articular cartilage during function--I. The formulation. J Biomech 1976; (9): 541-552.

Trickey W R, Vail T P, Guilak F. The role of the cytoskeleton in the viscoelastic properties of human articular chondrocytes. J Orthop Res 2004; (22): 131-139.

Uchio Y, Ochi M, Adachi N, Kawasaki K, Iwasa J. Arthroscopic assessment of human cartilage stiffness of the femoral condyles and the patella with a new tactile sensor. Med Eng Phys 2002; (24): 431-435.

van Susante J L C, Pieper J, Buma P, van Kuppevelt T H, van Beuningen H, van der Kraan P M, Veerkamp J H, van den Berg W B, Veth R P H. Linkage of chondroitin-sulfate to type I collagen scaffolds stimulates the bioactivity of seeded chondrocytes in vitro. Biomaterials 2001; (22): 2359-2369.

van Susante J L C, Buma P, Schuman L, Homminga G N, van den Berg W B, Veth R P H. Resurfacing potential of heterologous chondrocytes suspended in fibrin glue in large full-thickness defects of femoral articular cartilage: an experimental study in the goat. Biomaterials 1999; (20): 1167-1175.

Vasara A I, Jurvelin J S, Peterson L, Kiviranta I. Arthroscopic cartilage indentation and cartilage lesions of anterior cruciate ligament-deficient knees. Am J Sports Med 2005; (33): 408-414.

Venn M, Maroudas A. Chemical Composition and Swelling of Normal and Osteoarthrotic Femoral-Head Cartilage .1. Chemical Composition. Ann Rheum Dis 1977; (36): 121-129.

Wakitani S, Goto T, Pineda S J, Young R G, Mansour J M, Caplan A I, Goldberg V M. Mesenchymal Cell-Based Repair of Large, Full-Thickness Defects of Articular-Cartilage. J Bone Joint Surg 1994; (76A): 579-592.

Wakitani S, Imoto K, Yamamoto T, Saito M, Murata N, Yoneda M. Human autologous culture expanded bone marrow mesenchymal cell transplantation for repair of cartilage defects in osteoarthritic knees. Osteoarthritis Cart 2002; (10): 199-206.

Waldman S D, Spiteri C G, Grynpas M D, Pilliar R M, Hong J, Kandel R A. Effect of biomechanical conditioning on cartilaginous tissue formation in vitro. J Bone Joint Surg Am 2003a; (85A): 101-105.

Waldman S D, Spiteri C G, Grynpas M D, Pilliar R M, Kandel R A. Long-term intermittent shear deformation improves the quality of cartilaginous tissue formed in vitro. J Orthop Res 2003b; (21): 590-596.

Wayne J S, Woo S L, Kwan M K. Application of the u-p finite element method to the study of articular cartilage. J Biomech Eng 1991; (113): 397-403.

Wayne J S, Woo S L, Kwan M K. Finite element analyses of repaired articular surfaces. Proc Inst Mech Eng 1991; (205): 155-162.

Wei X C, Gao J Z, Messner K. Maturation-dependent repair of untreated osteochondral defects in the rabbit knee joint. J Biomed Mater Res 1997; (34): 63-72.

Whiteside R A, Bryant J T, Jakob R P, Mainil-Varlet P, Wyss U P. Short-term load bearing capacity of osteochondral autografts implanted by the mosaicplasty technique: an in vitro porcine model. J. Biomech 2003; (36): 1203-1208.

Wolfson J S, Hooper D C. Overview of Fluoroquinolone Safety. Am J Med 1991; (91): S153-S161.

Wouters D B, Bos R R, Mouton L J, van Horn J R. The meniscus Arrow or metal screw for treatment of osteochondritis dissecans? In vitro comparison of their effectiveness. Knee Surg Sports Traumatol Arthrosc 2004; (12): 52-57.

Yamamoto T, Bullough P G. Spontaneous Osteonecrosis of the Knee: The Result of Subchondral Insufficiency Fracture. J Bone Joint Surg 2000; (82 A): 858.

Yoshizumi Y, Sugita T, Kawamata T, Ohnuma M, Maeda S. Cylindrical Osteochondral Graft for Osteochondritis Dissecans of the Knee: A Report of Three Cases. Am J Sports Med 2002; (30): 441-445.

Ytrehus B, Carlson C S, Lundeheim N, Mathisen L, Reinholt F P, Teige J, Ekman S. Vascularisation and osteochondrosis of the epiphyseal growth cartilage of the distal femur in pigs - development with age, growth rate, weight and joint shape. Bone 2004; (34): 454-465.

Ytrehus B, Grindflek E, Teige J, Stubsjoen E, Grondalen T, Carlson C S, Ekman S. The effect of parentage on the prevalence, severity and location of lesions of osteochondrosis in swine. J Vet Med Series A-Physiol Pathol Clin Med 2004; (51): 188-195.

Ytrehus B, Haga H A, Mellum C N, Mathisen L, Carlson C S, Ekman S, Teige J, Reinholt F P. Experimental ischemia of porcine growth cartilage produces lesions of osteochondrosis. J Orthop Res 2004; (22): 1201-1209.

Zarzycki W. Evaluation of treatment results for osteochondritis dissecans of the knee joint. Chir Narzadow Ruchu Ortop Pol 2001; (66): 61-66.

Zhai G J, Stankovich J, Ding C H, Scott F, Cicuttini F, Jones G. The genetic contribution to muscle strength, knee pain, cartilage volume, bone size, and radiographic osteoarthritis - A sibpair study. Arthritis Rheum 2004; (50): 805-810.