From the Department of
CLINICAL SCIENCE, INTERVENTION AND TECHNOLOGY
Division of Orthopedics and Biotechnology
Karolinska Institutet, Stockholm, Sweden

Focal Knee Resurfacing

A translational study in sheep on the treatment of focal condylar cartilage lesions with metal implants

Nicolas Martinez Carranza

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To my grandmother Mercedez Alvarez Prado
and to my grandfather Héctor Martinez Carranza.

Thank you. So much.

Focal Knee Resurfacing

A translational study in sheep on the treatment of focal condylar cartilage lesions with metal implants

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

Nicolas Martinez Carranza

Huvudhandledare
Docent Hans Berg
Karolinska Institutet
Institutionen för klinisk vetenskap, intervention och teknik
Enheten för ortopedi och bioteknologi

Bihandledare
Professor Anne-Sofie Lagerstedt
Sveriges lantbruksuniversitet
Institutionen för kliniska vetenskaper
Enheten för hund, katt och andra smådjur

Med Dr Håkan Hedlund
Karolinska Institutet
Institutionen för klinisk vetenskap, intervention och teknik
Enheten för ortopedi och bioteknologi

Fakultetsopponent
Professor Per Wretenberg
Örebro universitet
Institutionen för medicinska vetenskaper
Avdelning för ortopedi

Betygsnämnd
Professor Kjell G Nilsson
Umeå universitet
Institutionen för kirurgisk och perioperativ vetenskap
Enheten för ortopedi

Professor Mats Trulsson
Karolinska Institutet
Institutionen för odontologi

Professor Stina Ekman
Sveriges lantbruksuniversitet
Institutionen för biomedicin och veterinär folkhälsovetenskap
Avdelning för patologi, farmakologi och toxikologi

”First they ignore you, then they laugh at you, then they fight you, then you win.”

Mahatma Ghandi

ABSTRACT

Background

The translational process of introducing a new treatment concept in medicine from innovation to clinical application is a challenging endeavour that often involves animal experiments. Focal cartilage lesion remains a clinical challenge and is considered to portend osteoarthritis (OA). For the last 30 years biological treatment strategies have aimed to regenerate a durable cartilage repair tissue. In the last decade a novel treatment strategy has been proposed by surgically replacing the void of the cartilage defect with a metal implant, focal knee resurfacing with metal (FKRM) implants.

Methods

We developed a double-radii, double-coated implant with an individualised instrumentation device for optimal positioning. This concept was tested in a larger animal model (sheep) evaluating the biological safety and efficacy of the implant. A total of 37 ewes were used in the experiments of this thesis. Implant position was measured using a developed laser-scanning protocol. General animal health, general cartilage health and macroscopic as well as microscopic cartilage evaluation were assessed according to a modified Mankin score as recommended by Osteoarthritis Research Society International (OARSI) for histological assessment of osteoarthritis in sheep. Osseointegration was evaluated histomorphometrically. A proposed classification of cartilage health adjacent to an implant was presented and implemented.

Results

Osseointegration was excellent, bone-to-implant contact was measured with a mean (95% confidence interval – CI) of 90.6% (79-102) at six months and 92.3% (89-95) at twelve months, respectively. Implant position correlated to opposing tibial cartilage damage, an implant should not protrude. Microscopic score as a function of implant position showed a linear relationship (P = 0.008) such that Mankin score increased by 4.3 units (95% CI: 1.5, 7.0) per each mm elevation in implant height. Using a dedicated guide the general implant position was consistent and adequate, and was on average 0.54 mm recessed (95% CI: 0.41, 0.67). Cartilage damage of the medial tibial plateau opposing the implant was increased compared to the non-operated knee by 1.77 units (P = 0.041; 95% CI: 0.08, 3.45) on a 0-27 unit scale. Remaining joint compartments were unaffected. Cartilage health adjacent to the implant was satisfactory and Hydroxiapatite (HA) improves chondrointegration. Untreated critical size cartilage defects did not heal at six-month follow-up.

Conclusions

Focal knee resurfacing with metal implants is a safe and viable treatment option for full depth focal condylar cartilage lesions. Cartilage damage evaluated microscopically was acceptable given implant position related to surrounding cartilage was optimal. Implant position is of utmost importance and individualised implants and instrumentations devices are recommended. An implant should never sit proud. Clinical studies of symptomatic focal cartilage lesions are recommended but prior to prophylactic treatment of asymptomatic patients more research is needed.

LIST OF SCIENTIFIC PAPERS

I. Focal knee resurfacing and effects of surgical precision on opposing cartilage. A pilot study on 12 sheep.
Martinez-Carranza N, Berg HE, Hultenby K, Nurmi-Sandh H, Ryd L, Lagerstedt AS.
Osteoarthritis Cartilage. 2013 May; 21(5): 739-45.

II. Fixation of a double-coated titanium-hydroxyapatite focal knee resurfacing implant: a 12-month study.
Martinez-Carranza N, Berg HE, Lagerstedt AS, Nurmi-Sandh H, Schupbach P, Ryd L.
Osteoarthritis Cartilage. 2014 Jun; 22(6): 836-44.

III. Treatment of full thickness focal cartilage lesions with a metallic resurfacing implant in a sheep animal model, 1-year evaluation.
Martinez-Carranza N, Ryd L, Hultenby K, Hedlund H, Nurmi-Sandh H, Lagerstedt AS, Schupbach P, Berg HE.
Osteoarthritis Cartilage. 2016 Mar; 24(3): 484-93.

IV. Cartilage health in knees treated with metal resurfacing implants or untreated focal cartilage lesions. A preclinical study in sheep.
Martinez-Carranza N, Hultenby K, Lagerstedt AS, Schupbach P, Berg HE.
In manuscript

CONTENTS

ABBREVIATIONS

AC	Articular Cartilage
ACI	Autologous Chondrocyte Implantation
B-SEM	Backscatter Electron Microscopy
CAD/CAM	Computer-Aided Design/ Computer-Aided Manufacturing
Co – Cr	Cobalt – Chrome
FEA	Finite Element Analysis
FKR	Focal Knee Resurfacing
FKRM	Focal Knee Resurfacing with Metal implants
GAG	Glycosaminoglycan
HA	Hydroxyapatite
ICRS	International Cartilage Research Society
LM	Light Microscopy
Micro-CT	Micro Computerised Tomography
MSC	Mesenchymal Stem Cells
OA	Osteoarthritis
OxZr	Oxidized Zirconium
PG	Proteoglycans
PMMA	Polymethylmethacrylate
SEM	Scanning Electron Microscopy
SPS	Spark Plasma Sintering

DEFINITIONS

Artefacts
Artefacts are structures or features in tissue that interfere with normal histological examination.

Bone ingrowth
New bone formation in intimate contact with the surface of the implant.

Critical size defect
Minimum cartilage defect (in diameter) that is repairable without intervention.

Finite Element analysis
Numerical technique for finding solution to partial differential equations. Widely used in science as a computational tool for engineering analysis such as deformation of cars due to crashes.

Histomorphometry
Is the quantitative study of the microscopic organisation and structure of tissues by computer-assisted analysis of images formed by microscopy.

Hydroxyapatite-calciumphosphate
The principal inorganic constituent of bone.

Implant
Medical device made from biomaterials, intended to use for repairing or replacing part of the body.

Osseointegration
Contact established between normal and remodelled bone and an implant surface without the interposition of non-bone or connective tissue, at the light microscopic level.

Osteolysis
Any focal area of bone loss adjacent to a prosthesis caused by the biological response to wear debris.

Press-fit
Insertion of an implant into an undersized premade space.

Uncemented
Implant designed for fixation by bone ingrowth.

Unipolar
Prosthesis used for hemiarthroplasties with no across the joint articulating component. A unipolar device can be of monoblock or modular design.

1 INTRODUCTION

1.1 BACKGROUND

1.1.1 Articular cartilage

The most common form of cartilage is hyaline (Greek: mirror like) or articular cartilage (AC) that in the adult is found in most synovial joints (Fig. 1)[1,2]. AC is a solid but flexible specialised connective tissue capable of enduring heavy loads over numerous and varying cycles throughout decades or even a lifetime. For instance the hip joint is loaded with approximately 250% of the body weight at normal walking speed[3]. On average, an adult walks 305 km per year, which corresponds to about halfway around the Earth during a lifetime[4]. AC has the appearance of a smooth, translucent and pearly bluish tissue, with a thickness that varies between species and different joints[5]. In humans AC is 2-4 mm thick[6]. The main function of AC is to provide a bearing surface that enables an almost frictionless movement between two opposing skeletal ends. Another function is to distribute transmitted loads towards the underlying bone during static loading as well as acting as a shock absorber under dynamic compression[7,8]. Also, fundamental for the protection against overload are the neuromuscular reflexes, the absence of which would be devastating for the joint as a consequence of stresses, strains and pressures of daily living[9]. The joint or articulation is a functional and structural unit situated at the point where two bones join for the purpose of body movement. Beside the two abutting bone ends the joint comprises of cartilage, capsule, ligaments and muscles (Fig. 2). The joint capsule has an inner layer, or synovial membrane, rich with blood and lymphatic vessels that serves as a diffusion membrane producing synovial fluid. The synovial fluid acts as a lubrication fluid further enhancing motion within the joint and serves as the only source of nutrition to the articular cartilage[10].

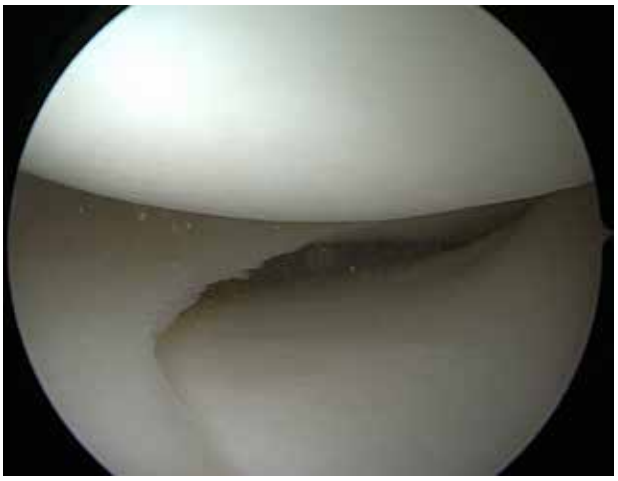


Figure 1. Normal articular cartilage in a human knee. Note the crescent-shaped fibrocartilaginous meniscus. Courtesy of Dr. Rikard Bessblad.

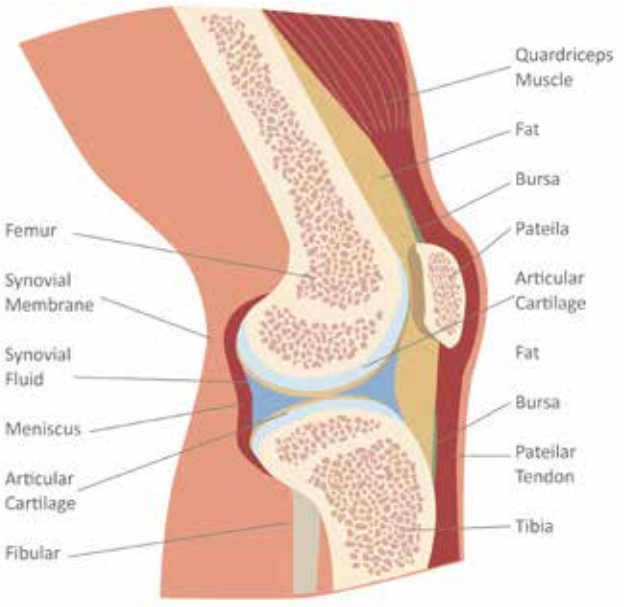


Figure 2. The joint – a functional unit designed for movement.

1.1.2 Histology

Cartilage tissue is composed of sparse chondrocytes entrapped in abundant extracellular matrix. In contrast to other connective tissues the cartilage is avascular, alymphatic and aneural, thereby described as an "isolated tissue deprived of communication with the vascular system"[11,12]. The physiological consequences of this are the lack of inflammation and pain in response to injury[13]. When observing AC with light microscopy (LM) it can be divided (for descriptive purpose) into three non-mineralised zones and one mineralised or calcified zone. The tidemark is seen as a wavy line that delineates where the non-calcified zone mineralises into the calcified zone. The superficial zone is thin, relatively cell rich with the collagen fibres oriented parallel to the articular surface. The intermediate zone is thick and the cells are less abundant than in the superficial zone, and here the fibres are oriented somewhat randomly in arcs. In the deep zone the cells and fibres are oriented perpendicular to the subchondral bone (Fig. 3)[1].This structural organisation is probably determined by the collagen fibres reflecting the tensile and compressive forces acting upon them (Fig. 4)[14]. The calcified zone enhances mechanical transition towards the underlying bone augmenting biochemical fixation and mechanical attachment between cartilage and bone[15]. This structural organisation of AC creates a stiffness gradient facilitating distribution of loads from the softer articular surface to the stiff cortical bone[14].

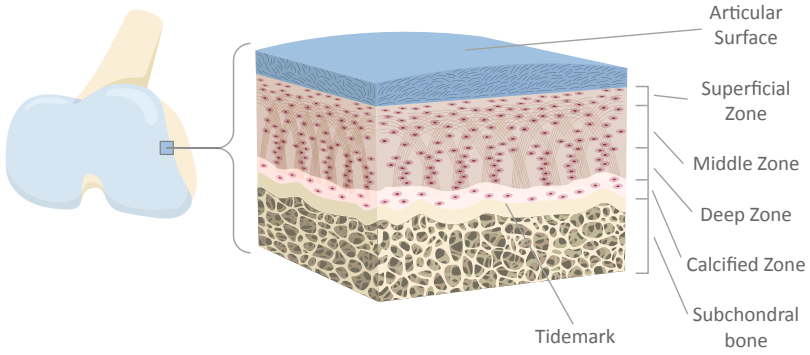


Figure 3. A schematic cross-section illustration of articular cartilage (AC).

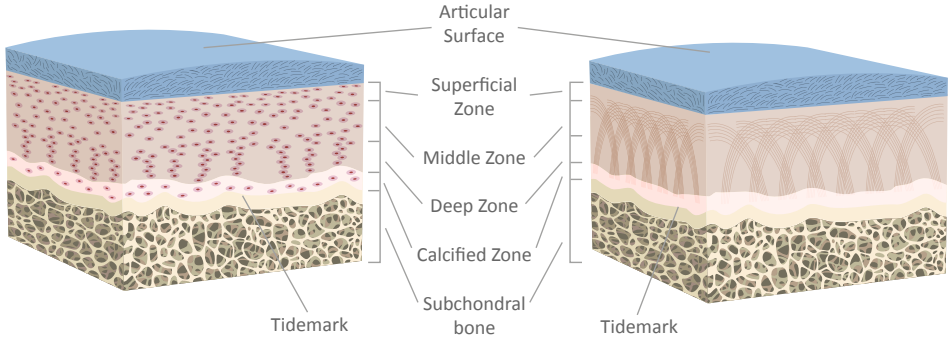


Figure 4. The architectural organisation of AC as a function of the loads acting on synovial joints.

1.1.3 Biochemistry of cartilage matrix

The matrix of the AC resembles a hyperhydrated gel composed mostly of water (65-80%) and a smaller proportion of solid mass. The matrix constituents are comprised mainly of collagens, proteoglycans (PG) and non-collagenous proteins (Fig. 5). The most abundant protein is the fibrillar type II collagen that enmeshes large aggregating hyalorunic proteoglycans. The PG´s are the second largest group of macromolecules in the AC composed by up to 100 aggrecans attached to a single hyaluronic strand via link proteins. The aggrecan consists of a core protein to which glycosaminoglycans (GAG) chains are attached. These chains are composed of 30-100 keratan sulfates and chondroitin sulfates that are repulsed from each other by their negative charges (Fig. 6). This high density in negative charges attracts massive volumes of water producing a swelling of the matrix that is only counteracted by the tensile properties of the collagen fibres[14]. This complex interaction is what enables the AC matrix to withstand the compressive forces acting on it while providing resilience and the capacity to recover shape when deformed[16]. The remaining AC constituents are smaller amounts of other components such as non-aggregating PG, non-collagenous proteins, minor collagens, lipids and minerals. Some of these plays a role in the interaction with collagens, fibrillogenesis and interfibrillar communication, while the function of the others are not completely understood[7].

Figure 5. Distribution of water and solids (left) and main components (right) of AC.

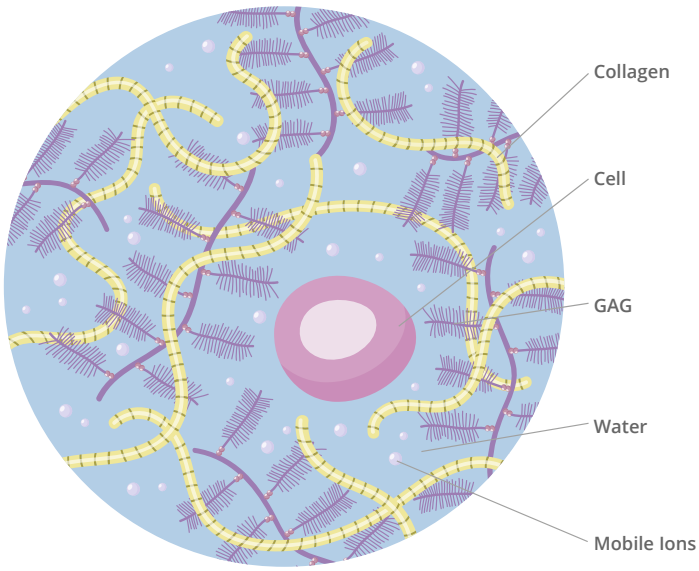


Figure 6. Molecular ultrastructure of articular cartilage.

1.1.4 Cartilage metabolism

The chondrocyte within the matrix is a metabolically active cell albeit the inert appearance of cartilage tissue[11]. AC turnover is a function of the synthesis of matrix molecules that are lost through degeneration or injury. Unfortunately the healing power of AC is remarkably ineffectual considering the harsh biomechanical environment acting upon it[13]. Needless to say, the most limiting factor in this meagre healing process is the intrinsic avascular nature of AC. The necessary nutrients for restorative endeavour must therefore reach the chondrocyte by diffusion from the synovial fluid. As the chondrocyte is entrapped in the pericellular matrix, it is unable to migrate and therefore the nutrient must traverse the matrix to reach the chondrocyte[17]. The passage of nutrients to the cells is restrained by size, charge and configuration of the matrix leaving an estimated pore size of 6 nm[7,18]. Pressure loads acting upon the AC creates mechanical, electrical and chemical signals that helps the chondrocyte direct the synthesis of the matrix (Fig. 7)[19]. With ageing, the matrix composition changes and the chondrocytes lose the ability to respond to these stimuli[20].

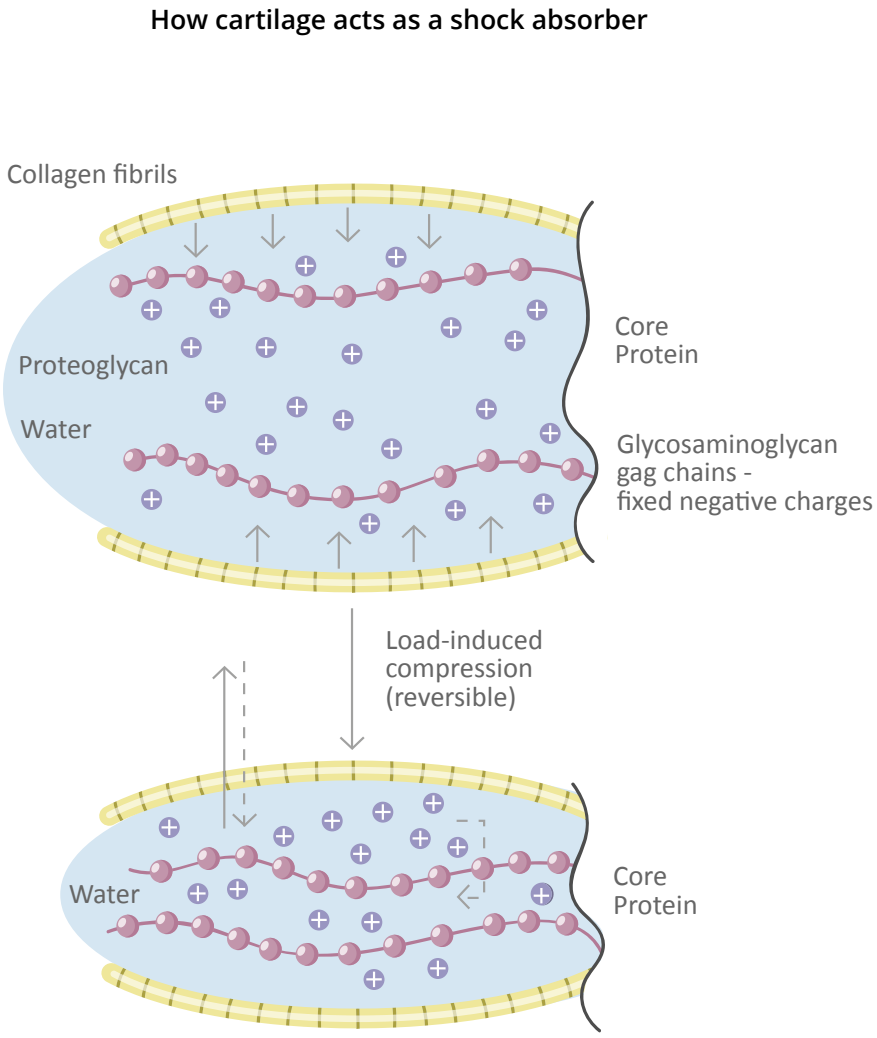


Figure 7. Cartilage metabolism directed by forces acting on the tissue.

1.1.5 Cartilage injury and repair

AC endures incredibly high mechanical stresses such as those caused by gravity and muscle contraction. It has been stipulated that human articular cartilage resists impacts in the range of 25 N/mm^2 (2.5 kg/mm^2) without apparent damage[21]. When impacts to the articular surface exceed the AC capacity to absorb the load (by deformation, dampening and distribution of fluids) it eventually ruptures. Typically, like in the situation of degenerative joint diseases, changes in matrix composition, loss of molecules and metabolic imbalance between degradation and synthesis precede any visual sign of injury (Fig. 8). This process, when it develops, appears to be driven by a nucleus of chondrocytes in the affected area that starts to express an alternative part of the genome and produce matrix degrading enzymes, collagenases and metallo-proteases[22]. These enzymes also leach out into the joint fluid affecting parts of the joint distant from the original injury. The ability of the chondrocytes to maintain the extracellular matrix depends on their capability to detect changes in matrix composition and to respond by synthesis and assembly of the components necessary for the restoration[23]. The success rate of this healing process is correlated to the size of the lesion and the age of the individual[20,24]. The newly formed AC is unfortunately often fibrocartilaginous in nature and rarely replicates the elaborate structure of the original macromolecular network. The biomechanical consequence is augmented permeability hence increased vulnerability to repeated trauma, which probably is responsible for the long-term deterioration and OA as an end result[25]. As early as 1743, Hunter observed that cartilage '(…) once destroyed it is not repaired' [26]. This principle has not changed since.

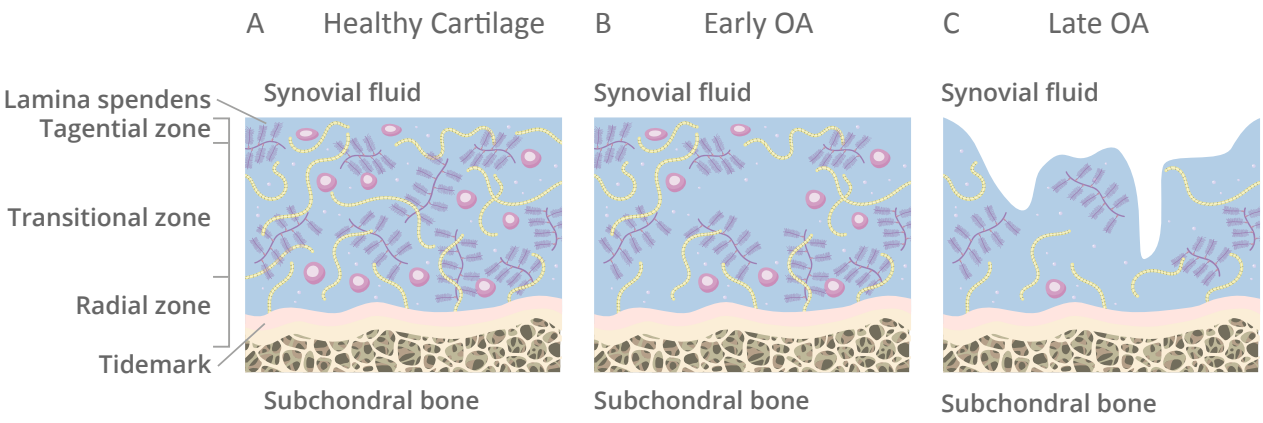
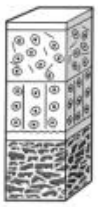


Figure 8. Cartilage degeneration and loss of macromolecules as a portend to OA.

1.1.6 Prevalence and classification of cartilage lesions

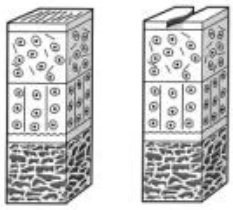
The aetiology and natural history of AC injuries are not completely understood. Also, the difficulty in diagnosing these injuries makes it problematic to establish their relationship to the development of OA. Acute or repetitive trauma can cause mechanical disruption such as fissures, tears and focal cartilage defects[27]. Sports injuries as well as an increased activity level in an aging population have put this pathology on the rise. AC lesions can be divided in partial and full thickness injuries depending on the depth of the cartilage injury. Contained, or focal AC lesions can further be classified arthroscopically according to Outerbridge or the International Cartilage Research Society (ICRS) score (Fig. 9)[28]. Briefly, grades 1-2 are superficial lesions and generally do not require surgical treatment. Grades 3-4 are deep fissures or exposed bone respectively and thereby potential candidates for surgical repair (Fig. 10).

ICRS Grade 0 - Normal



ICRS Grade 1 – Nearly Normal

Superficial lesions. Soft indentation (A) and/or superficial fissures and cracks (B)

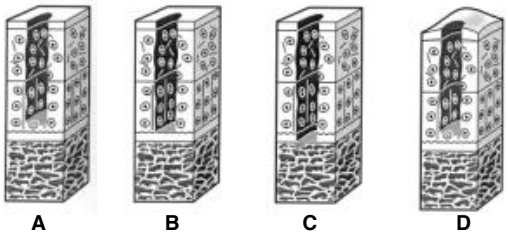


ICRS Grade 2 – Abnormal

Lesions extending down to <50% of cartilage depth

ICRS Grade 3 – Severely Abnormal

Cartilage defects extending down >50% of cartilage depth (A) as well as down to calcified layer (B) and down to but not through the subchondral bone (C). Blisters are included in this Grade (D)



ICRS Grade 4 – Severely Abnormal

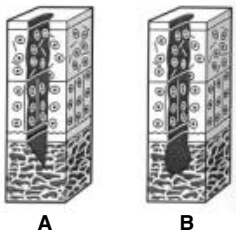


Figure 9. ICRS cartilage injury classification. Reprinted with permission from ICRS.

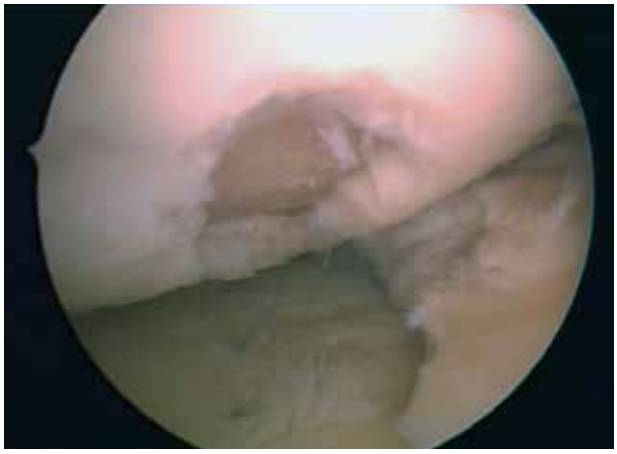


Figure 10. A full depth cartilage lesion in a femoral condyle. Courtesy of Dr. Leif Ryd.

Cartilage repair surgery is most suitable for patients younger than 40 and up to 50 years with a single focal lesion grade 3-4 of an area of 1 cm² or more[29]. The few existing epidemiological studies on cartilage injuries provide data on the incidence of cartilage lesions, indicating the approximate number of candidates for cartilage repair[29-32]. In these studies based on 1,000-31,000 arthroscopies the prevalence of chondral lesions of any type was reported to be about 60% of all arthroscopies performed for any reason. The most common reason for the arthroscopy was trauma (58-61%). Associated injuries such as meniscal tear or ligament injury were present in about 60-70% of all cartilage lesions. Totally 20-30% of all cartilage lesions were single focal lesions, the remaining were multiple lesions. The medial femoral condyle was the most frequent localisation (34-58%), and the second most frequent localisation was the patella-trochlear region. The size of the focal chondral lesions ranged from 0.5-1 cm^2 in about 25% of the cases, between 1-2 cm^2 in 29-42% of the cases, and lesions larger than 2 cm^2 were reported in 7-12% of the cases. The most common type of lesion was partial thickness grade 1-2 found in approximately 40-65% of the cases. Grade 3-4 accounted for 36-60% of the lesions (Table 1).

In summary, focal cartilage lesions grade 3-4 with an area of at least 1 cm² in patients under the age of 40 years and up to the age of 50 years accounted for 5-7% and 9-13% of all arthroscopies respectively. As aforementioned these lesions left untreated do not heal or only partially heal, and even after various kind of treatment they might progress to OA. Hence, it is generally accepted that these lesions portend to the origination of OA.

Table 1 - Summary of cartilage lesions at arthroscopy	
Frequency	Cartilage lesions were found in approximately 60% of all arthroscopies.
Indication for arthroscopy	The cause of arthroscopy was trauma in about 60%.
Associated injuries	These were found in about 60% of the cases.
Type of lesion	Focal lesions accounted for approximately 30% of the cartilage lesions.
Localisation	The most frequent localisation was the medial femoral condyle.
Size of the lesion	The most frequent size was an area of about 1-2 cm^2 (30%).
Classification	Superficial lesions accounted for 50% of the cases.

Table 1. Characteristics of lesions encountered in arthroscopies performed for any reason.

1.1.7 Biological treatment

A better understanding of the limitations in the natural repair process has contributed to the interest during the last three decades in the repair and regeneration of articular cartilage tissue. There is scientific evidence that transplanted chondrocytes, perichondrium, periosteum, growth factors and artificial matrices can stimulate neoformation of cartilaginous tissue[27].

1.1.7.1 Abrasion chondroplasty, Pridie drilling and microfracturing

Abrasion chondroplasty, Pridie drilling and microfracturing are three methods which all disrupt the subchondral bone plate (Fig. 11), permitting a healing process stimulated by the vascular system[13,33-35]. The cartilage defect is filled with a reparative tissue that undergoes a maturation process to reconstitute a hyaline-like fibrocartilaginous tissue[36]. This reparative tissue is composed of larger proportions of type I collagen and less PG than normal hyaline cartilage[36]. The success rate of this healing tissue is highly variable depending on the severity of the lesion, age of the patient, physical activity and follow-up time[32]. These techniques have been advocated mainly for patients younger than 40 years and with rather small lesions[37].

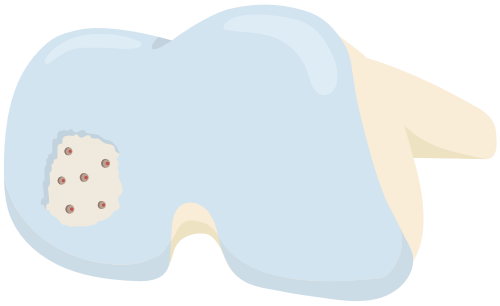


Figure 11. A focal cartilage lesion treated with perforation of the subchondral bone plate; microfracturing.

1.1.7.2 Osteochondral autografting and allografting

Autologous mosaicplasty is more commonly used than allogeneic techniques. It implicates resurfacing a defect with multiple osteochondral plugs (Fig. 12)[38,39]. The main drawback is donor site morbidity since the plugs are surgically removed from an already diseased joint, and given their size it is unlikely that the donor site will heal[8]. The viability of the plugs, particularly at the border, is possibly impaired and further jeopardised by the hammering process to press-fit them into the defect[40,41]. Once in place the plugs resemble a cobblestone surface with questionable nutritional capacity to mutually adhere and with a considerable height mismatch relative to surrounding and opposing cartilage surfaces[42].

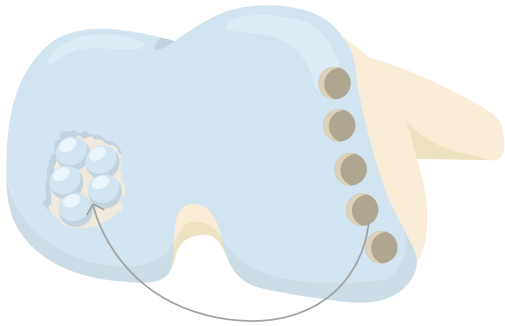


Figure 12. Mosaicplasty of a chondral lesion. Note donor site morbidity.

Allographic osteochondral plugs are similar to autologous plugs but are intended to act as a mechanical substitution of the defect as these do not stimulate a cartilage repair response. These can be used to restore bone stock in complex salvage procedures[43,44]. This method has performed well in clinical settings even at long-term follow-up but might have the drawback of immunological reaction that *in vivo* is less extensive when compared to experimental settings[45,46]. Another disadvantage of a more generalised implementation of this technique is the shortage and storage inconvenience of the cryopreserved osteochondral plugs.

1.1.7.3 Autologous chondrocyte implantation

In autologous chondrocyte implantation (ACI), healthy cartilage is obtained arthroscopically and subsequently enzymatically digested to release chondrocytes, which are expanded in culture for about 11-21 days. This will generate approximately a 10-fold increase in the number of cells obtained

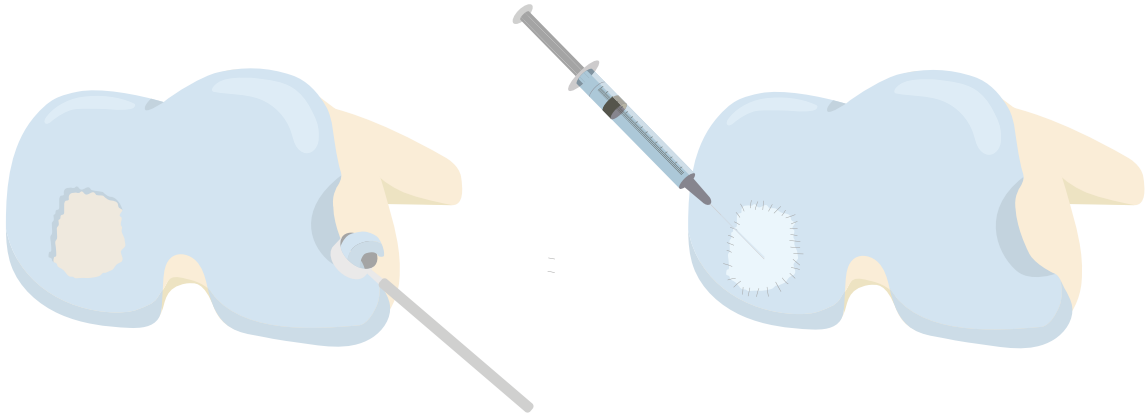


Figure 13. A schematic illustration of the harvesting and implantation of autologous chondrocytes.

from the biopsy. A suspension with about 2.5-5 million cells is then transplanted directly into the defect beneath the coverage of a sutured-periosteal flap (Fig. 13)[46,48]. A matrix-associated variant (MACI) was introduced to ACI, where the chondrocytes are embedded in a gel-like matrix rather than a suspension and thereby avoid the need for periosteal flaps[49]. More recently these methods have been refined by the selection of a subgroup of chondrocytes from the harvested cell (characterised), which has a high chondrogenic capacity, so-called characterised chondrocyte implantation (CCI)[50].

In a Cochrane review from 2011 six heterogeneous randomised trials compared ACI to autologous mosaicplasty (three trials) and to microfracture (three trials). The authors concluded that there was insufficient scientific evidence to state whether ACI is superior to the other cartilage repair interventions[51]. Nevertheless there is some evidence from a well-performed RCT that CCI combined with MACI performs better than microfracture at two-year follow-up in terms of pain and function[52].

1.1.7.4 Tissue engineering strategies

With the three key constituents cells, matrix scaffold and signalling molecules, tissue-engineering researchers aim to regenerate functional and enduring cartilage tissue[53,54]. Most of the research so far has been conducted in a laboratory setting, although some work with animals and humans has been conducted. Bone marrow-derived *mesenchymal stem cells* (MSCs) can differentiate to chondrocytes in the presence of stimulation factors or signalling molecules such as growth factors[16]. A *cell carrier or matrix* (scaffold) enhances the process of cartilage tissue growth by force of its peculiar geometrical configuration, stimulating cell attachment and binding of growth factors[55]. *Signalling molecules* specifically guide the course of differentiation in the desired (chondrogenic) direction[56]. These building blocks can be assembled and fully differentiated *in vitro* and the final implant can then be transplanted to the defect. The drawback is the difficulty to fixate and fit the implant to the natural curve of the condyle. The basic building blocks (matrix, cells and signalling molecules) can be produced *in vitro* and thereafter assembled and differentiated *in vivo* (in the cartilage defect). This will more likely adhere and follow the natural curve of the condyle but will differentiate less accurately to the desired tissue. At last, growth factors (no cells) in a matrix can be applied *in situ* to a defect stimulating migration and proliferation of MSCs[57].

1.1.8 Focal knee resurfacing with metal implants

Biological treatment methods are less effective with increasing patient age and they all claim a maturation process of 12-14 months[58]. Primary joint replacement is not ofthen indicated for the middle-aged patients with cartilage lesions. Moreover, joint replacement in the middle-aged patient with early OA has shown inferior results with increased risk for revision surgery and high morbidity[59]. Therefore there is a treatment gap for the middle-aged patients with symptomatic full depth focal cartilage lesions[59,60]. An attractive alternative treatment strategy is to resurface the cartilage defect with a metal implant (FKRM). When we started this project in 2007 we were aware of only two other groups in the world investigating this concept[61,62]. FKRM can be regarded as the final attempt at joint preservation, and consequently the salvage procedure for a failed resurfacing implant is a joint replacement. A provisional treatment with FKRM will hopefully delay or indefinitely postpone the need for arthroplasty. There are three clinical trials (level of evidence IV) performed where cartilage defects are resurfaced with metal implants showing promising results in terms of improved pain and function[63-65]. A worrisome revision rate of 28% has been reported that was primarily caused by progression to OA indicating that FKRM does not halt OA development in all cases[66]. Hence, caution is warranted when selecting patients for this treatment, moreover patients should be properly informed on the possible temporary effect of this procedure[64]. Finally, resurfacing a full thickness focal cartilage lesion in the knee with a metallic implant articulating against the opposing tibial cartilage (unipolar) requires three fundamentals to succeed. First, the implant must bond to the host bone. Second, the surrounding cartilage should not be damaged but rather adhere to the implant. Third, it is imperative that the opposing cartilage withstands the new biomaterial over time (Fig. 14).

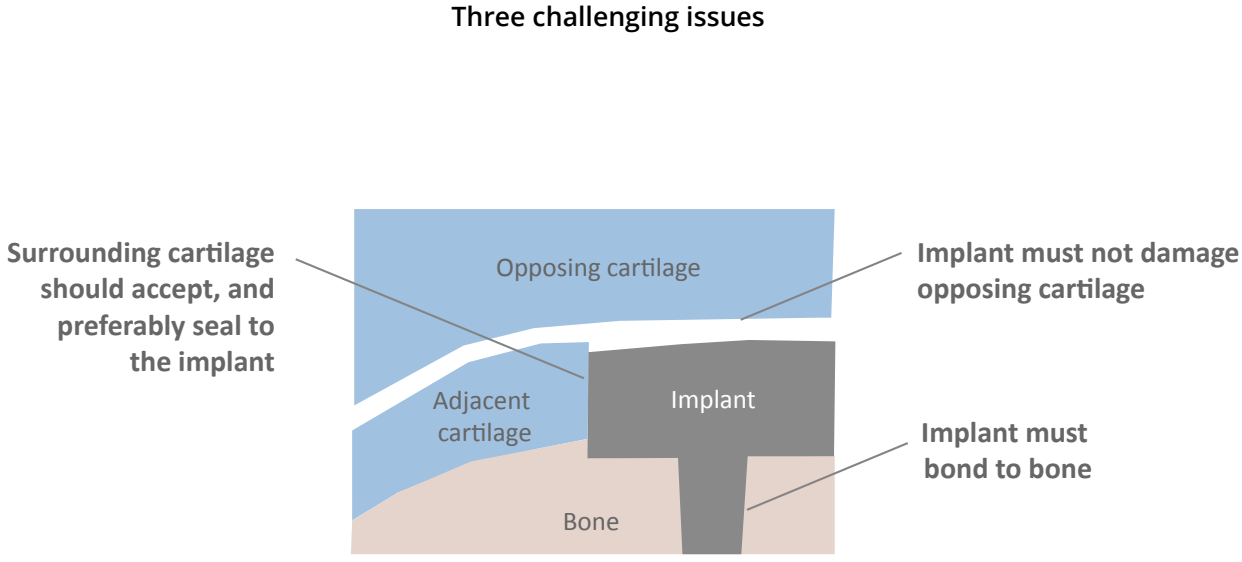


Figure 14. A schematic illustration showing the three major challenges when treating full depth focal cartilage defects with metal implants.

2 GENERAL AIMS

1. Develop a new treatment concept including an implant and instrumentation device for the treatment of femoral condylar full depth focal cartilage lesions.

2. Bridge the gap from technological development to clinical application by assessing the biological safety of the implant using a sheep animal model.

3. Provide scientific data for the decision to enter clinical trials using FKRM implants with the aim to reduce pain and restore function in an active middle-aged patient group with symptomatic deep focal femoral condylar lesions, this remains a clinical challenge.

3 HYPOTHESES

1. Osseointegration is obtained for an uncemented press-fit implant with a double coated Ti and HA layer on a Co – Cr implant that is immediately loaded post-operatively.

2. Cartilage remains healthy adjacent to the implant while full depth untreated cartilage defects do not heal.

3. Cartilage damage of the surface opposing a metal implant of optimal fit and ideal position is acceptable.

4. Joint cartilage homeostasis is not disturbed in knees treated with a metal implant.

4 IMPLANT, ANIMALS AND METHODS

4.1 IMPLANT

A medical and technological interdisciplinary team was created to develop a new treatment concept for sealing focal cartilage defects with metal implants (Fig. 15). The introduced (first generation) implant (Fig. 16) was designed as a spherical monobloc implant of functional gradient material (FGM). FGM is characterised by the combination of different materials with a gradient across different levels. Specifically, the articulating superficial layer was made of pure stainless steel (316L) that was gradually replaced with bioactive hydroxyapatite (HA) towards the bone. Because HA is brittle, a blend of 50% HA and 50% stainless steel achieved mechanical stability although remaining osteoconductive. This characteristic allows the implant to benefit from a specific feature of each material according to its particular purpose. The first FGM implant was manually manufactured using the Spark Plasma Sintering (SPS) technique characterised by sintering of the metal powder by high pressure in combination with direct electrical current at high voltage. The outer diameter of the implant was 10 mm and the peg measured 10 mm in length and 2 mm in diameter. The radius of the curvature was 17 mm in all planes. The articulating surface of the implant was manually polished to a roughness (Ra) < 0.03 (Fig. 16). The first generation FGM implant was successful from a fixation point of view according to pull-out tests (unpublished results) performed at the Royal Institute of Technology, Stockholm (KTH). However, the FGM implant development was not practical from a manufacturing point of view as they were

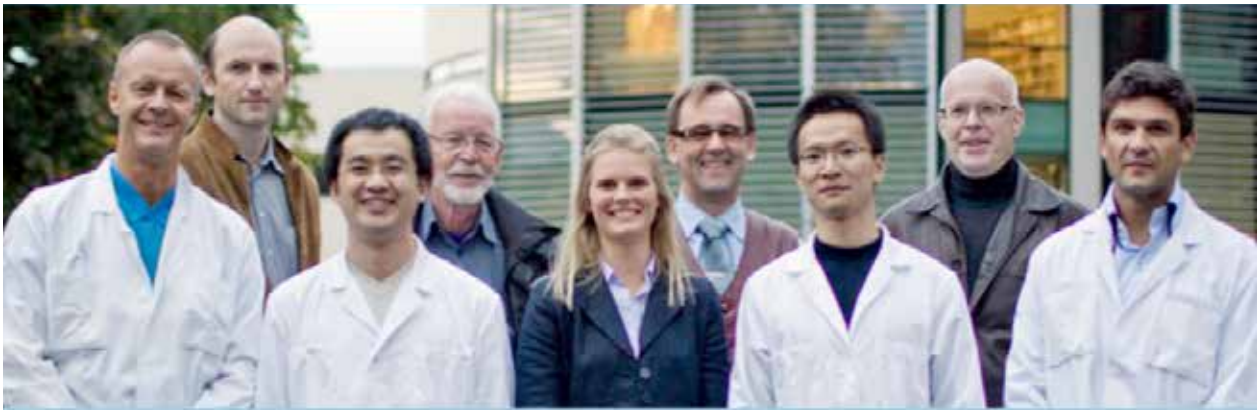


Figure 15. A medical-technological interdisciplinary team consisting of engineers, scientists and staff from Episurf Medical AB.

First generation

Episealer ® implant

Figure 16. Pictures showing the first generation (left) and second generation (right) Episealer ® implant. Courtesy Episurf Medial AB, Stockholm.

Figure 17. Immersion test performed at KTH showing galvanic corrosion of the implant (to the left).

manually produced and polished. Additionally, the SPS machine is only available for research-scale productions. Hence it was decided to abandon the FGM material in favour of time-honoured cobalt – chrome (Cr – Co) alloy.

After completing the first part of our pilot study we observed some yellow secretion on the deeper part of the firts generation implant. Analyses performed at KTH (unpublished results) showed galvanic corrosion, which occurs when two different metals are combined in a corrosive electrolyte (Fig. 17).

The second-generation implant was designed as a double-curved monobloc Cr – Co implant. The monobloc core was plasma sprayed with a double-coating of titanium (Ti) and HA for long-term fixation purposes. First we used a layer of commercially pure titanium (60 um) onto which a second layer of HA (60 um) was plasma sprayed (Plasma Biotal Ltd., Buxton, GBR). These implants were manufactured with a computer-aided design and manufacturing (CAD/CAM) technique modelled from one "standard" sheep knee as captured from CT DICOM-files. The second generation FKR implant had radii of 12 and 19 mm that followed the sagittal and transversal condylar curvature (respectively) of the standard sheep. The hat of the implant had a diameter of 7.5 mm. For primary fixation the implant had a peg that measured 10 mm in length and 2.0 mm in diameter, intended to be press-fit into an undersized drill hole of 1.8 mm (Fig. 16).

4.1.1 Titanium and hydroxyapatite coating

A prerequisite for the long-term success of orthopaedic and dental implants is the permanent fixation to the bone without intervening soft-tissue, so called osseointegration, which was first described by Brånemark[67]. Prosthesis and implants can be implanted into the body either with cement using polymethylmethacrylate (PMMA) or without PMMA (cementless fixation). We used the latter since it has been suggested that PMMA is not suitable for fixation in young patients (< 50 years) who require a more stable fixation where bone ingrowth is desirable. Drawbacks with PMMA such as cell necrosis, endosteal bone resorption and cement failure have been reported[68]. HA or calcium phosphate ($Ca_{10}(PO_4)_6(OH)_2$) is a bone mineral and constitutes 50% of the human bone volume and this molecule can be synthetized artificially. HA has an osteoconductive property that enhances the bonding of bone (osseointegration) and is therefore widely used in grafts for bone repair or coatings on metal implants (Fig. 18)[69,70].

However, HA might resorb or degrade in biological settings and subsequent disintegration could compromise implant fixation[71]. Chemical composition, mechanical characteristics, crystallinity, porosity, thickness and roughness as well as the material and texture of the implant will influence HA´s performance in terms of osseointegration. It is recommended to have as high calcium phosphate purity as possible (95-97%) with a Ca/P ratio of

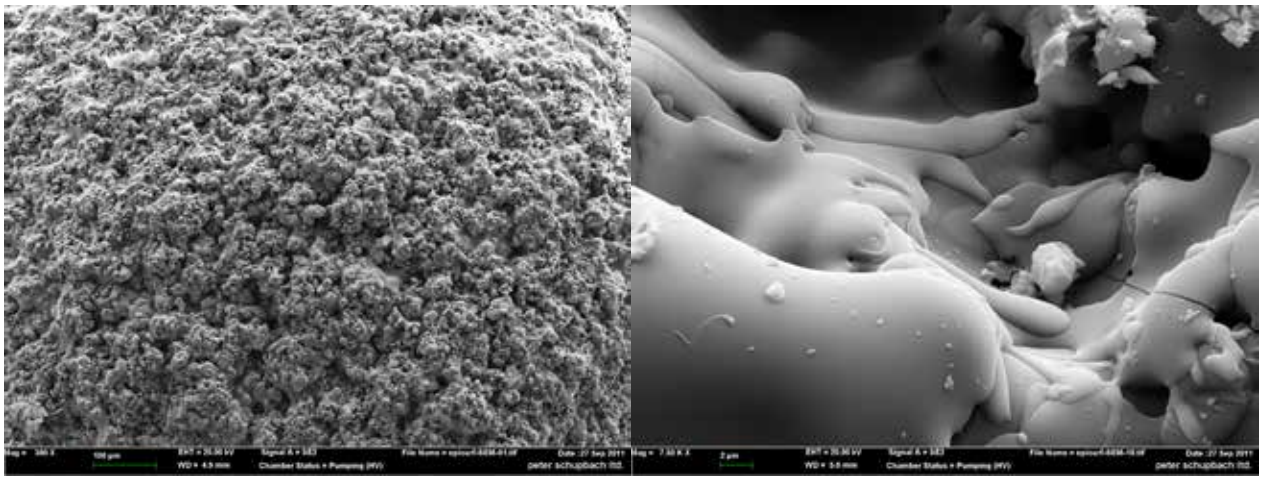


Figure 18. A scanning electron microscopy picture showing the Episealer® HA layer (left) at higher magnification (right).

1.76. We followed international standards; HA purity was in the order of 95%, Ca/P ratio was 1.67 and cristallinity 60% (ASTM 88). High crystallinity with low porosity is mechanically stable but less bioactive (less osteoconductive) and might induce delamination of larger HA fragments that cannot be dissolved by phagocytosis. This could locally decrease pH further stimulating HA dissolution or hypothetically migrate to the joint, and potentially act as third-body wear particles. A thicker coating larger than 100 μm might also be predisposed to mechanical fatigue stress, therefore a 50 μm thick coatings has been recommended[72]. Also, high surface roughness increases contact with body fluid enhancing bioactivity through dissolution and apatite precipitation. Finally, the choice of implant material and texture is of outmost importance for osseointegration. The most common biomaterials used in orthopaedics are Co – Cr or Ti alloys as they are biocompatible with favourable corrosion properties and fatigue strength. Both are stiffer than bone but Ti alloys produce less stress shielding and have a higher bonding strength compared to HA[73]. The surface of the metal used for uncemented fixation can be treated either by grit-blasting or beading to promote osseointegration. A porous coating can induce osseointegration *per se* and is therefore more effective than grit-blasting. Nevertheless, it has been shown that a porous coating alone without HA can induce a fibrous fixation rather than bone ingrowth especially if gaps and micro-motion are present at the implant-bone interface[74]. HA limits the formation and stimulates the conversion of fibrous tissue to bone even in the presence of gaps up to 1 mm, both in unloading and loading conditions[75-77]. This circumferential bone apposition (sealing effect) prevents polyethylene, metal debris or other fluids to penetrate along the bone-implant interface[78,79].

4.2 ANIMAL MODELS

Translational research bridges basic science with clinical trials and often involves animal studies to evaluate the safety and efficacy of novel treatment methods as well as the optimisation of existing ones before application in humans. The common descent of all living organisms makes this strategy possible in combination with the conservation of metabolic and developmental pathways as parts of the evolutionary principle. Studying model organisms can be informative, but care must be taken when extrapolating from one organism to another[80]. For ethical reasons and to optimise the development of new treatment possibilities, animal studies are critical in knee pathophysiology research[81]. Each animal model has its own advantages and draw-backs. The cartilage of small animals (mice, rats and rabbits) is very thin making these animals inferior for use in the study of surgical implants. Large animal models offer thicker cartilage but require more logistical, financial and ethical considerations. Dogs are well suited for specific rehabilitation protocols such as treadmills but exhibit thin cartilage and require more ethical considerations due to their status as companion animals. Pigs are similar to humans with respect to weight-bearing conditions and cartilage thickness but their large size and aggressiveness makes them difficult to handle in research facilities. Within the horseracing industry, much research has been done on cartilage lesions[82]. While cartilage defects with a similar volume as in humans can be obtained, the loading condition of the stifle joint will however expose the knee to loads greater than in humans.

4.2.1 Sheep

Sheep (Ovis aries) are quadrupedal mammals commonly kept as livestock. They re-chew plant matter to stimulate digestion, a process called rumination. The height and weight of sheep depends on the breed. Ewes (adult female sheep) typically weigh 45-100 kg and rams (adult male sheep) 45-160 kg. They generally live for 10-12 years, although some sheep live as long as 20 years (Fig. 19). Sheep and goats are commonly used models when studying chondral repair of partial and full thickness cartilage lesions. They have the advantage of being large animals with a similar knee anatomy to humans. In addition, the biomechanics are comparable to humans regarding weight-bearing, joint size and cartilage thickness (0.5-1.5 mm). Also, they have limited intrinsic healing capacity (a critical size defect is approximately 6 mm). The caprine model allows surgically created defects with a diameter of 4-15 mm, but not larger-sized lesions that are considered relevant for humans. Goats have a thicker cartilage layer, but on the other hand they may develop OA by the age of 2 years[61,83,84], which might jeopardise the evaluation of treatment effects. Finally, another benefit of using sheep is that there are well-established protocols for anaesthesia and analgesia. Together with simple housing conditions, they make well-powered studies possible[85]. We chose Swedish landrace ewes between 2-6 years (skeletally mature "middle-age" animals) for the experiments in our studies.

Figure 19. A sheep, operated unilaterally with a FKRM implant in the right stifle joint (note bandage).

4.2.2 Ethics

In 1822, the British Parliament enacted the first law for animal protection preventing cruelty to cattle (British Animal Protection Legislation), being the oldest known debate on ethics when using animals in research. In Sweden, a local ethics committee, which serves directly under The Swedish Board of Agriculture, upholds guidelines and standards for research which intends to use animals. Research must prove the potential for benefit to human health, the minimisation of pain and distress, and timely and humane euthanasia. In addition, experimenters must justify their protocols based on the principles of Replacement, Reduction and Refinement[86]. Replacement refers to efforts to engage alternative methods to animal use. For instance, this could include the use of computer models, non-living tissues and cells, and when animal models cannot be avoided, replacement of "higher-order" animals (primates and mammals) with "lower" order animals (e.g. cold-blooded animals, invertebrates, bacteria). Reduction refers to efforts to minimise the number of animals used in an experiment, as well as prevention of unnecessary replication of previous experiments. Finally, refinement refers to efforts to make experimental designs as painless and efficient as possible. We obtained ethical committee approval by Uppsala Djurförsöksetiska Nämnd (Uppsala Animal Experiments Ethical Board, Dnr. C271/8 and C135/10).

4.2.3 Summary of all animals

Thirty-seven female sheep were used in this thesis. The animals were operated with an implant in the weight-bearing surface of the medial femoral condyle in one or both knees. In the pilot study they were operated bilaterally with implants. In the last study (Paper IV) they were operated bilaterally, with an implant in one knee and with an iatrogenic full-depth lesion as a control defect in the other knee. The remaining sheep (Paper II-III) were operated unilaterally using the contralateral knee as a control. The first 28 sheep came from one breeder and the remaining nine sheep came from two other breeders. The age of the sheep ranged from 2-7 years and weighed in the range of 60-99 kg. All animals were housed at the department of Clinical Science, Swedish University of Agriculture Sciences (SLU) in Uppsala, Sweden. They were kept indoors in stables in groups of three, and for the long-term studies (Paper II-IV) they were kept in an outdoor grass paddock with a windbreak after the first two weeks. Food was given twice a day and water was freely available. The animals were well acquainted with the persons handling them and they were observed daily to monitor their general condition, signs of pain and lameness (Fig. 20).

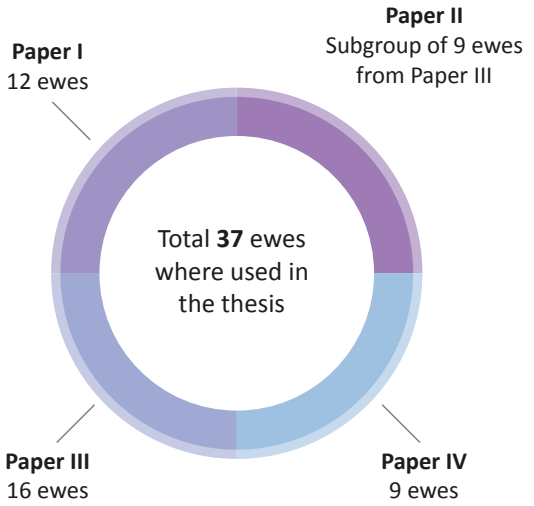


Figure 20. Summary of all animals used in this thesis.

4.2.3.1 Paper I (pilot study)

Twelve healthy Swedish landrace ewes from the same breeder were operated bilaterally with an implant between March and December 2009. The mean age and weight of the sheep were 5 years (range 5-7) and 82.5 kg (range 70-99). The animals were operated in two different time periods. The first batch of six animals, batch 1, were operated bilaterally with first generation implants in March 2009 and euthanized in April and June 2009 respectively. Three of them were euthanized six weeks after surgery and three were euthanized at three months after surgery. The second batch of six sheep, batch 2, were operated bilaterally with second generation implants in December 2009. One died immediately postoperatively, thus five sheep were euthanized six weeks after surgery in January 2010.

In summary, a total of eleven sheep operated bilaterally (22 implants) were included in the analysis. Of these, eight animals were sacrificed six weeks after surgery (16 implants) and three animals were sacrificed three month after surgery (six implants). These animals were pooled together as one group and presented in Paper I (Fig. 21).

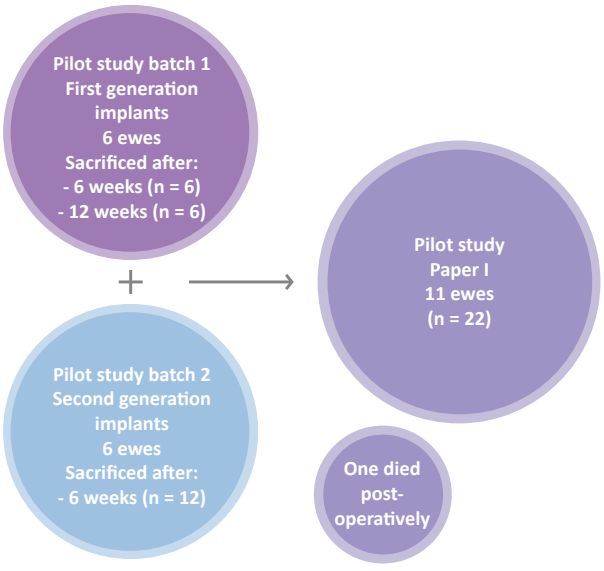


Figure 21. Animals used in Paper I (n = implant).

4.2.3.2 Paper II-III (preclinical studies)

Sixteen healthy Swedish landrace ewes from the same breeder were operated unilaterally with a second-generation implant using the opposite knee as the control knee, between June and December 2010. The mean age and weight of the sheep were 4 years (range 2-6) and 82.5 kg (range 70-99), respectively. These animals were also operated in two different time periods. The first batch of ten animals was operated in June 2010. One died two months postoperatively. From the first batch three animals were sacrificed six months after surgery, and the remaining six animals were euthanized one year after surgery. The second batch of six animals was operated in December 2010. From the second batch, two animals died and the remaining four were euthanized six months after surgery.

In summary, 16 ewes were used for Paper III, out of which three died. One died immediately postoperatively and two were sacrificed at two months due to pneumonia and septic arthritis respectively. Thus, thirteen sheep operated unilaterally using the contralateral side as a control were used in the analysis. Of these thirteen animals, seven were sacrificed six months postoperatively and six animals were sacrificed one year postoperatively. The animals were pooled together as one group and presented in the analysis of Paper III (Fig. 22).

A subgroup of nine animals from Paper III (batch 1) was used in Paper II (Fig. 23).

4.2.3.3 Paper IV (comparative study)

In Paper IV, nine healthy Swedish landrace ewes from two different breeders were operated bilaterally with an implant in one knee and with an iatrogenically produced full-depth lesion (the size of the implant, 7.5 mm) as a defect control in the other knee. Special care was taken not to penetrate the subchondral bone when producing the full-depth focal lesion. The nine animals were operated in April 2011. One of the nine ewes died immediately postoperatively and two others were sacrificed two months after surgery due to pneumonia and severe limp, respectively. The remaining six animals were euthanized at six months and used for the analysis of Paper IV (Fig. 24).

In addition to these six ewes, six matched ewes from Paper III were included for analysis of cartilage health adjacent to the implant (n = 12).

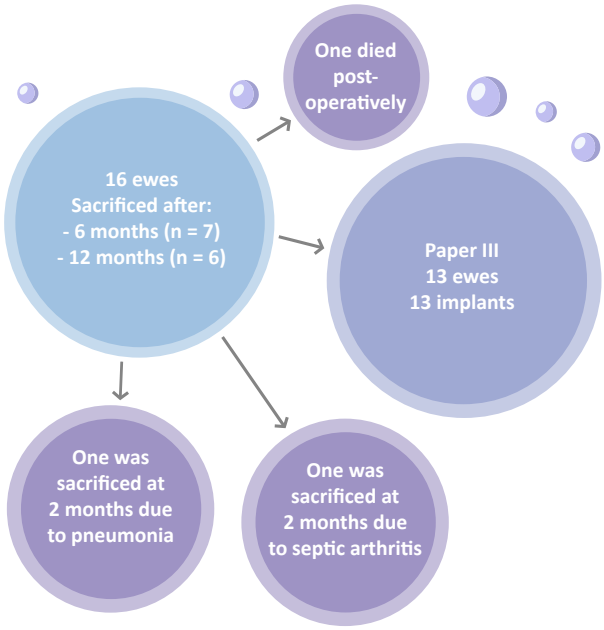


Figure 22. Animals used in Paper III (n = number of animals).

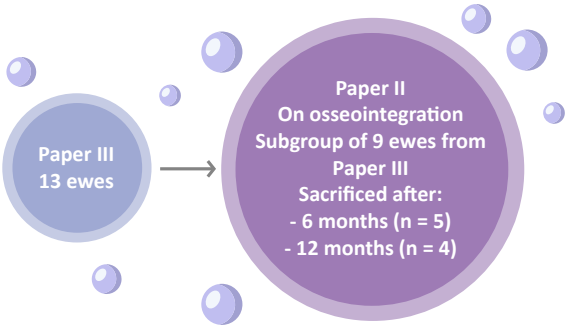


Figure 23. Animals used in Paper II (n = number of animals).

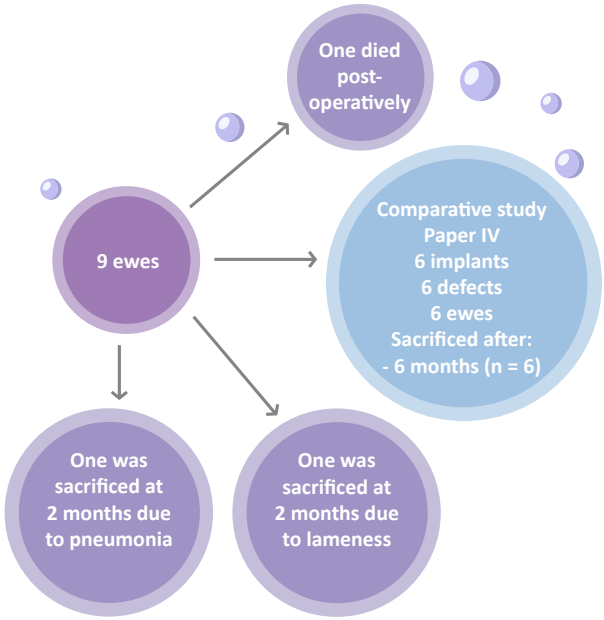


Figure 24. Animals used in Paper IV (n = number of animals).

4.3 METHODS

4.3.1 Anaesthesia in sheep

There are some anaesthesiological difficulties in sheep and other ruminants that are not encountered in simple stomached animals that require special consideration. The rumen content may be regurgitated during anaesthesia since it cannot be emptied by pre-operative starvation. Hence, precautions must be taken to ensure that rumen contents are not aspirated. Also, gas will build up in the rumen inducing swelling that reduces lung capacity and impairs ventilation. Sheep salivate constantly during general anaesthesia; therefore endotracheal intubation with a cuffed tube is essential whenever general anaesthesia is induced in adult ruminants. Additionally a gastric tube should be placed to avoid distension and regurgitation. Risks of regurgitation, salivation, aspiration and ruminal distension are also present during recovery (Fig. 25). For anaesthesia protocol see Methods in Paper I-IV.

Figure 25. Professor Anne-Sofi Lagerstedt providing anaesthesia to a sheep.

4.3.2 Surgery

All operations were carried out under aseptic conditions by the same surgeons (HNS, NMC and LR). The medial femoral condyle was exposed through a medial parapatellar 5-6 cm incision through skin and subcutaneous tissue. After inspecting and evaluating the general health of the knee according to the modified O´Driscoll score (Table 2) the operation was carried out using a set of specially designed instruments. First, a centralising aiming guide with a built-in guiding tube, adapted to the contour of the weight-bearing condylar surface was applied and fixed to the condyle by means of three pins engaging the metaphysis outside the articulating cartilage (Fig. 26). Through the guiding tube, sitting perpendicular to all tangents of the articulating surface, a specially designed drill was used to cut the cartilage and the underlying bone in a way to exactly correspond to the shape of the implant (Fig. 26).

According to the pilot study (Paper I), we aimed to position the implant 0.5 mm recessed below the surrounding cartilage (Paper II–IV). The depth of the implant level3 was incrementally increased by 0.01 mm by turning the guide tower clock-wise one step until a satisfactory level was achieved (Fig. 27). A slightly smaller testing device with identical articulating contour as the final implant was used iteratively to control the position in height relative to the surrounding cartilage before finally inserting the implant (Fig. 27).

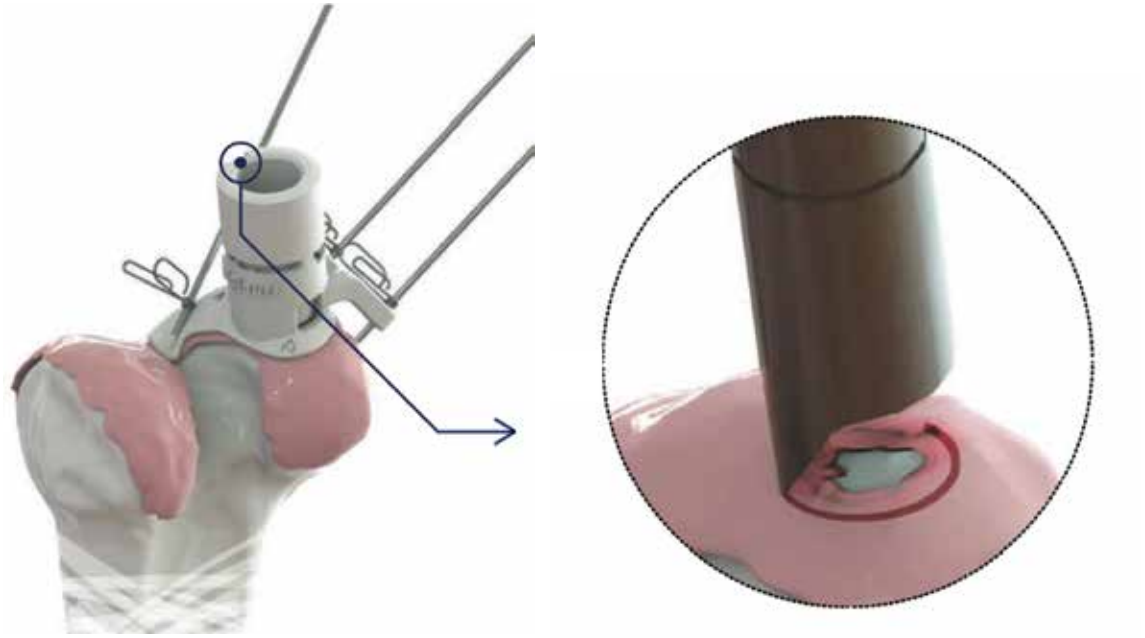


Figure 26. Individualised aiming tube (left) perpendicular to the condylar surface and cartilage cutting device (right). These pictures show instruments sized for human use, and shown on a human femoral model.

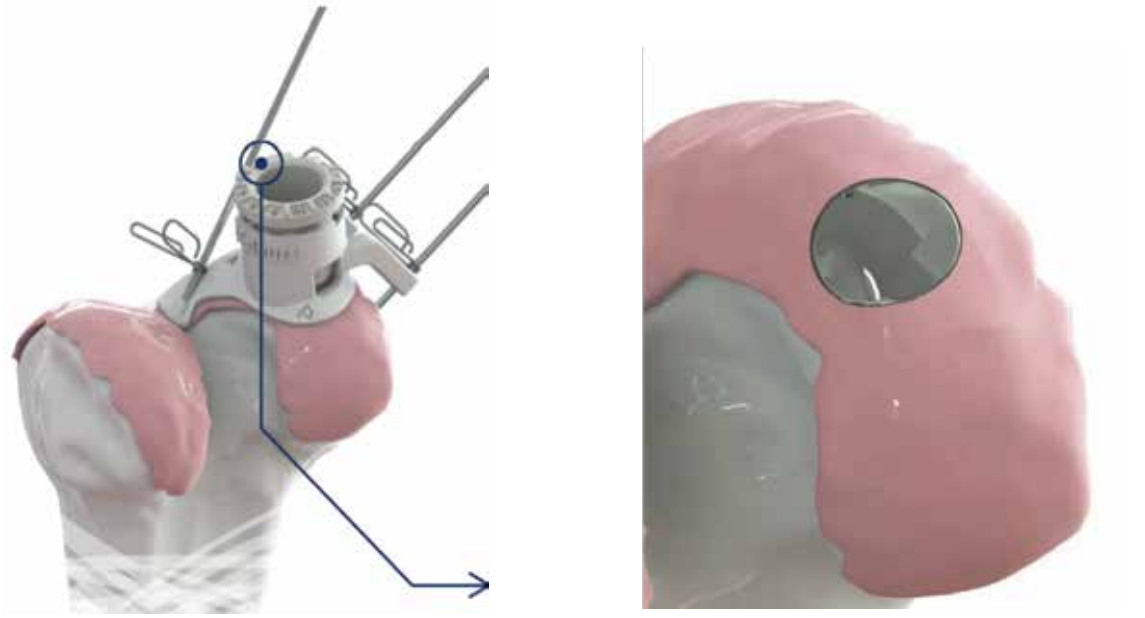


Figure 27. The Epiguide® tower clock that regulates depth of iatrogenic cartilage defect (left), final implant position (right). These pictures show instruments sized for human use, and shown on a human femoral model.

Finally, the joint capsule was sutured in a continuous pattern using polydioxanone (PDS®, Ethicon) and the subcutaneous tissue and skin were closed in a similar pattern using polygliglecaprone 25 (Monocryl®, Ethicon). No surgical complications occurred during the operations. The sheep were extubated in their stables and under continuous observation and regained consciousness within one-hour postoperatively.

4.3.3 Laser measurements

A total of 38 implants were laser-scanned and analysed for implant position in terms of height relative to surrounding cartilage. Eighteen of these were additionally analysed regarding tilting in relation to cartilage level. Five implants were lost for analysis due to technical problems related to either the negative print, scanning of the print or software used for analysis. In Paper I 20 implants were analysed in terms of height (out of 22 implants operated). In Paper III twelve implants were analysed for both height and tilting (out of 13 implants operated). Finally, in Paper IV 6 implants were analysed for height and tilt in 6 animals using the laser scanning method. The first step was to take a negative print of the medial femoral condyle (housing the implant) using an alginate plaster (Hydrogym; Zhermack, Badia Polesine, Italy) (Fig. 28). This negative print was subsequently analysed using a high precision (< 1 µm) laser-scanning device (www.nikonmetrology.com; LK, Scandinavia, Stockholm, Sweden). The contour of the femoral condyle including the implant was digitised using a specific software program (Metris Focus Inspection 9.2) (Fig. 28).

This software calculated the radius of the condyle curvature in both the sagittal and coronal planes using a set of data points from the surrounding cartilage (Paper I). In the remaining papers (III and IV) the radius of the condyle curvature was pre-set to the radius of the implant. The surface of the implant was then marked with five different reference points (centre, anterior, posterior, medial and lateral). From these landmarks the implant height (mm) relative to the surrounding condylar radii at the cartilage surface plane was calculated (Fig. 29).

Using the relative height (h) and the inter-distance (d) of the antero-posterior or medio-lateral data points, respectively, the angulation (tilt) of the implant relative to the surrounding condylar surface was calculated by the appropriate trigonometric equation (Arctan (h/d)).

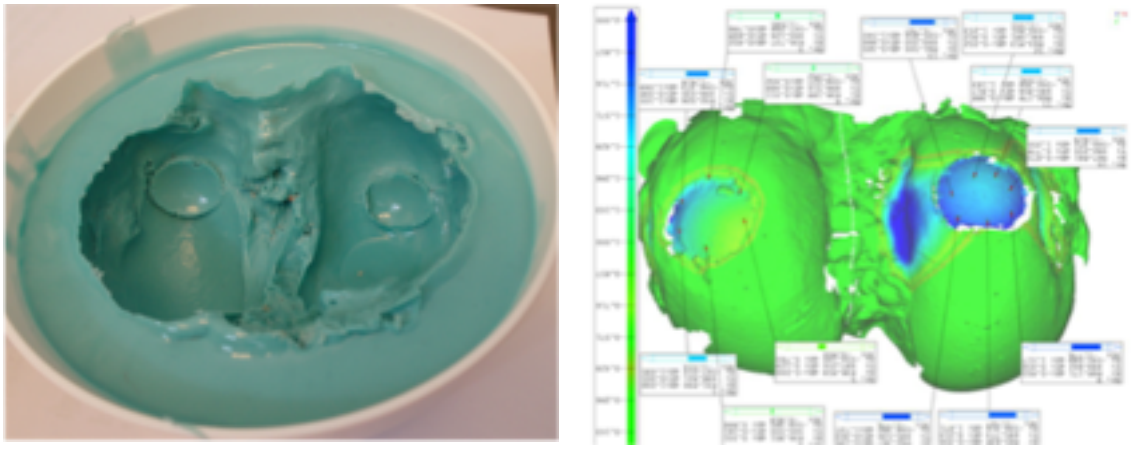


Figure 28. The negative print in alginate plaster (left) and the digitised image (right).

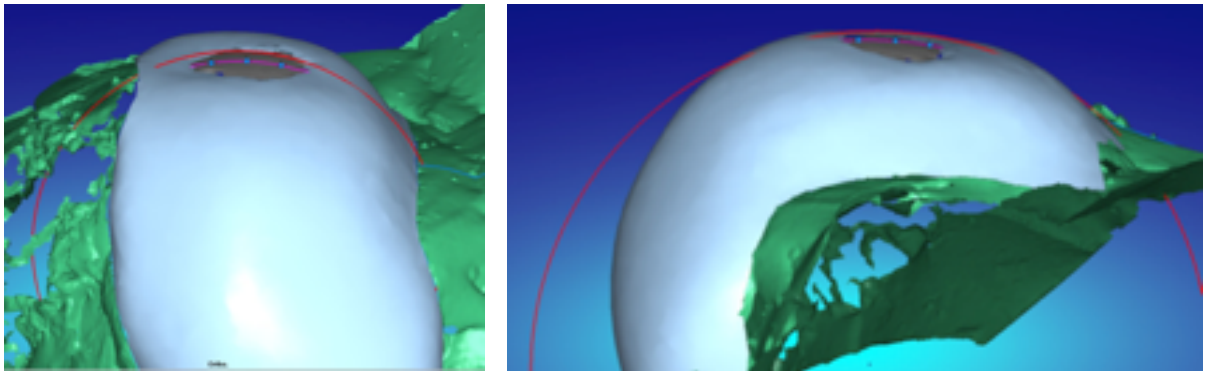


Figure 29. Postoperative laser measurements used to evaluate implant position (Courtesy of LK Skandinavia AB).

4.3.4 Macroscopic cartilage and joint health

After euthanasia the animals were autopsied at the National Veterinary Medical Institute (SVA) according to their general routine and analysed for general diseases that could possibly influence the outcome. There was not sign of toxic reaction that could be caused by the implant material. The results were noted in autopsy protocols. The general joint health was evaluated according to a modified O´Driscoll score (0-6 points instead of 0-10 as the parameter restoration of contour and cartilage erosion of the graft was not possible to evaluate) (Table 2)[87,88]. As the joint had been opened a sample of joint fluid was taken. Both tibial and femoral condyles were dissected and high-resolution photographs (Canon EOS 450D, EF-S 17-55 mm f/2.8 IS USM lens fixated at a distance of 0.3 m, using 35 mm focal length) were taken to enable macroscopic evaluation according to the ICRS score. Articular cartilage lesions were thus classified according to a scale 0-4, where grade 0 is normal, grade 1 is fibrillation (softening not possible to evaluate on photograph), grade 2 is superficial fissures (not reaching the subchondral bone), grade 3 fissures to the subchondral bone and grade 4 exposed subchondral bone[28,89].

Table 2 - Joint health evaluation according to the modified O´Driscoll score

CONTRACTURE	**Score**
None	2
Partial	1
Complete	0
ADHERENCE	
None	2
Minimum	1
Large	0
GENERAL CARTILAGE APPEARANCE	
Translucent	2
Opaque	1
Discoloured	0

Table 2. Classification used to evaluate general joint health intra-operatively and post mortem.

4.3.5 Histology

Histology is the microscopic study of cells and tissues. In order to be able to microscopically evaluate the tissue or sample it has to be prepared. Sample preparation consists of fixing, processing, embedding, sectioning and staining. *Fixing* uses chemical fixatives to maintain the structure of cells and to preserve the tissue from degradation. The most commonly used fixative is 10% neutral buffered formalin. *Processing* consists of dehydration, clearing and infiltration of tissues. The goal is to replace the water in the tissue with a substance that hardens (most commonly paraffin) permitting thin sections to be cut. To remove the water from the tissue, the samples are transferred between baths of progressively more concentrated ethanol (dehydration). Then the alcohol is removed from the tissue (clearing) using a hydrophobic agent such as xylene. Finally, the xylene is replaced with molten paraffin wax (infiltration). *Embedding*; samples are placed into moulds with the embedding material that hardens after cooling. These blocks are then ready for sectioning. *Sectioning*; a microtome is used to cut about 5 micrometre thick tissue samples for light microscopy. The most commonly used direction to cut the sample is perpendicular to its surface (cross section). *Staining*; this enhances the ability to visualise or identify microscopic structures (since biological tissues have almost no contrast in the light microscope). There are many different types of stains, and among them safranin and toluidine blue are used to identify cartilage. These stains are basic or cationic dyes that bond to the strongly acidic

(anionic) glycosaminoglycans staining the tissue red (metachromasia). This reaction between laboratory chemical and tissue is termed histochemistry and is frequently used in qualitative cartilage evaluation. *Artefacts* are features in the tissue that interfere with normal histological examination. These can be introduced prior to the collection of the tissues or can result from tissue processing. Processing can lead to changes like shrinkage, colour changes and structure alterations in the tissue.

4.3.5.1 Cartilage evaluation of joint surfaces

In Paper I samples were prepared and evaluated as follows: the medial tibial surfaces were dissected and placed in 2% glutaraldehyde 1% paraformaldehyde in 0.1 M sodium cacodylate buffer, pH 7.4 and refrigerated. Areas chosen to represent cartilage facing the implants were cut and rinsed in 0.1 M phosphate buffer, pH 7.4 post-fixed in 2% osmium tetroxide 0.1 M phosphate buffer, pH 7.4 at 4 °C for 2 h, dehydrated in ethanol followed by acetone and embedded in LX-112 (Ladd, Burlington, Vermont, USA). Semi-thin sections were cut and stained with toluidine blue and used for light microscopic analysis. Digital images were taken by using a Morada camera (Olympus Soft Imaging Solutions, GmbH, Münster, Germany). Damage to the cartilages were evaluated according to a modified Mankin score (the smear layer and calcified zone is not evaluated); grade 0-12: where grade 0 is normal cartilage and grade 12 is totally deranged cartilage (Table 3)[90,91]. In Paper III-IV, samples were prepared similar to Paper I while stained with toluidine blue or safranine blue for light microscopic analysis. Damage to the articular cartilages was evaluated according to a modified Mankin score as recommended by the Osteoarthritis Research Society International (OARSI) for histological assessment of osteoarthritis in sheep (Table 4)[83].

Table 3 - Histological evaluation according to Mankin

		Score
STRUCTURE	Normal	0
	Surface irregularities	1
	Pannus and surface irregularities	2
	Clefts to transitional zone	3
	Clefts to radial zone Clefts to calcified zone	4 5
	Complete disorganisation	6
CELLS	Normal	0
	Diffuse hypercellularity	1
	Cloning	2
	Hypocellularity	3
SAFRANIN STAINING	Normal	0
	Slight reduction	1
	Moderate reduction	2
	Severe reduction	3
	No staining	4
TIDE MARK	Intact	0
	Crossed by blood vessels	1

Table 3. The histological-histochemical grading system according to Mankin.

Table 4 - Modified Mankin according to ICRS recommendations		
Microscopic scoring of cartilage		**Score**
STRUCTURE (score the worst area in field of view)	Normal	0
	Slight surface irregularities (surface barely disturbed)	1
	Moderated surface irregularities (surface roughened)	2
	Severe surface irregularities (disruption, fissuring/ fibrillation to less than 10% depth)	3
	Fissures to transitional zone (1/3 depth)	4
	Fissures to radial zone (2/3 depth)	5
	Fissures to calcified zone (full depth)	6
	Erosion or severe fibrillation to mid-zone (1/3 depth)	7
	Erosion or severe fibrillation to deep-zone (2/3 depth)	8
	Erosion or severe fibrillation to calcified-zone (full depth)	9
	Erosion or severe fibrillation to subchondral bone	10
CONDROCYTE DENSITY (average score for whole field of view in non-calcified cartilage)	Normal	0
	Increased or slight decrease	1
	Moderate decrease	2
	Severe decrease	3
	No cells	4
CELL CLONING (score the whole field of view)	Normal	0
	Several doublets	1
	Many doublets	2
	Doublets and triplets	3
	Multiple cell nests or no cells in section	4
	Interterritorial Tolouidine blue	5
INTERTERRITORIAL TOLOUIDINE BLUE (score the worse area in field of view working from AC surface down)	Intact	0
	Decreased staining to mid-zone (1/3 depth)	1
	Decreased staining to deep-zone (2/3 depth)	2
	Decreased staining to calcified-zone (full depth)	3
	No staining	4
TIDEMARK (score the worst area in field of view)	Intact subchondral bone plate + single tidemark	0
	Intact subchondral bone plate + duplicated tidemark	1
	Blood vessel penetrate thru subchondral bone plate to calcified cartilage	2
	Tidemark penetrated by blood vessels	3

Table 4. Histological classification used to evaluate cartilage damage of joint compartments.

TABLE 5 - Cartilage health adjacent to the implant		
MACROSCOPIC EVALUATION		**Score**
Cartilage Abutting Implant	Complete	2
	Disrupted < 50%	1
	Disrupted > 50%	0
Abuttting Cartilage Appearance	Smooth	2
	Fibrillations	1
	Erosions	0
Cartilage Colour Abutting the Implant	Bluish hyaline-like	2
	White	1
	Yellow	0
Cartilage Flow	Yes	1
	No	0
MICROSCOPIC EVALUATION		**Score**
Cartilage Integration with Implant	Complete integration	3
	Demarcation line < 50%	2
	Demarcation line > 50%	1
	Gap or cyst	0
Implant Level with Surrounding Cartilage	Recessed < 25%	2
	Recessed < 50%	1
	Protruding or recessed to subchondral bone	0
Degenerative Change of Adjacent Cartilage	Normal cellularity, no cluster, normal staining	2
	Moderate cellularity, some cluster, moderate staining	1
	Severe hypocellularity, many clusters, severe destaining	0

Table 5. Score developed to assess cartilage health surrounding the implant.

4.3.5.2 Cartilage health surrounding the implant

To evaluate the health of the cartilage bordering the implant a score was formed using both macroscopic and microscopic parameters (Table 5). Parameters were modified from existing cartilage repair classifications[92-94]. For details please see Paper IV. We propose that a score of 10-14 points suggests satisfactory result, 5-9 points moderate result and a score of 0-4 point denotes unsatisfactory chondrointegration (Table 5).

4.3.5.3 Evaluation of cartilage defect

The defects were evaluated macroscopically using high-resolution photographs according to ICRS cartilage repair score (Table 6) and microscopically according to O´Driscoll cartilage repair score (0-24)[28,93].

TABLE 6 - Cartilage repair assessment		
		Score
DEGREE OF DEFECT REPAIR	In level with surrounding cartilage	4
	75% repair of defect depth	3
	50% repair of defect depth	2
	25% repair of defect depth	1
	0% repair of defect depth	0
INTEGRATION TO BORDER ZONE	Complete integration with surrounding cartilage	4
	Demarcating border < 1 mm	3
	3/4 of graft integrated, 1/4 with a notable border > 1 mm width	2
	1/2 of graft integrated, 1/2 with a notable border > 1 mm width	1
	From no contact to 1/4 of graft integrated with surrounding cartilage	0
MACROSCOPIC APPEARANCE	Intact smooth surface	4
	Fibrillated surface	3
	Small, scattered fissures or cracks	2
	Several, small or few but large fissures	1
	Total degeneration of grafted area	0
Overall Repair Assessment		**Score**
Grade I	Normal	12
Grade II	Nearly normal	8-11
Grade III	Abnormal	4-7
Grade IV	Severly abnormal	0-3

Table 6. ICRS Cartilage Injury Evaluation Package. Used for the evaluation of untreated cartilage defects.

4.4 Bone quality and osseointegration

4.4.1 Micro-CT

Micro-computed tomography (CT) is x-ray imaging in 3-D, similar to regular CT used in hospitals but with significantly higher resolution (Fig. 30). Like 3-D microscopy small-scale structures (in the micron range) can be visualised non-invasively. As the object rotates producing hundreds of different angular views a computer produces virtual cross-sectional slices. The region of interest can then be 3-D analysed in several morphometric parameters such as bone architecture, density and quality[95,96].

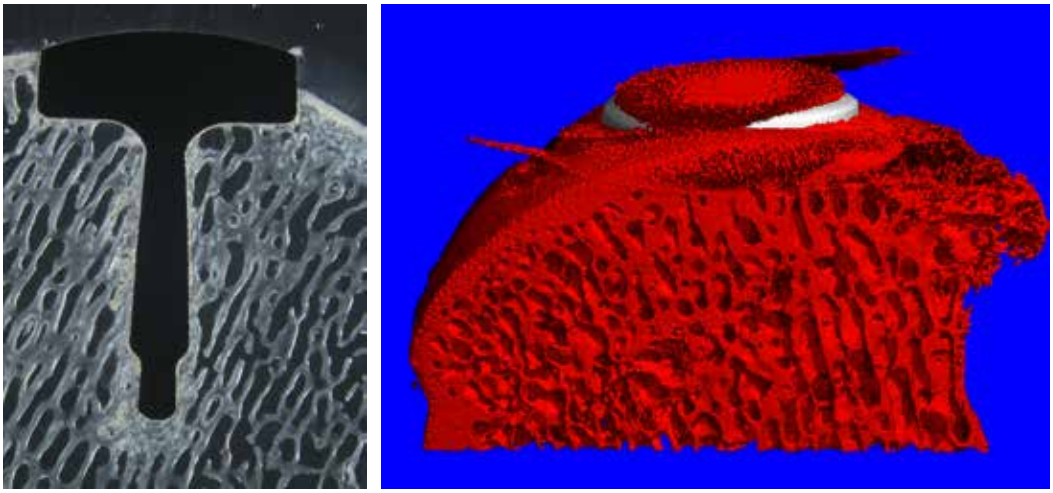


Figure 30. A photograph showing a micro-CT image (left) of an implant inserted into the medial femoral condyle and a 3-D reconstruction (right) useful to evaluate bone features.

4.4.2 Bone histomorphometry

While histology is commonly descriptive, quantitative measurements are of importance when evaluating bone tissue. Bone histomorphometry offers quantitative study of the microscopic organisation and structure of bone through computer-assisted analysis of images formed by microscopy. Quantitative analysis of bone architecture provides valuable information on the amount of bone and its cellular activity. In our experiment several bone parameters were evaluated such as osteolytic bone areas, stress shielding, osteid bone, bone density and bone to implant contact.

For methods on the evaluation of osseointegration and data in detail see Methods in Paper II. In summary, the medial femoral condyle containing the implant was prepared for light microscopy, according to the ground sectioning technique by Donath and Breuner and embedded in plastic (not paraffin)[97]. The sections were stained with Sanderson's RBS stain and counter stained with acid fuchsin (both Dorn & Hart, USA). The specimens were examined with a Zeiss Supra VPN-40 field cathode scanning electron microscope using the backscatter detector described in the following section. The resulting images were evaluated using ImageAccess (Imagic, Switzerland) software. The measurement was started from the first bone to implant contact at the left side of the hat around the body of the implant to the first bone contact at the right side. The amount of the bone-to-implant contact was measured in percent.

4.4.3 Backscatter electron microscopy

Backscatter electron detectors are commonly used in association with scanning electron microscopy. The detector is placed above the sample in a chamber and detects the scattered electrons from the interaction of the accelerated electron beam with the sample (atoms), explained like "billiard balls" (electron) colliding with larger particles (atoms). This produces a scatter that is proportional to the

atomic size: thus larger atoms produce brighter backscatter electron intensity and particles while a smaller atomic size produce darker images. Backscatter electron detectors provide high-resolution compositional images of a particular sample (Fig. 31)[98].

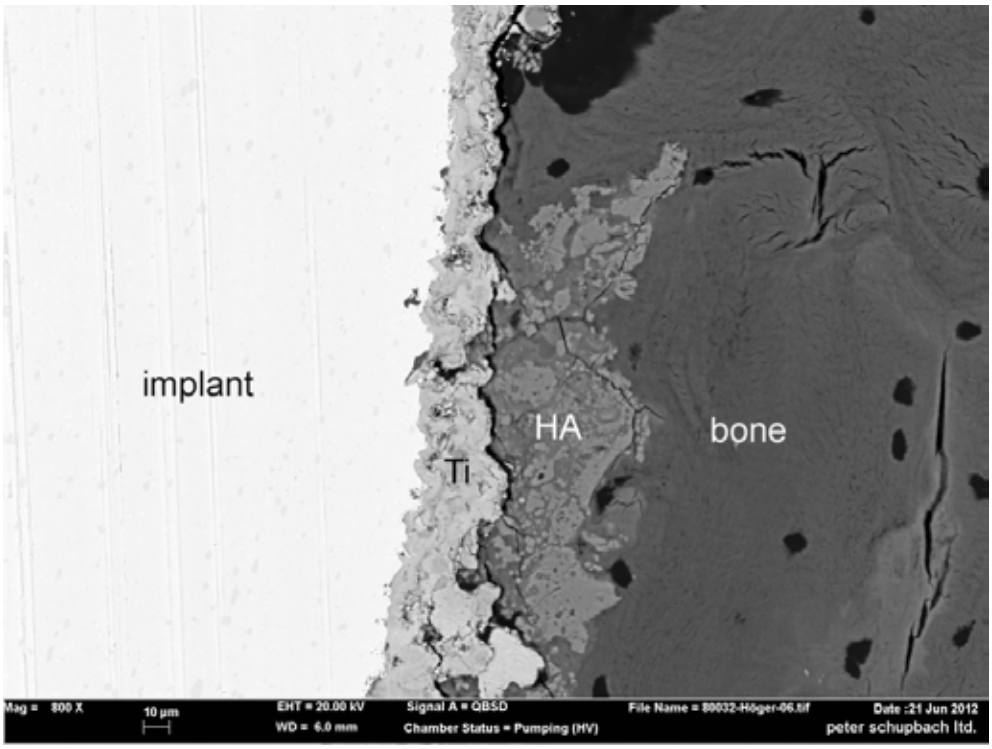


Figure 31. Backscatter electron detectorphotograph showing a compositional image. Metal produces brighter image.

4.5 STATISTICAL METHODS

"Your sample size is small, your standard deviation is large, your conclusions mean nothing"

Homer Simpson

Data are presented as means with their range, standard deviation (SD), estimated 95% confidence intervals or shown in box-plots. A two-factor analysis of variance (ANOVA) model was used to compare cartilage damage related to operative treatment and across time (Paper III) or to compare cartilage damage related to operative treatment and joint location (Paper IV), and interactions between those factors. Independent samples Student´s t-test was used to compare means between groups. For non-parametric values the Mann-Whitney U test for ranked values was used to compare results at different time points and Wilcoxon signed ranked test for matched pairs, respectively. Fischer's exact test was used to compare counts of individuals with or without significant changes. Linear regression was used to assess the relationship between implant position and cartilage damage; means for each animal were used as independent samples (Paper I) or to assess the relationship between cartilage damage and age (Paper III). P-value was set at 0.05. Calculations were performed using STATA v12.1 or SPSS 15.0 for Windows statistical package.

5 RESULTS

5.1 PAPER I

Twenty-two knees in eleven animals were available for macroscopic and microscopic evaluation, and because two samples could not be digitised, 20 knees remained for laser measurement. No histological differences in opposing tibial cartilage were seen between the two types of implants used (P = 0.82; from 10 samples of comparable implant heights). Mankin score after twelve weeks did not grossly differ from six weeks (mean 6.7, 5.5, respectively, P = 0.61; batch 1). Therefore data are presented as one pooled group of implants.

5.1.1 Implant position

Height of implants (n = 20) as assessed by the mean of the three transversal data points from laser scans averaged standard deviation (SD) -0.20 (0.66) mm in the group aimed at flush position, -0.33 (0.18) in the group aimed for 0.3 mm recessed and -0.76 (0.12) in the group aimed for 0.7 mm recessed, respectively. Implant position expressed with the aimed offset level subtracted, averaged for the merged group, was -0.12 (0.47) mm. In fact 80% of the implants were placed somewhat lower than aimed for but some implants protruded up to 0.7 mm above the intended position.

5.1.2 Macroscopic cartilage evaluation

Macroscopic score (n = 11) averaged (range) 1.7 (1-3). Macroscopic score as a function of implant position showed a linear relationship (P = 0.01) such that International Cartilage Research Society (ICRS) score increased by 1.2 (95% conf. int: 0.4, 2.0) units per each mm increase in implant height.

5.1.3 Microscopic cartilage evaluation

Histological preparation showed a varying degree of surface damage. Modified Mankin score (n = 11) averaged (range) 4.8 (1-10), and protruding implants showed higher Mankin score (P < 0.01). Microscopic score as a function of implant position showed a linear relationship (P = 0.008) such that Mankin score increased by 4.3 (95% conf. int: 1.5, 7.0) units per each mm elevation in implant height.

5.2 PAPER II

Joint health as indicated by the modified O'Driscoll score showed no changes in range of motion (ROM), fibrosis or cartilage appearance (average 0.0 out of maximum 6 points at 6 or 12 months). Likewise ICRS macroscopic score of the tibial surface showed no damage (average 0.0 points out of maximum 4 points) at 6 months and 0.67 (95% CI: -0.16, 0.51) points following 12 months indicating no statistical significance (Z = 0.77; <1.96).

5.2.1 Micro-CT evaluations

At 6 months one specimen out of three showed small osteolytic areas beyond the hat, whereas the peg was always firmly osseointegrated. The other two specimens were completely osseointegrated. At 12 months, two specimens out of six showed small osteolytic areas at the hat. One specimen showed enlarged bone marrow chambers. The remaining three specimens were completely osseointegrated.

5.2.2 Histomorphometry

Stained ground sections cut longitudinally through the centre of the peg showed functionally osseointegrated implants after both 6 and 12 months, respectively. At both time points, a high bone-to-implant contact was measured with a mean of 90.6% (95% CI: 79.4, 101.9) at 6 months and 92.3% (95% CI: 89.5, 95.2) at 12 months, respectively, indicating no statistically significant difference (Z = 0.00; <1.96). The mean bone density in a 500 micron wide band adjacent to the implant surface was 72.5% (95% CI: 60.0, 85.0) at 6 months and 75.4% (95% CI: 67.3,

83.5) following 12 months, respectively indicating no statistically significant difference (Z = 0.60; < 1.96). In the corresponding section of normal host bone distant to the implant the mean bone density was 63.0% (95% CI: 58.1, 68.0) at 6 months and 48.7% (95% CI: 43.3, 54.2) following 12 months, respectively.

5.3 PAPER III

Joint health as indicated by the modified O'Driscoll score showed no changes in ROM, fibrosis or cartilage appearance (average 0.0 out of maximum six points at 6 or 12 months).

5.3.1 Implant position

Height of implants (n = 12) as assessed by the mean of three transversal and three anteroposterior data points in the implant measured from laser scans, averaged 0.54 mm recessed (95% CI: 0.41, 0.67) with a standard deviation (SD) of 0.23 mm for the whole group aimed at 0.5 mm recessed. Further, the mean frontal (transversal) and sagittal (anteroposterior) tilt was 0.03° (95% CI: -2.08, 2.14) (SD 3.73) and 0.25° (95% CI: -2.09, 2.60) (SD 4.15), respectively.

5.3.2 Macroscopic cartilage evaluation

The macroscopic evaluation (Outerbridge 0-4) of the medial tibial cartilage surface showed modest cartilage damage both in the surface opposing the implants 0.45 (range 0-3) and in the control knee 0.50 (range 0-2) with no statistically significant difference between sides (Z = -0.14). Lateral tibia and femoral surface showed no or minor damage. Likewise, the tibial surface opposing the implant showed no damage across time; average 0.29 (range 0-1) at 6 months; and 0.67 (range 0-3) points following 12 months indicating no statistical significance (Z = 0.43; < 1.96).

5.3.3 Microscopic cartilage evaluation

The tibial plateaus of both the operated and the control knee showed a varying degree (range 0-17 units) of articular cartilage damage evaluated according to the Modified Mankin score as recommended by OARSI (0-27 units). Cartilage damage of the medial tibial plateau showed a linear increase with age.

Cartilage damage of the medial tibial plateau opposing the implant was increased compared to the medial tibia of the non-operated knee by a statistically significant difference of 1.77 units (P = 0.041; 95% CI: 0.08, 3.45). Tibial Mankin score of the 12-month group (n = 6) was 1.85 units higher when compared to the 6-month group (n = 7), however this did not reach statistical significance (P = 0, 42; 95% CI: -2.98, 6.68). Also, no interaction between implant and time was observed (P = 0.94). The tibial cartilage of the lateral compartment was substantially less damaged than the medial compartment in both the operated knee, by 4.2 units (P = 0.007; 95% CI: 1.38, 7.13), and the non-operated knee by 2.40 units (P = 0.03; 95% CI: 0.29, 4.50). There was no difference in tibial cartilage damage of lateral compartments between operated or non-operated knees (0.09 units, P = 0.86; 95% CI: -1.02, 1.19). Five out of 13 (38%) animals showed a difference of 4 or more Mankin units between their medial tibial surfaces. This did not reach statistical significance (Fischer's exact test; P = 0.59).

5.4 PAPER IV

Joint health as indicated by the modified O´Driscoll score showed no changes in ROM, fibrosis or cartilage appearance (average 0.0 out of maximum six points).

5.4.1 Implant position

Height of implants (n = 6) as assessed by the mean of three transversal and three antero-posterior data points in the implant measured from laser scans, averaged 0.60 mm recessed (range 0.16-0.82) with a standard deviation (SD) of 0.23 mm for the whole group (aimed at 0.5 mm recessed). Further, the mean frontal (transversal) and sagittal (anteroposterior) tilt was 3.7° (range 0.4-7.3) and 2.6° (range 0.8-4.6).

5.4.2 Macroscopic cartilage evaluation

The macroscopic cartilage evaluation (Outerbridge 0-4) of the medial tibial cartilage surface showed modest cartilage damage both in the surface opposing the implants 1.6 (range 0.5-4) and in tibia opposing the untreated defect 0.9 (range 0-1.5) with no statistically significant difference between sides (Z = 0.707, P = 0.480). Lateral tibia and femoral surface showed no or minor damage macroscopically.

5.4.3 Microscopic cartilage evaluation

The opposing tibial plateaus of both the treated and untreated defect showed a varying degree (range 1-15) of articular cartilage damage evaluated according to the Modified Mankin score as recommended by OARSI (0-27 units).

Cartilage damage of the medial tibial plateau opposing the implant showed no statistically significant difference (P = 0.51; 95% CI: -3.7-6.5) compared to the medial tibial plateau opposing the non-treated defect of the contralateral knee. Using repeated measures ANOVA we showed a statistically significant difference in cartilage damage between different joint compartments. The medial femoral compartment was significantly less damaged compared to medial (P = 0.02; 95% CI: 0.45-8.14) or lateral tibial compartments (P = 0.004, 95% CI: 1.59-5.00). The tibial cartilage of the lateral compartment showed no statistically significant difference compared to the medial compartment (P = 0.51, 95% CI: -4.61-2.61). Differences between compartments were not related to treatment with implant or left untreated (P = 0.38). A post-hoc comparison in a pooled group of twelve young sheep showed no statistically significant difference (P = 0.32) between medial and lateral compartment in knees treated with implants.

5.4.4 Cartilage health adjacent to the implant

Cartilage health adjacent to the implant scored macroscopically (n = 12) averaged 6.5 (range 5.0-7.0) with a SD of 0.72 and averaged microscopically (n = 10) 5.5 (range 3.0-6.5) SD (1.01). When pooled together (n = 10) the total score averaged 11.3 (range 9-13) with a SD of 1.46.

5.4.5 Cartilage repair of the defect

Cartilage repair of untreated defects scored macroscopically (n = 6) on average 5.0 (range 0.5 – 5.0) and microscopically (n = 6) on average 8.0 (range 2.0-14).

5.4.6 Histomorphometry

Bone-to-implant contact (BIC) averaged 79.3% (range 41.9-98.8).

6 DISCUSSION

The treatment of deep focal condylar lesion of a volume of approximately 550 mm^3 (area of 2-4 cm^2) in the middle-aged patient remains a clinical challenge[8]. For the last three decades the gold standard approach to treat these lesions has been biological treatment. The aim is to reconstitute a long-lasting structural and functional cartilage tissue that resembles natural hyaline cartilage. However, it has not been possible to entirely reproduce the complex structure of articular cartilage, specifically the collagen and proteoglycan interaction. Therefore instead of the biological approach we opted for a biomechanical strategy by resurfacing cartilage lesions with a metal implant (FKRM). Similar to the treatment of tooth caries we aimed to fill the cartilage void with inorganic material. This method bridges the gap between biological treatment at a younger age and joint replacement at the end of the OA road.

6.1 IMPLANT DESIGN

Co – Cr material has been a widely used biomaterial for orthopaedic implants in the last decades proving its efficacy and biological safety. Also, in order to avoid drawbacks of modular systems such as corrosion and fatigue failure at the junction between the hat and the peg, we needed a strong material that permitted building the implant as a monobloc. Co – Cr alloys have proven to have excellent fatigue strength and corrosion resistance even in a chloride environment (such as body fluids) when compared to other materials[99].

The contour of the implant was our next concern. Based on mathematical calculations (finite element analysis; FEA), we concluded that an implant should follow the contour of the femoral condyle and be somewhat recessed in relation to surrounding cartilage[100,101]. It is easier to achieve this goal if the articulating surface is double-contoured, as it more accurately mimics the natural curves of the femoral condyle in all planes. A single radius contour would require an excessive recession in order not to protrude at any point of its surface. All implants were circular mainly because of the difficulty to ream individualised shaped defects perpendicular to the condyles in all planes.

Friction of the articulating surface was another concern. Hyaline cartilage provides an almost frictionless surface with a coefficient of friction in the order of 0.0025 (compared to rubber on concrete 1.0, Teflon on Teflon 0.04, or ice on ice 0.02). Biomaterials regularly used in orthopaedics are not close to matching the cartilage friction coefficient. For example polished steel on ultra high molecular weight polyethylene (UHMWPE) has a friction coefficient of 0.05. A five-axial milling device was used to shape the double-curved contour of the articulating surface, leaving a stepwise structure when observed at higher magnification. The surface was therefore highly polished to a Ra of 0.04 μ, meeting the required International Organization for Standardization (ISO) standard of Ra of 0.05 for Co – Cr alloys used in articulating orthopaedic implants.

The fixation of the non-cemented implant to underlying bone is vital for the success of this concept. Primary fixation was intended by a slender press-fit peg into an undersized predrilled hole. Others used a modular system with a large screw anchoring to the underlying bone[61,64]. We tried an alternative design with a thread-like shape of the peg to increase the contact peg-bone area similar to others[88]. In our series however, this design offered no benefit but rather decreased the percentage of implant that was ossoeintegrated. Long-lasting secondary fixation was intended by use of a double-coated plasma sprayed Ti – HA layer.

6.2 CONTACT PRESSURES AND BIOMECHANICS

Fundamental for successfull result when resurfacing a full depth focal condylar lesion is that the implant must not increase the peak contact pressure causing damage to opposing tibial cartilage. Becher et al. showed that a proud implant would increase the contact peak pressure on opposing tibia by approximately 200% compared to an untreated defect. An implant seated flush did not show increased contact peak pressure[102]. The presence of a meniscal tear increased the peak contact pressures by about 80% compared to an implant seated flush with normal meniscus[103]. Furthermore, when tested dynamically they concluded that an FKRM implant seated recessed did not produce deleterious effect on opposing cartilage[104]. To study the behaviour of the cartilage surrounding the implant Manda et al. developed a finite element mathematical model and tested both the spherical first generation implant and the double contoured second-generation Episealer® FKRM. Both implants showed favourable effect compared to an untreated defect when seated recessed (0.3 mm) regarding rim stresses. However if the implant is seated too deep it may also lead to high rim stresses[100,101]. We therefore aimed implants 0.5 mm recessed (Paper III-IV) to accommodate for surgical imprecision and cartilage compression under dynamic loading (Paper I).

6.3 INSTRUMENTATION

We realised that optimal implant positioning was of utmost importance for the well-being of neighbouring cartilage. A protruding implant would plough opposing cartilage whereas an implant which was too recessed would not impede the apposing cartilage wall from collapse. An optimally positioned implant is dependent on the shape of the implant and similar to the knee prosthesis, depends on the used instrumentation and guides. A first generation aiming device was developed using an external hat that aimed to place a guiding pin perpendicular to the cartilage surface, similar to others[64]. It was however not possible to obtain the desired position accurately and consistently as demonstrated in Paper I. In this pilot study a significant imprecision was noted showing a standard deviation from the intended position of 0.47 mm. Based on the concept of patient-specific instrumentation (PSI), an individualised second-generation aiming guide was developed (Fig. 32). Individualised instruments adapted to the sheep condyle showed accurate and consistent implant placement as demonstrated in Paper III.

Figure 32. Individualised set of instruments used to achieve accurate implant position similar to the set of instruments used for the animal studies. Courtesy of Episurf Medical AB.

6.4 IMPLANT POSITION IN RELATION TO SURROUNDING CARTILAGE

We found that cartilage damage correlated strongly with implant position showing a linear relationship such that the Mankin score increases by 4.3 units per each mm elevation in implant height (Paper I). There is enough evidence from FEA, biomechanical tests and preclinical studies to state that an implant should not protrude[62,102-106]. There is however no consensus on the ideal FKRM implant position and most authors aim for implants seated flush[62,64]. The use of an 'external' guide system in Paper I showed significant imprecision and when aimed flush, in fact some implants protruded causing devastating effects on opposing cartilage. I therefore recommended, in accordance with FEA and our preclinical results, to aim implants at a somewhat recessed position (0.3-0.5 mm). An individualised instrumentation guide based on each particular anatomy is recommended for optimal implant fit. Accordingly, accurate and reproducible measurements of implant position play a key role in both development and concept evaluation ensuring reliable and repeatable performance. Laser scanning technology is widely used for this purpose in industry and has a resolution (< 1 μm) well beyond the requirements for this application. By digitising the entire condyle including the implant, the geometry and the implant position within the condyle can be evaluated on the basis of graphic colour diagrams (Fig. 33). Among the difficulties in measuring implant position in terms of height related to the cartilage level is the fact that the cartilage above the implant is missing. Therefore the radii of the condyle have to be calculated from the cartilage that surrounds the implant. Because the curvature of the condylar surface is not spherical (constant) the radii are difficult to calculate[107]. Two different measurement strategies were used to calculate this virtual cartilage plane. First (Paper I), the sagittal and transversal radii of the individual condyle were estimated using a large portion (20 mm) of the surface bordering the implant. The difference in height between these constructed circles and the implant surface as determined from five data points on the implant was used to describe implant position in terms of height (Fig. 33). While the condylar radius in the transversal plane is near spherical, it is actually J-shaped in the sagittal plane (the radius decreases posteriorly). Therefore a radius averaged in the sagittal plane corresponds less precisely with the true cartilage level. The second measurement strategy (Paper III-IV) used the radii of the implant to construct a cap, which was positioned at the cartilage margins to represent the missing cartilage level.

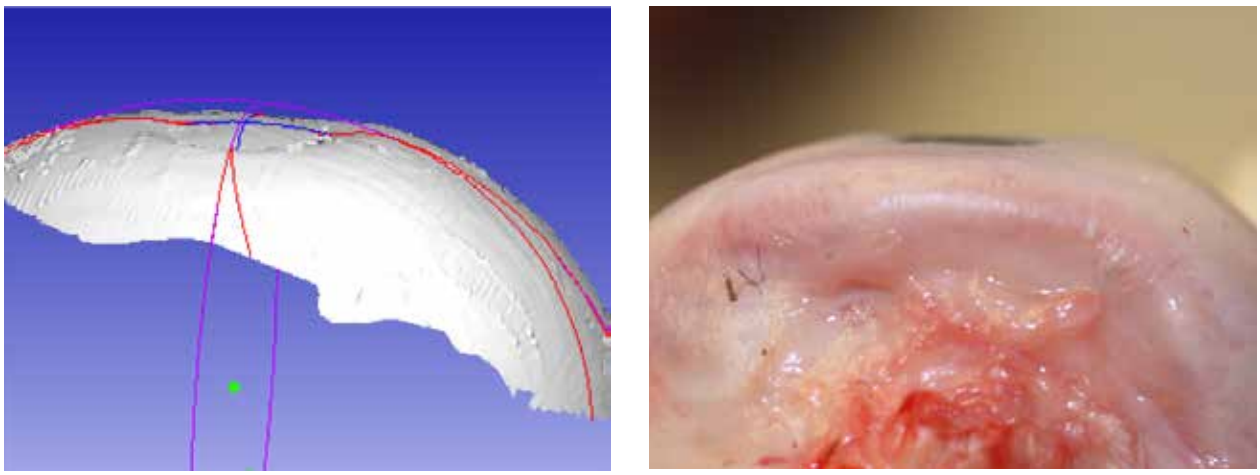


Figure 33. This shows the radii at the missing cartilage level (in violet) based on the curvature (red) of the condyle (left). Photograph of an adequate implant position (right).

6.5 OSSEOINTEGRATION

The slender monobloc implant was designed in order to preserve as much bone as possible, in case of need for future surgical interventions. For fixation purposes a press-fit peg was deemed sufficient to achieve primary fixation and then supplemented with a double-coating layer (Ti – HA) for secondary long-lasting fixation. Titanium has proven osteoconductive properties and does not dissolve over time, providing osseointegration if there are no gaps at the bone-implant interface[108]. In some cases osteolysis was found at the hat (Paper II) and it was speculated that synovial fluid from the joint penetrated the hat-cartilage interface at insertion. This interface was subsequently sealed by HA, by converting fibrous tissue into bone (circumferential bone apposition). HA has a filling effect of a gap up to 1 mm in both loading and unloading conditions[77]. Moreover, a frame of newly produced trabecular bone surrounded these lytic areas indicating a positive trend towards bone formation. A combination of these two compatible osteoconductive materials (Ti – HA) would promote osteoactivity in the presence of gaps regardless of loading condition. A double coating with an underlying layer of titanium secures long-lasting fixation, should the first HA layer dissolve. In fact, a main result of this study was the excellent osseointegration of the double-coated Ti – HA implant (Paper II). The osseointegration was about 90% (average) of the implant surface in contact with bone and never less than 40%, which is high compared to any other animal or human studies[61,84,109]. Hence, the concept of a double-layer on a slender monobloc Co – Cr core proved its efficacy (Fig. 34).

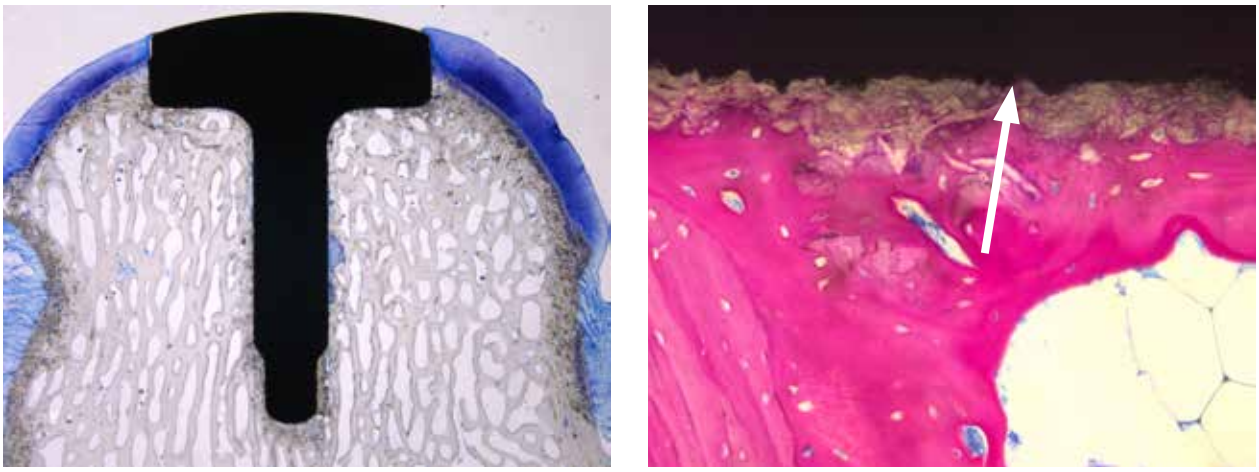


Figure 34. A microphotograph of an osseointegrated FKRM implant (left) and the intimate bonding between bone (red) to HA (brown) and at some points to the Ti-layer (arrow) in areas where the HA have dissolved (right).

6.5 CARTILAGE HEALTH AND CHONDROINTEGRATION AT THE IMPLANT

A prerequisite for FKRM implants to succeed is that the host tissue must accept the implant. As aforementioned the bone bonded with the implant excellently, as shown using accepted histomorphometric methods. There are however no methods to assess bonding between implant and surrounding cartilage, or an accepted score to quantify cartilage damage when adjacent to a metal implant. Kirker-Head et al. described an absence of cracks or fibrillations and cartilage flow over the implant in their original pilot study[61]. In our first experiments we noticed that cartilage as well as bone adhered to the HA covered perimeter of the hat and a primary evaluation showed that most (85%) of the cartilage in contact with the HA adhered (Fig. 35, 36). Based on this finding the implants were modified in the

subsequent experiments to cover the entire perimeter of the implant with HA, a concept not described before. We attempted to construct a classification system based on parameters validated for cartilage repair (Table 4) to evaluate the efficacy of the cartilage to accept the implant. Furthermore, we suggested a grading score (see Methods in Paper IV) where 10-14 points suggests satisfactory cartilage health and bonding to the implant. All animals evaluated according to our method showed satisfactory results (n = 10; Paper IV). Additionally, it can be hypothesised that the implant prevents collapse at the rim of the defect by supporting the walls, as also suggested by the absence of fibrillation around the implant[110]. Similar to other researchers, we observed a metachromatic membrane partially covering the implant, a positive phenomenon described as matrix flow (Fig. 35, 36)[61,110-112]. Finally, the sealing effect of HA might not only apply to bone but also to cartilage, further preventing synovial fluid from penetrating to the subchondral bone and potentially producing cysts.

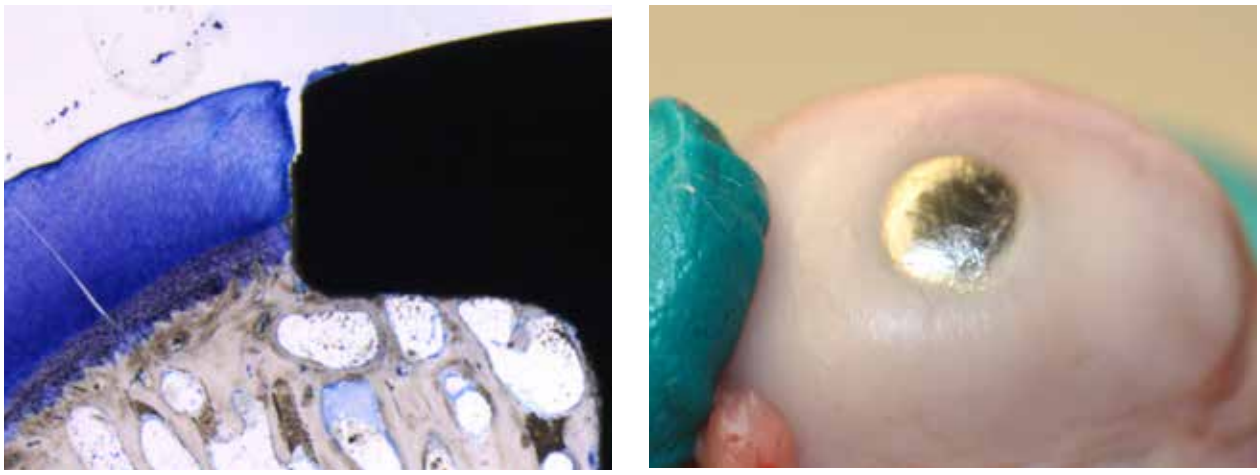


Figure 35. Picture of a cross-sectional histological specimen showing proper attachment of cartilage to the HA covered perimeter of the hat and a gap between the cartilage and the uncoated perimeter of the implant (left). High-resolution photograph showing a macroscopic image of healthy cartilage adjacent to the implant (right).

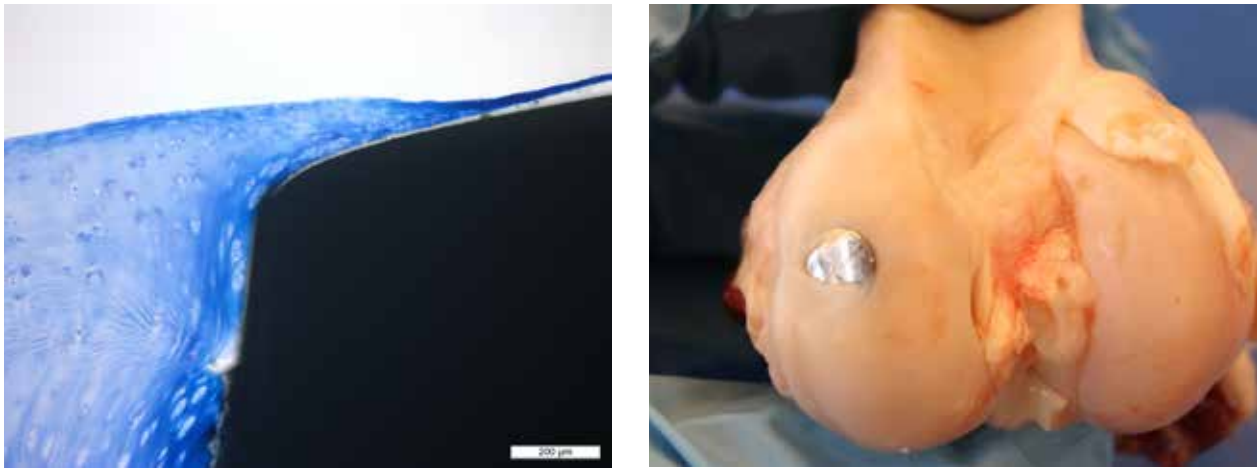


Figure 36. Note the metachromatic cartilage flow on top of the implant (left), and shown macroscopically on a high-resolution photograph (right).

6.7 CARTILAGE DEFECTS

It is acknowledged that focal cartilage defects can cause pain and dysfunction. According to Heir et al. these patients reported more discomfort than patients with anterior cruciate ligament-deficiency, and their quality of life was just as affected as in patients scheduled for knee replacement[113]. However, it should be remembered that articular cartilage defects may also be asymptomatic and the likelihood of a defect becoming symptomatic is unknown[114-116]. Shelbourne et al. assessed the natural history of focal deep lesion grade III–IV left untreated and compared them to a matched control group. After 6.5-year follow-up the patients with defects had slightly but significantly lower subjective scores than controls, and 79% returned to jumping or pivoting recreational sports. The authors concluded that most patients had few symptoms and the results could be used to compare results from procedures treating articular defects[117]. It has been recognised that full depth and even superficial cartilage lesions do not heal and might progress to generalised OA[57,118,119]. In Paper IV it was established that a created full depth cartilage lesion ICRS grade IV does not heal (Fig. 37). There was no evidence of repair according to the evaluation using a validated repair score and some defects showed signs of rim collapse. Thus the experimental model comparing untreated to defects treated with FKRM implants is motivated.

In Paper IV we hypothesised that an untreated defects would disturb joint homeostasis while a defect treated with a FKRM implant would interrupt the OA progression. In our paper we could not show this progression and we believe this was due to the small numbers of animals and the short six-month follow-up time.

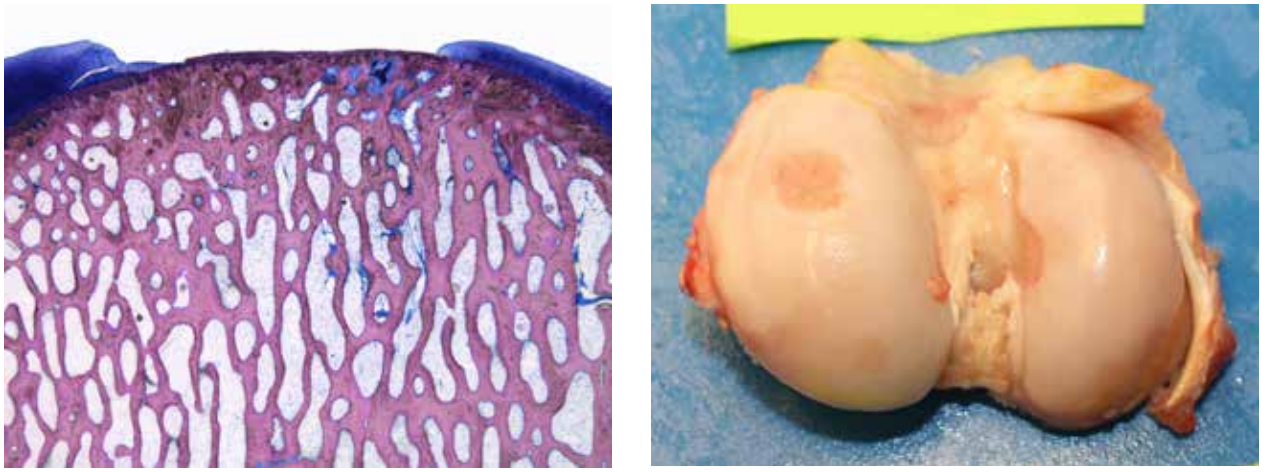


Figure 37. Picture of a cross-sectional histological specimen showing an unhealed cartilage defect at six months (left). High-resolution photograph showing macroscopic view of the same defect (right).

6.8 OPPOSING AND DISTANT CARTILAGE HEALTH

In a pioneering pilot study, Kirker-Head et al. noted fibrillation and erosions of the goat tibia articulating towards a Co – Cr FKRM implant[61]. However, they noted fibrillation and erosion in the contralateral non-operated knee as also observed in other species[120]. It was argued that cartilage degeneration was related to implant elevation but quantification of implant position or histological cartilage scores was not reported. However the authors concluded that screw-anchored FKRM (Hemicap® Arthrosurface) implants caused less cartilage damage when compared to other studies on biological

resurfacing methods[61]. In two other studies evaluating the effects of FKRM implants on opposing tibia, Custers et al. reported significant cartilage degeneration[84,106]. In their first study, histological cartilage degeneration of the tibia cartilage opposing an oxidized zirconium (OxZr) implant scored eight units (scale 0-24) compared to twelve units in a group that had a focal defect microfractured[106]. In the subsequent study they reported cartilage damage to opposing tibia corresponding to twelve units (scale 0-24) regardless of the bearing material (Co – Cr or OxZr) or if the defect was left untreated. These data were compared to eight units of cartilage degeneration in a healthy non-operated control group (surprisingly these data were from a separate unpublished study). In our studies we also found a statistically significant but modest difference in cartilage degeneration of opposing tibia by two units (scale 0-27) compared to the non-operated control knee (Fig. 38), despite accurate implant position (Paper III). The results from Custers et al. could be considered satisfactory considering no use of instrumentation guide was reported. Cartilage damage to opposing tibia could in fact be secondary to implant malposition such as tilt or protrusion. Furthermore no postoperative measurements of implant position were reported[84,106]. Interestingly, all research groups report certain damage to cartilage surfaces within the knee regardless if defects were treated or left untreated and even in non-operated healthy controls. Additionally, almost no human or animal studies of femoral cartilage defects and their biological treatment report data on opposing tibia. Custers et al. found a similar amount of cartilage degeneration opposing defects treated with implants or left untreated. In Paper IV we compared defects treated with FKRM implants or left untreated and observed a similar amount (six units in a 0-27 scale) of damage to opposing tibia. Unfortunately, implants were at a slightly inaccurate position either by tilt or by over-recessing, with one tilted implant causing devastating effects on opposing tibia. Summarising our data (Paper III-IV), cartilage degeneration of opposing tibia averaged 7-9 units regardless of implant position or age. This should be compared to a 5-8 unit cartilage degeneration of opposing tibia in healthy controls or untreated defects, respectively. These differences in cartilage degeneration were considered acceptable considering that full depth cartilage lesions remain a clinical challenge in the symptomatic middle-aged patient group where an alternative treatment option is lacking.

It can be hypothesised that FKRM treatment hampers the progression to generalised OA. This is not what other authors have found who showed progression of degenerative changes to lateral compartment in knees treated with implants or microfracture[84,106]. In these studies defects were treated after 10 weeks by either an implant or microfracture, similar to the clinical situation where treatment delay is common. It can be speculated that progression to the lateral compartment seen in their studies can be the consequence of this delay. We, however, did not observe progression of degenerative changes in our non-treated defects after treatment with FKRM implants or when leaving defects untreated. The short follow-up time of untreated defects (Paper IV) might have obscured long-term cartilage degeneration. Moreover, it was suggested from our studies that degenerative changes on cartilage articulating against a FKRM implant was mechanical and had a local effect on opposing tibia, whereas remaining joint surfaces were unaffected. Additionally, cartilage degeneration on opposing tibia did not progress over time (Paper III). Thus, having an implant in the medial femoral condyle did not disrupt the panarticular joint homeostasis.

It is recognised that goats as young as two years old show signs of early OA but this has not been shown in sheep. In the studies performed by Custers and Kirker-Head only young goats (2-3 years old) were used and therefore their results were not influenced by age[61,84,106]. In Paper III we used skeletally mature ewes (2-6 years old) and observed a varying degree of cartilage degeneration in both operated and non-operated knees. In a post-hoc analysis we suggested that there was a linear relationship correlating cartilage damage to age in sheep. This should be considered when evaluating results of cartilage defects studies. Consequently, in Paper IV only young

ewes (2-3 years old) were used and no difference in cartilage damage of the tibia articulating against a metal implant or an untreated defect was observed. Furthermore, there was no difference in cartilage damage between medial to lateral compartments, in contrast to what was observed in Paper III. We suggested that this could be age-related. When performing a post-hoc comparison using all twelve young sheep no difference in cartilage damage between medial and lateral compartment was observed. In Paper IV, the lateral compartments showed cartilage degeneration similar to those seen earlier (Paper III) in non-operated healthy control knees. Older ewes show more cartilage degeneration, often more pronounced in the medial tibia, whereas younger ewes show similar amount of cartilage degeneration in the whole knee. We speculated that this minor cartilage damage, often fissures, represented the young ewes' cartilage healing capacity to maintain joint homeostasis.

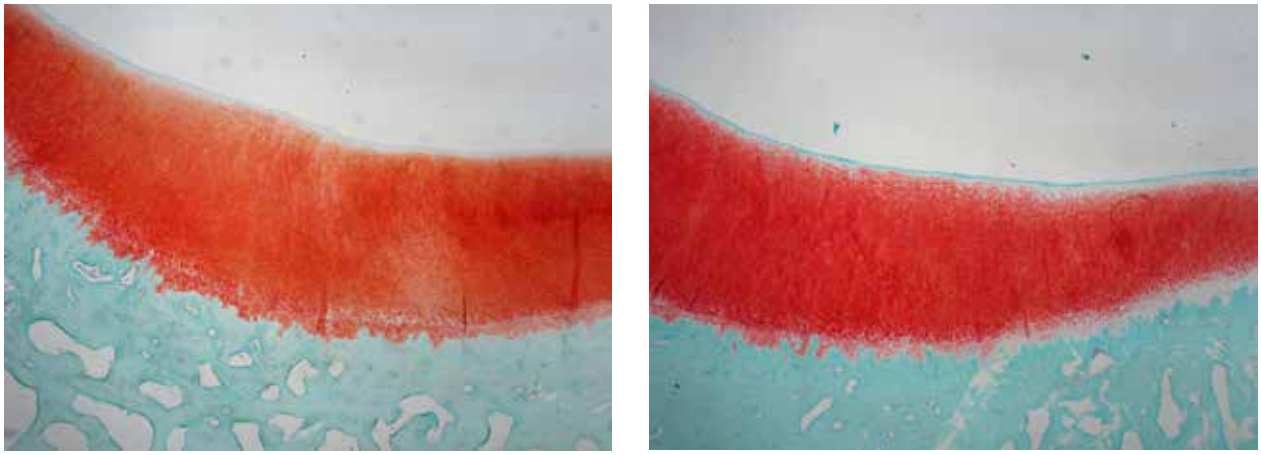


Figure 38. Cross-sectional histological photographs. Note minor differences between the tibial cartilage opposing an implant (left) and the tibial cartilage opposing the non-operated control knee (right).

6.9 SIMILAR CONCEPTS

The concept of a unipolar metal implant articulating against cartilage tissue is not new. Hodge presented a case where a moulded vitallium distal femur arthroplasty, inserted cementlessly, withstood the test of time and was still functioning after 30 years[121]. Harrington presented (1992) 28 patients treated with resurfacing arthroplasty using the McKeever prosthesis for advanced arthritis of the patella. Seventeen rated good or excellent at five years according to a patella score[122]. Another concept that has reached acceptance is minimally invasive knee arthroplasty, which offers the advantages of reduced pain, hospital stay, morbidity with faster rehabilitation and increased range of motion compared to standard knee arthroplasty[123,124]. Siguier et al. reported (2001) the results using a partial surface replacement for osteonecrosis of the femoral head with a minimal soft tissue disruption and bone resection in 33 patients followed 40 months. Nine failures were attributed to expansion of the osteonecrosis, and 24 of 28 surviving implants rated excellent or good according to a hip score[125]. The authors concluded that surgery was relatively simple, with minimised soft tissue damage and preserved bone stock allowing for immediate weightbearing. Should failure occur, a conversion to hip arthroplasty was deemed simple considering the preserved tissues[125]. A low profile modular unipolar focal knee resurfacing implant was presented by Hemicap® (2003) and a first pilot study in animals and humans showed promising results[64,61]. Concomitantly, Custers et al. presented a similar concept using a monobloc unipolar focal knee resurfacing

implant that was tested in animal models. The authors showed cartilage degeneration following treatment with the metal implant and research in humans was not conduct[88]. The last two methods have been discussed elsewhere throughout the thesis. A flurry of different implant choices is progressively being developed such as UniCap® (similar to HemiCap) with an additional polymer tibia inlay, ConforMIS® (personalised knee resurfacing prosthesis), Orthoglide® (Co – Cr tibia inlay only), just to mention some. The combination of minimally invasive knee surgery techniques with focal metal implants seems an attractive treatment option, but still preclinical and clinical studies are scarce.

6.10 LIMITATIONS

The major limitation of this study was the small number of animals available; this was both for ethical, and economical reasons. For instance, the use of a bilateral model in the pilot study increased the number of knee samples, although we were aware that they were not independent individuals. One post-hoc analysis included animals from two experimental series. Also, some animals were lost to follow-up, which might become critical in large-animal studies with often reduced number of animals.

The short follow-up time (Paper IV) limited the comparison and assessment of OA progression in knees with untreated defects. However, previous studies have shown OA progression with a similar amount of animals.

Two slightly different histological scores were used to evaluate cartilage damage in Paper I and Paper III-IV, which limited the possibility to compare results. There was no defined strategy on how to analyse cartilage health adjacent to the implant. Moreover, there is still no standardised score for evaluating cartilage adjacent to an implant. The proposed macroscopic and microscopic evaluation score is not validated.

The animals were operated with a guide standardised for one sheep, rather than individualised for each animal, for practical and economical reasons. This could result in a somewhat less optimal implant position and negatively affect the results. We made no comparative study with other treatment models such as allogenic mosaicplasty (which is the most similar to our method) or the standard treatment (microfracture).

Translating results from animal studies to humans is a delicate matter. Sheep have thinner cartilage layers, and defects are possible to make only in the lower range of what is judged clinically significant for humans. Also, they cannot report subjective data and functional outcome measures cannot be assessed.

6.11 CONLUSIONS

A monobloc double-curved and double-coated (Ti – HA) Co – Cr implant is presented. Both implant and instrumentation for the treatment of full depth focal cartilage lesions in the femoral condyle was developed successfully for testing in a translational sheep animal model.

A firm and consistent bond to bone under weight-bearing conditions in all of the evaluated cases was obtained. Therefore, the results of this study support the use of this double-coated construct. Moreover, we noted signs of adherence between cartilage and the HA-covered area of the implant, indicating a possible bonding between HA and cartilage. In an attempt to evaluate the health of the cartilage that surrounded the implant we proposed a score and showed satisfactory results in terms of integration between host-tissue and implant. We confirmed that untreated defects do not heal. However, our study did not have the power to prove OA progression of untreated cartilage defects to distant compartments. Implant precision is hard to achieve and damage to opposing tibia is distinctively sensitive to implant position, and it is emphasised that implants should never protrude. With 'individualised' aiming guides an accurate and consistent implant position was achieved. Using this position there was limited damage to opposing tibia that did not increase over time. Having an implant did not disturb the general joint cartilage homeostasis; hitherto the effects of an implant on cartilage degeneration are acceptable.

The biological safety of a novel FKRM implant in a large animal model and its efficacy in terms of fixation, integration to host tissue and cartilage joint homeostasis was studied. Based on these scientific data, human studies aimed to evaluate treatment of symptomatic middle-aged patients with full-depth focal cartilage lesion in the medial femoral condyle are motivated. However, the clinical and natural history of these lesions is not completely understood and we could not show OA progression at short-term. Hence, we can not advice for prophylactical treatment of asymptomatic patients with focal cartilage lesions until further research data is at hand.

6.12 SAMMANFATTNING PÅ SVENSKA

Bakgrund: Knästukningar vid idrott, i arbetsliv eller trafik leder ofta till invalidiserande broskskador. Trots modern biologisk behandling kan dessa skador utvecklas till smärtsam degenerativ ledsjukdom (artros), som på sikt kan kräva inoperation av konstgjord led (knäprotes). Genom ett tvärvetenskapligt medicintekniskt samarbete har en behandlingsmetod med syftet att försluta centimeterstora broskdefekter med metallimplantat vidareutvecklats. Ett nytt implantat med instrumentering och de resulterande effekterna på knäleden har sedan studerats genom djurexperiment hos får. Ett övergripande mål är att lindra belastningssmärta samt förbättra funktion och livskvalité efter knäskador med broskdefekter. Speciellt medelålders patienter som ännu ej är lämpliga för knäprotes saknar behandlingsalternativ. På sikt är förhoppningen att artrosutvecklingen kan bromsas.

Hypotes: Med exakt kirurgisk insättning av metallimplantat kan beninväxt uppnås samt broskskador förseglas utan att omgivande brosk i leden skadas.

Metod: Ett dubbelkurverat metallimplantat (Co – Cr) med undersidan belagd av titan-hydroxyapatit (Ti – HA) opererades in i mediala femurkondylen hos totalt 37 tackor i tre experimentserier. Beninväxt mättes med histomorfometri. Implantatposition i form av höjd och vinkel uppmättes genom laserscanning. Broskkvalitet och defekter bedömdes makroskopiskt och mikroskopiskt. Fårens hälsa bedömdes av veterinär.

Resultat: En pilotstudie påvisade varierande implantatposition och att broskskadan på motstående tibiayta var linjärt korrelerad till höjdfelställningen, och att ett implantat inte skall sticka utanför ledbroskytan. En serie av andra generationens individanpassade metallimplantat kunde placeras med hög precision; 0.54 mm nedsänkt, och att broksslitaget då var acceptabelt efter första året. Likaledes var brosket intill implantatet oskadat och vidhäftigt på implantatets HA beklädda yta. Broskkvaliteten i knäets övriga ledytor visade inga tecken på slitage i denna långtidsstudie. En obehandlad broskdefekt läkte inte och visade jämförbar skada på motstående brosk jämfört med implantatbehandling. Alla implantat växte fast. Får utvecklar broskskada med ålder, speciellt på knäets insida (mediala tibia).

Slutsatser: Det utvecklade implantatsystemet visade sig säkert och pålitligt för reparation av små fokala ledskador hos får. Slitage på motstående ledbrosk kan accepteras om implantaten placeras i optimal position. Speciell intrumentering rekommenderas för detta, eftersom ett implantat aldrig får sticka ut. Kliniska studier av patienter med smärtsymptom rekommenderas. Ytterligare långtidsstudier av får eller patienter behövs innan profylaktisk operation kan rekommenderas.

6.13 FUTURE PERSPECTIVES

"Fiction of today is the science of tomorrow"
Nicolas Martinez Carranza

Every 45 second a total knee arthroplasty (TKA) is performed in the US, and by the year 2030 it is predicted that 3.5 millions TKAs will be performed each year in the US alone[126]. Another trend is that younger patients are receiving knee joint arthroplasty (unicompartmental or total), and this group performs inferiorly in terms of pain, function, morbidity and prosthesis survival[127-129]. It is recognised that there is no correlation between pain and OA but there is a correlation between the different stages of OA and patient satisfaction after knee replacement[130]. Hence, patients with early OA perform worse with knee arthroplasty (unicompartmental or total) compared to patients with advanced OA. Patients with early OA are often middle-aged and many of them have focal lesions with disabling symptoms similar to those scheduled for TKA[113]. These patients are 'caught' in a treatment gap: they are considered too young for TKA, and too old for biological treatments[22]. These patients present with symptoms that need to be treated, and their progression to OA needs to be stopped. The envisaged individualised patient oriented modular treatment of focal cartilage lesion of traumatic or degenerative origin proposed is an FKRM implant. Hopefully this could halt the OA progression, but should the disease progress, conversion to UKA is still possible. Should the OA progress a primary TKA would be the solution in non-responsive cases, and hopefully without requiring further revisions.

In order to treat younger patients with limited cartilage lesions or early OA by using FKRM implants more research is recommended. First, ethical permission to define the normal cartilage condition in different joint surfaces and at different ages in sheep has been obtained. Data should be used to better understand and define the origin of focal lesions. Second, a well-powered long-term animal study is needed to assess the natural evolution regarding cartilage degeneration in the different knee compartments, and analyse if early implant treatment could delay OA progression. Third, this treatment concept needs to be compared to other proven methods such as allographic osteochondral plugs, chondrocyte transplantation, tissue-engineered cartilage resurfacing and common microfracturing. Fourth, a system to assess cartilage health adjacent to an FKRM implant should be further developed and validated. Fifth, the assessment of the efficacy of FKRM implants in humans in terms of improved pain and function, and the biological safety as determined by the long-term survivorship by entering standard national registries is needed. Finally, randomised clinical studies should compare FKRM results to non-treated lesions and other treatments modalities in terms of pain, restored function and OA progression.

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