Treatment of full thickness focal cartilage lesions with a metallic resurfacing implant in a sheep animal model, 1 year evaluation

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SUMMARY

Background: Full depth focal cartilage lesions do not heal spontaneously and while some of these lesions are asymptomatic they might progress to osteoarthritis. Treatment for these lesions is warranted and the gold standard treatment at younger age remains biological healing by cell stimulation. In the middle-age patient the success rate of biologic treatment varies, hence the surge of non-biological alternatives. Our objective was to evaluate the efficacy and safety of a metallic implant for treatment of these lesions with respect to the long-term panarticular cartilage homeostasis.

Methods: The medial femoral condyle of 16 sheep was operated unilaterally. A metallic implant was inserted in the weight-bearing surface at an aimed height of 0.5 mm recessed. Euthanasia was performed at 6 or 12 months. Implant height and tilt was analyzed using a laser-scanning device. Damage to cartilage surfaces was evaluated macroscopically and microscopically according to the Osteoarthritis Research Society International (OARSI) recommendations.

Results: Thirteen sheep were available for evaluation and showed a varying degree of cartilage damage linearly increasing with age. 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. Implant position averaged 0.54 recessed (95% CI: 0.41, 0.67).

Conclusions: Our results showed a consistent and accurate placement of these implants at a defined zone. At this position cartilage wear of opposing and surrounding joint cartilage is limited. Thus expanded animal and human studies are motivated.

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Introduction

The treatment of a focal cartilage lesion grade 3–4; according to the International Cartilage Research Society (ICRS) score remains a clinical challenge. These lesions are often associated with other sports injuries and the increased activity levels at older age coupled with a demand on higher quality of life puts this pathology on the rise. Because cartilage injuries show a decreasing healing capacity with age and a focal cartilage lesion left untreated might progress to osteoarthritis, treatment is warranted. Various types of biological treatments have proven valuable but are either technically demanding, require sophisticated laboratory resources or proved less effective with increased age.

In the last decade a novel surgical strategy has been proposed using focal knee resurfacing metal implants (FKRM). The most commonly described indication is for the symptomatic middle-aged (35–60 years) active patient where biological treatments have failed or shown to be less effective. This method can be regarded as the final attempt at joint preservation in the younger
pain and lameness (grade 0 was normal gait and 1
them, and observed daily to monitor general condition, signs of
available. They were well acquainted with the person handling
in stables in groups of three the
respectively at veterinary examination were excluded. Animals were
presented general disease or lameness as determined preopera-
(evenly distributed; range 2
Animals that did not meet these characteristics, or
5 kg (range 5.5

Animals

Sixteen healthy female sheep (Swedish landrace) from the same
breeder were used. Mean age and weight of the sheep were 4 years
(evenly distributed; range 2–6) and 82.5 kg (range 70–99),
respectively. Animals that did not meet these characteristics, or
presented general disease or lameness as determined preopera-
tively at veterinary examination were excluded. Animals were
housed at the Department of Clinical Sciences, Swedish University
of Agriculture Sciences (SLU) in Uppsala, Sweden, and kept indoors
in stables in groups of three the first weeks and thereafter in an
outdoor stable. Food was given twice a day and water was freely
available. They were well acquainted with the person handling
them, and observed daily to monitor general condition, signs of
pain and lameness (grade 0 was normal gait and 1–4 was mild,
moderate, major and severe lameness respectively)17. Euthanasia
was randomly performed at 6 months (7 animals) or after 12
months (6 animals) using an overdose pentobarbital (100 mg/ml)
after securing blood samples. Three animals were lost for follow-up
(see below). The knees were prepared for further investigations as
described below. Animal Ethics Committee, Uppsala Sweden,
approved the protocol.

Implant

The implant (diameter 7.5 mm) had a double-curved (radii 19
and 12 mm) articulating surface modeled after computer tomog-
raphy (CT) scans of a “standard” sheep knee, and was manufactured
from implant-grade cobalt-chrome by a computer aided design and
manufacturing (CAD/CAM) process. Implants were coated with
commercially pure titanium (60 μm) on which a layer of hydroxy-
apatite (HA; 60 μm) was plasma sprayed (Plasma Biotal Ltd., GBR).
The articulating surface was then polished to a roughness (Ra) < 0.03 μm. The monobloc implant (Fig. 1a) had a 10 mm peg
(diameter 2 mm) introduced into an undersized (diameter 1.8 mm)
drill hole in the bone for primary interference fit. Implants were
manufactured and provided by Episurf AB, Sweden.

Anesthesia

The animals were anaesthetized by an intravenous injection in
the jugular vein of xylazine (Rompun® vet, Bayer Animal Health,
Denmark) 0.11 mg/kg and ketamine (Ketaminol® vet, Intervet,
Sweden) 2.2 mg/kg and then intubated. The anesthesia was
maintained with isoflurane (IsoFlo® vet, Orion Pharma Animal
Health, Sweden) 1.5–3% in 100% oxygen. After anesthetization,
blood samples were taken from the cephalic vein. The animals were
given antibiotics, cefuroxime (Cefuroxim, Farmaplus, Norway)
22 mg/kg IV and analgesic, carprofen (Rimadyl® vet, Orion Pharma
Animal Health, Sweden) 4 mg/kg SC, buprenorphine (Temgesic®,
Scering-Plough, Sweden) 0.01 mg/kg IM and glucopyrrolate
(Robinul®, Meda, Sweden) 0.25 ml/10 kg SC.

Surgery

Surgery was performed on one knee; randomly prepared closed
envelope determined the side. 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 para-
tellar 5–6 cm incision through skin and subcutaneous tissue.
After inspecting the knee the operation was carried out using a set
of specially designed instruments: First, a centralizing 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 articu-
lar cartilage (Fig. 1b and d). Through the guiding tube, sitting
perpendicular to the articulating surface, a specially designed drill
was used to cut the cartilage and the underlying bone. According to
previous studies15, we aimed to position the implant at a level
0.5 mm recessed below the surrounding cartilage (Fig. 1c). A testing
device with identical articulating contour as the final implant was
used iteratively to control the position in height relative to the
surrounding cartilage. The level of the implantation depth was
incrementally increased by 0.01 mm by turning the guide tower
clock-wise one step until a satisfactory level was achieved before
finally inserting the implant. Finally, the joint capsule was sutured
using polydioxanone (PDS®, Ethicon), subcutaneous tissue and skin
were closed in a similarly using poliglecaprone 25 (Monocryl®,
Ethicon). No surgical complications occurred during the operations.
The sheep were extubated in their stables under continuous
observation and regained consciousness within 1 h post surgery.

Laser measurements of implant position

The medial femoral condyle with the implant was used for analysis. A negative print was taken using an alginate plaster
(Hydrogym; Zhermack, Italy), which was subsequently scanned
using a high precision (<1 μm) laser-scanning device (www.
The contour of the femoral condyle and implant was digitized using a specific software program (Metris Focus Inspection 9.2) and the radius of the condyle curvature was determined in both the sagittal and coronal planes. The surface of the implant was then marked with five different reference points (center, anterior, posterior, medial and lateral). From these landmarks the implant height (mm) relative to the surrounding cartilage surface was calculated (Fig. 2). 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 could be calculated by the appropriate trigonometric equation (Arctan (h/d)). For technical reasons one implant could not be analyzed.

Macroscopic cartilage evaluation

The joints were inspected macroscopically, according to Outerbridge (0–4) and 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). High-resolution photographs (Canon EOS 450D, EF-S 17–55 mm f/2.8 IS USM lens fixed at a distance of 0.3 m, using 35 mm focal length) were taken of all condyles (Fig. 3). Two blinded independent observers (NMC & HB) evaluated the photographs of each tibia plateau separately.

Articular cartilage lesions were classified according to a scale 0–4, 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.

Microscopic cartilage evaluation

After removal of soft tissues and photography, the articular cartilage of the tibia was dissected and placed in 2% glutaraldehyde +1% paraformaldehyde in 0.1 M sodium cacodylate buffer, pH 7.4 and stored in refrigerator. Segments of cartilage facing the implants and from the anterior and posterior third of the joint including the control medial tibial plateau, lateral tibial condyles and from the lateral femoral condyle 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, USA). Semithin sections were cut and stained with toluidine blue and used for light microscopic analysis. These specimens were blinded and scored at random by three observers (NM, HB, HH). Damage to the articular cartilages was evaluated according to a modified Mankin score as recommended by Osteoarthritis Research Society International (OARSI) for histological assessment of osteoarthritis in...
This scoring system assesses five different parameters; structure (0–10), chondrocyte density (0–4), cell cloning (0–4), interterritorial toluidine blue (0–4) and tidemark (0–3), ranging from 0 to 27 were 0 is pristine articular cartilage and 27 is complete destruction. The inner, middle and outer section of each segment was analyzed and the most severe lesion was used for scoring. Differences of 4 Mankin units were used as an arbitrary clinical threshold. The scores of the observers were averaged. Outliers with a difference of more than three points were scored again until consensus was reached.

Histomorphometric analyses of osseointegration

For methods on evaluation of osseointegration and data in detail see Martinez-Carranza 2014. In summary, the medial femoral condyle containing the implant of a subset of nine specimens was prepared for light microscopy, according to the ground sectioning technique by Donath and Breuner. The sections were stained with Sanderson’s RBS stain and counter stained with acid fuchsine. The specimens were examined with a Zeiss Supra VPN-40 field cathode scanning electron microscope using the backscatter detector. The resulting images were evaluated using ImageAccess (Imagic, Switzerland) software. The measurements started from the first bone-to-implant contact at one side of the hat and all along the implant to the last bone to the other side. The amount of the bone-to-implant contact was measured in percentage.

Statistical methods

Data are presented as means with their range or estimated 95% confidence intervals, and shown in box-plot. A two-factor analysis of variance (ANOVA) model was used to compare cartilage damage related to operative treatment or across time, respectively, and

Fig. 2. Postoperative laser measurements used to evaluate implant position. The red circles show the radius of the original cartilage (white color), purple line show the radius of the implant surface (silver). The difference between these radii at the marked data points on the implant (blue dots) are then used to calculate the average implant height (μm) and tilt in relation to surrounding cartilage in the (a) frontal and (b) sagittal plane. (Courtesy of LK Skandinavia AB).

Fig. 3. High-resolution photographs used to evaluate macroscopical cartilage damage in two implanted knees (left panel) and the non-operated control knee (right panel). Observe the modest difference in gross macroscopical appearance between a case with minor histological changes (upper row) and a case with histologically more severe cartilage damage (lower row).
interactions between those factors. 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, where a test statistic (Z-score) exceeding 1.96 indicates statistical significance. 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 cartilage damage and age. p-value was set at 0.05. Calculations were performed using the SPSS 15.0 for Windows statistical package.

**Results**

**General and joint health of the animals**

The general health of the sheep was good prior to operations. One sheep died immediately after extubation and two sheep were sacrificed after 8 weeks, one due to pneumonia and the other due to septic arthritis. The wounds healed without complications. During the first week all sheep had various degrees (2–3°) of limp (scale 0–4). The lameness decreased gradually and after 4 weeks, 3 sheep showed a minor limp (1–2°). These 3 sheep showed no limp respectively at 5, 9 and 12 weeks postoperatively. 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).

**Implant height and tilt**

Height of implants (n = 12) as assessed by the mean of three transversal and three antero-posterior 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 (Fig. 5a). Further, the mean frontal (transversal) and sagittal (antero-posterior) 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 (Fig. 5b and c).

**Macroscopic cartilage evaluation**

The macroscopic evaluation (Outerbridge 0–4) of the medial tibial cartilage surface (Fig. 3) 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).

**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 (Fig. 6) evaluated according to the Modified Mankin score as recommended by OARSI (0–27 units). Data are visualized in a box-plot (Fig. 7). Cartilage damage of the medial tibial plateau showed a linear increase with age (Figs. 6 and 8).

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 = 0.59; 95% CI: −0.29, 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).

Averaged wear of all compartments are presented in Fig. 9. 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).

**Histomorphometric analyses of bone ingrowth**

In a subset of nine animals the percentages of bone-implant contact, marrow-implant contact and connective-tissue-implant contact was measured. These data has previously been presented in detail (NMC2014). At both time points, a high bone-to-implant contact was measured with a mean ± SD of 90.6 ± 9.9% at 6 months and 92.6 ± 2.8% at 12 months, respectively (Fig. 10; one animal at 12 months).

**Discussion**

The main finding of this study was the minor amount of cartilage damage of the articular surface opposing the implant. Articular damage to the operated knee compared to the control knee was less...
than 2 Mankin units (0–27 unit scale). Although this difference reached statistical significance we question the clinical relevance, as this would probably not compromise the articular cartilage healing capacity of a young ewe. Interestingly, 38% of the animals showed a difference between operated and control knee of 4 Mankin units (no animal showed a difference larger than 5 Mankin units). It is not fully understood however, at which stage cartilage damage is irreversible and relentlessly starts to breakdown (pre OA)7,8,25. OARSI recommended an OA grading system in order to increase simplicity and utility in cartilage damage evaluation. A difference of 4 Mankin units would imply structural as well as cellular or biochemical damage, hence corresponding to at least one grade difference according to the OARSI cartilage OA grading system26. Although the fraction of animals presenting such a difference did not reach statistical significance it might suggest that some individuals are negatively influenced by the implant. It should be remembered, however, that there is poor correlation between histopathological or radiographic changes and clinical outcome27.

The other major finding of this study was that the cartilage damage was localized to the opposing tibia whereas remaining surfaces were unaffected; thus having an implant in the medial femoral condyle did not disrupt the panarticular joint homeostasis. We therefore suggest that this metal implant has a mechanical impact on opposing cartilage surface rather than a biological effect.

Supporting this view we did not find any deleterious process on cartilage health over time when comparing 6 to 12 month data. Contrary to this some authors suggest that the repair tissue after biological treatment might degenerate over time28,29 potentially progressing to panarthritis4,50.

Sheep and goats are commonly used animals models for the study of OA, as they, beside the advantage of being large, have anatomy and biomechanics comparable to humans. Other researchers used the goat model to test the safety and efficacy of FKR implants although it is known that goats develop spontaneous OA as young as 2 years old13,21,22. Thus to avoid compromising the results we used female sheep (ewes) between 2 and 6 years (skeletally mature “middle age”). The animals were operated unilaterally using the contralateral unoperated side as a control group for comparison. They were kept indoor the first weeks and thereafter in an outdoor stable, yet allowed to “exercise” outside in a standardized fashion to limit random lesions that could have compromised the results. As histopathology remains the gold standard for evaluation of cartilage injuries we focused on this method and followed the recommendations of the OARSI group for cartilage studies in sheep and goat animal models21. Interestingly, we observed a wide span of cartilage damage of both operated and non-operated knees whilst the differences between knees in the same animal were moderate. Furthermore, in a post-hoc analysis we showed a strong correlation between age and cartilage damage suggesting that also sheep show signs of early OA (Figs. 6, 8). Researchers should consider this when planning studies on treatment of cartilage injuries using a sheep animal model.

A weakness of this study could be the wide age range among individuals with relatively large variances in cartilage damage. This might obscure differences in response to treatment between implanted and non-implanted specimens. Similarly, the small number of individuals in each age group restrains the possibility of statistical analysis when evaluating the cartilage damage at each time point (Fig. 8). Consequently, from our limited data we could not state that older individuals are more vulnerable to cartilage wear by opposing implants although an age dependent cartilage response could be implied. Cartilage with early OA changes might have less healing capacity when mechanically stressed. Hence, caution should be taken when inserting implants in condyles of older individuals showing signs of early OA.

To avoid failure of the FKR implant a long lasting secure fixation to bone is imperative. We have in a previous report shown the excellent osseointegration of the FKRM implant, sealing femoral cartilage defects in sheep51. The double coating of a Co–Cr implant, first with a pure layer titanium and then with a layer of hydroxyapatite, showed 90% bone ingrowth, which was in fact high when compared to any human or animal data13,19,22,31. Osseointegration was not impaired over time when comparing 6–12 month post-operative data. When fixating an FKR implant it is vital to spare bone for future intervention should this be needed and for this reason both large anchoring screws and cement should be avoided. Also, a firm fixation warrants a tight sealing between implant and surrounding tissue, as penetration of joint fluid into the implant–bone interface is prone to induce osteolysis and subsequently implant loosening. In our study we noted signs of adherence between cartilage and the HA covered area of the hat and speculated that this would further prevent joint fluids from penetrating the metal–bone interface.

We have previously shown the significance of implant positioning in terms of level of implantation using a laser-scanning device12. We showed a significant implant positioning inaccuracy that correlated to cartilage damage, nevertheless when seated optimally (0.5 mm recessed) mechanical damage to opposing cartilage was acceptable. A protruding implant on the other hand...
Fig. 6. Cross-sectional histological pictures (safranin blue) of the tibial cartilage opposing the implant (left panel), and opposing the non-operated control knee (right panel). Scale bar denotes 1 mm. Rows represent 2–6 year old sheep. Observe the increasing degree of cartilage damage of both operated and non-operated knees with age.
might severely damage the opposing cartilage surface. In this study we demonstrated that the technical development of the instrumentation, using a “custom-made” guide system, proved effective to place all implants in the suggested “safe zone”; thus the positioning of these implants was much improved relative to our previous study. Tilting of implants however has not been reported in the literature before, although this might also cause deleterious effects to surrounding cartilage. The refined laser measurement protocol allowed calculation of implant angulation in relation to the cartilage surface, both in the sagittal and frontal plane. For our particular sized implant seated at a recessed height of 0.5 mm we calculated that an angulation of $8^\circ$ could be allowed before any spot of the implant would protrude. Three implants showed a posterior (sagittal) tilt between 5 and $8^\circ$ whereas the transversal (frontal) angulation seemed negligible. Those implants
had one spot (out of five) that protruded 0.1 mm each, however these individuals were not outliers in terms of cartilage wear. These data together with the accurate seating, on average 0.54 mm recessed, make us conclude that implant positioning was satisfactory using the developed guiding instrumentation. For human knee implants there is a similar need to confirm the final overall implant position. Implant design is yet another important factor when trying to preserve the opposing cartilage surface. Previously described FKRMs used a single-radius articulating surface that will not match the different sagittal and frontal radii of the femoral condyle. A single-radius (spherical) implant inserted using a basic positioning device aimed at a flush position might in fact protrude or tilt, causing severe damage to the opposing cartilage. We chose a double-curved implant with radii similar to the surrounding condyle in order to maximize implant-cartilage fit and integration while minimizing the need for surgical margins. In our study we showed little tilt, thus optimal final overall position. This proved to be a successful design and these results would warrant a similar design to be used clinically in patients. To obtain the requested height and tilt that follows the curvature of the native condyle, a patient-specific implant and guiding system could be recommended.

The treatment of mid-size (2–3 cm²) full thickness focal cartilage lesions in middle age patients (35–60 years) remains a clinical challenge. Our studies have shown excellent osseochondrointegration in a sheep animal model. Consistent and accurate position of FKR implants at a defined safe zone (about 0.5 mm recessed and less than 8° of tilting for this particular implant) was reached. At this position cartilage wear of opposing and surrounding joint cartilage is limited. We believe that this FKR method should be compared to biological resurfacing methods such as osteochondral autologous grafting (mosaicplasty) with regard to cartilage damage. Thus expanded animal and human studies are motivated.

### Contribution of authors

NM-C: Study design, performance of all surgeries, data analyses, writing manuscript, LR: study design, performance of most surgeries, KH and HH: histological preparation evaluation, HN-S: performance of all surgeries and care of animals, A-SL: performance of most surgeries, care of animals, writing part of the manuscript, PS data analysis, histometric preparation and evaluation, and HB: study design, data analyses and writing manuscript. All authors: review of the manuscript.

### Disclosure

Dr. Martinez-Carranza reports grants from Stockholm County Council, Karolinska Institutet, Karolinska University Hospital, and from Episurf Medical AB. Stockholm, Sweden during the conduct of the study. Dr. Berg reports grants from Stockholm County Council, Karolinska Institutet, and grants from Episurf Medical AB. Stockholm, during the conduct of the study. Dr. Lagerstedt and Dr. Nurmi-Sandh report non-financial support for laboratory facilities from Episurf Medical AB. Stockholm, during the conduct of the study. Dr Schupbach reports non-financial support for laboratory facilities from Episurf Medical AB, Stockholm during the conduct of the study.

### Conflicts of interests

Dr. Ryd reports financial support as a board member from Episurf Medical AB, Stockholm. For this reason he did not participate in the analysis of data.

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