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Perilesional changes of focal osteochondral defects in an ovine model and their relevance to human osteochondral injuries

P. Hepp,
G. Osterhoff,
M. Niederhagen,
B. Marquass,
T. Aigner,
A. Bader,
C. Josten,
R. Schulz

*From University of
Leipzig, Leipzig,
Germany*

■ P. Hepp, MD, Assistant
Professor, Orthopaedic
Surgeon
■ G. Osterhoff, MD, Research
Fellow
■ B. Marquass, MD,
Orthopaedic Surgeon
■ C. Josten, MD, Professor and
Chairman
Department of Trauma and
Reconstructive Surgery
University of Leipzig,
Liebigstrasse 20, 04103 Leipzig,
Germany.

■ M. Niederhagen, MD,
Pathologist
Department of Pathology
University of Munich (LMU),
Thalkirchner Strasse 36, 80337
Munich, Germany.

■ T. Aigner, MD, MSc,
Professor of Histopathology
Department of Pathology
University of Leipzig,
Liebigstrasse 26, 04103 Leipzig,
Germany.

■ A. Bader, MD, Professor and
Chairman
■ R. Schulz, PhD, Head of
Cartilage Research Group
Department of Cell Techniques
and Applied Stem Cell Biology
University of Leipzig, Center of
Biotechnology and
Biomedicine, Leipzig, Germany.

Correspondence should be sent
to Dr P. Hepp; e-mail:
pierre.hepp@medizin.uni-
leipzig.de

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Perilesional changes of chronic focal osteochondral defects were assessed in the knees of 23 sheep. An osteochondral defect was created in the main load-bearing region of the medial condyle of the knees in a controlled, standardised manner. The perilesional cartilage was evaluated macroscopically and biopsies were taken at the time of production of the defect (T0), during a second operation one month later (T1), and after killing animals at three (T3; n = 8), four (T4; n = 8), and seven (T7; n = 8) months. All the samples were histologically assessed by the International Cartilage Repair Society grading system and Mankin histological scores. Biopsies were taken from human patients (n = 10) with chronic articular cartilage lesions and compared with the ovine specimens. The ovine perilesional cartilage presented with macroscopic and histological signs of degeneration. At T1 the International Cartilage Repair Society 'Subchondral Bone' score decreased from a mean of 3.0 (SD 0) to a mean of 1.9 (SD 0.3) and the 'Matrix' score from a mean of 3.0 (SD 0) to a mean of 2.5 (SD 0.5). This progressed further at T3, with the International Cartilage Repair Society 'Surface' grading, the 'Matrix' grading, 'Cell Distribution' and 'Cell Viability' grading further decreasing and the Mankin score rising from a mean of 1.3 (SD 1.4) to a mean of 5.1 (SD 1.6). Human biopsies achieved Mankin grading of a mean of 4.2 (SD 1.6) and were comparable with the ovine histology at T1 and T3.

The perilesional cartilage in the animal model became chronic at one month and its histological appearance may be considered comparable with that seen in human osteochondral defects after trauma.

Owing to its avascularity, articular cartilage shows no significant capacity to recover after injury or destruction.¹ Common clinical treatment such as microfracture drilling² or osteochondral autologous transplantation (OATS) attempts to restore the damaged cartilage tissue.^{3,4} Microfracture drilling produces fibrocartilage of inferior quality with less mechanical load capacity and OATS has the disadvantage of the morbidity related to harvesting.⁵ Since the first description of autologous chondrocyte implantation (ACI) for deep cartilage defects in the human knee,⁶ the analysis of tissue engineering systems has grown in importance.⁷ Preclinical studies using large animal models with comparable stresses and strains reflecting the complex surgical site are indispensable.^{7,8} Previously, many studies dealing with cartilage regeneration used an acute cartilage defect in which generation and reconstruction of the defect were conducted at the same time.⁹⁻¹⁶ This practice does not represent the clinical situation. Most patients have a lengthy course following trauma to the cartilage, leading to chronic defects. This results

in altered intra-articular homeostasis.^{17,18} Therefore, the encouraging and reproducible data obtained in experimental studies *in vivo* have been unattainable in the clinical setting.¹⁹⁻²³ Although assessment of the biochemical and biomechanical alterations of the cartilage surrounding a defect has been performed in a semi-qualitative manner,²⁴ quantitative and reproducible data are not yet available.

This study assessed changes in the perilesional cartilage over time in focal osteochondral defects of the load-bearing area of the medial femoral condyle in an ovine knee using established histological scores. Biopsies of human cartilage adjacent to focal cartilage lesions were evaluated to assess whether the changes were comparable with the clinical appearance and to find when there was maximum morphological similarity between ovine and human perilesional cartilage.

Materials and Methods

A total of 23 female Merino-mix sheep, with a mean age of 22.3 months (SD 3) and a mean body weight of 65 kg (SD 63) were included in

Fig. 1a

Fig. 1b

Fig. 1c

Fig. 1d

Photographs showing preparation of the defect. After marking the defect with a sharp tube (a), cartilage can be harvested by a curette (b). Under guidance of the tube, the subchondral endplate is penetrated by a drill (c), resulting in an osteochondral defect (d).

the investigation. The experiment was approved by the local legal representative (TVV33/04, State Directorate Leipzig, Germany) and performed in accordance with the guidelines of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes.²⁵

Two operations were performed in each animal. After fasting for 12 hours, the animals were anaesthetised by intramuscular administration of 11.0 mg/kg ketamine, 0.22 mg/kg xylazine and 0.02 mg/kg atropine.^{26,27} After endotracheal intubation, anaesthesia was maintained by continuous intravenous administration of ketamine (8.25 mg/kg/h) and xylazine (0.165 mg/kg/h).²⁸ Analgesia was supplemented by fentanyl (0.1 mg bolus intravenously)

prior to surgery,²⁹ and as needed during the operation. During anaesthesia, supplementary oxygen (flow: 8 l/min to 10 l/min) was given, and in the event of capillary saturation falling below 90%, ventilation was assisted manually. The knee joint was prepared in a standard sterile manner, the medial condyle was exposed by a small parapatellar medial arthrotomy, and the articular surface was assessed before and after creating the defect.

A standardised circular osteochondral defect 7 mm in diameter was created in the main load-bearing region of the medial condyle, extending for 2 mm into the subchondral bone (Fig. 1). First, a circular cartilage lesion 7 mm in diameter was made with a tube harvester (Arthrex Inc., Naples, Florida) without penetrating the subchondral bone. The

Fig. 2

The scheme for assessment of the sheep with osteochondral defects.

cartilage was carefully excised manually down to the calcified cartilage layer with a curette. Before drilling, and in order to minimise damage to the perilesional cartilage, a sharp tube was pressed on to the osteochondral border³⁰ as a guide for the 7 mm cylindrical flat-tipped drill (B. Braun Melsungen AG, Melsungen, Germany). The depth of the osteochondral defect was controlled by a custom-made drill stop on the tube. There was bleeding at the base of the defect in all cases.

A 1.8 mm diameter osteochondral biopsy specimen was harvested 2 mm from the edge of the cartilage lesion of the left knee in eight sheep using a 13-gauge Jamshidi bone marrow biopsy needle (Cardinal Health Inc., McGaw Park, Illinois).

One month after the first operation, a second procedure was performed (T1). The knee was opened in the manner previously described and a 1.8 mm diameter biopsy specimen was obtained as before.

All the wounds were sutured and covered with a spray bandage. The animals received metamizol (Novaminsulfon, Ratiopharm, Ulm, Germany) as analgesia for the first seven days. Immediately after operation, they were allowed to move freely and bear weight fully without restriction. They were monitored at all times by a veterinary surgeon. At three months after the first operation eight animals were killed (T3), a further eight after four months (T4) and seven after seven months (T7) (Fig. 2).

At the time of the two operations and after killing, the appearance of both the osteochondral lesion and the marginal area of the defect was rated according to the International Cartilage Repair Society Cartilage Injury Classification (ICRS-CIC),³¹ and possible abnormalities within the joint were documented.

The biopsy specimens of the perilesional cartilage of the sheep were prepared as described below for the human tissue samples. After removing the left femur, osteochondral cylinders (15 × 15 × 15 mm) containing the defect and the surrounding cartilage were obtained using a water-cooled precision-saw (Exakt-Trennschleifsystem, Exakt Apparatebau GmbH,

Norderstedt, Germany), the tissue samples fixed in 4% buffered formalin for one to three days. The specimens were then decalcified in a 20% EDTA solution at pH 7.4 for 16 weeks. They were then dehydrated in a graded series of ethanol, embedded in paraffin, and sectioned at a thickness of 3 µm. After dewaxing the slices, a described staining protocol for Safranin O, haematoxylin and eosin (HE)³² was followed to enable histological examination. In some cases an additional toluidine blue staining was performed to provide better contrast. These two or three stains were necessary to enable precise histomorphological analysis of the biopsies.^{33,34} Histological scoring of the perilesional cartilage was undertaken using the Mankin score³⁵ and the ICRS Visual Histological Assessment Scale (ICRS-VHS).³³

Human biopsies. A total of ten patients were recruited after appropriate Institutional Board Review approval and giving their informed consent. Inclusion criteria were age between 16 and 65 years, and radiological (MRI) evidence of a focal grade IV osteochondral lesion, according to the ICRS classification, requiring arthroscopy.³¹ The presence of any cartilage lesion, including the size and the grade, was recorded.

The patients underwent diagnostic arthroscopy and routine exploration of the joint cavity. Abnormal findings were recorded and graded using ICRS-CIC.³¹ The surgeon then obtained a 1.8 mm diameter biopsy specimen as described for the animal model above.³⁶ Any relevant surgical therapeutic procedures necessary were then performed and microfracture² was undertaken.

The biopsy specimens were fixed in 4% buffered formaldehyde for a maximum duration of six to nine hours. After fixation, decalcification using 20% EDTA pH 7.4 was undertaken for at least 12 to 15 hours. Afterwards the specimens were dehydrated, embedded in paraffin, and sectioned at a thickness of 2 µm with a conventional microtome. The sections were stained with Safranin-O, HE and toluidine blue, and graded using the histological scoring system described by Mankin et al.,³⁵ with reliability testing according to van der Sluijs et al.³⁷

Statistical analysis. Data were expressed as mean with SD, their normal distribution being examined by the Kolmogorov-Smirnov test and for homogeneity using Levene's test. For the animal model a Kruskal-Wallis test was used for each parameter against time. If significant findings were detected, the Mann-Whitney U test was applied for data which were not normally distributed to selectively analyse differences that were considered statistically significant if the p-value was < 0.05. Correlations were calculated using Spearman's rank correlation coefficient, and the correlation was considered significant for values of $r > 0.05$ and $p < 0.001$. Statistical analysis was performed with SPSS 14.0 software (SPSS Inc., Chicago, Illinois).

Results

Animals. All animals tolerated anaesthesia and their surgical procedures well. Although some sheep limped for the initial two to three days after operation, normal gait soon

Fig. 3a

Fig. 3b

Fig. 3c

Fig. 3d

Fig. 3e

The image sequence shows the macroscopic appearance of the defect and the perilesional cartilage (a) immediately after creation of the defect (T0), (b) after one (T1), (c) three (T3), (d) four (T4) and (e) seven (T7) months.

returned and no swelling of the joints was observed in any animal prior to the creation of the defect, interval biopsies and euthanasia.

According to the ICRS-CIC, the cartilage defects were grade IV lesions when they were produced (T0). Although all (23 of 23) defects still extended into the subchondral bone after one month (T1) (mean 4, SD), a filling of the defect after three months (T3) with a mean grade of 3.5 (SD 0.5, at four months (T4) with a mean grade of 2.6 (SD 0.5) and at seven months (T7) with a mean grade of 2.6 (SD 0.5). There was a statistically significant change in the mean grade from T1 to T3 ($p = 0.037$) as well as from T4 to T7 ($p = 0.009$, Mann-Whitney U test). In all groups, the defects were distinguishable from the surrounding tissue (Fig. 3). A lucid softening of the tibial cartilage was seen opposite the femoral defects in all animals.

Macroscopically, the perilesional cartilage showed time-dependent changes. No damage was seen after the initial defect (T0). At four weeks (T1) the border showed mainly fibrillation with slight softening and additional superficial lacerations and fissures, corresponding to ICRS-CIC grades Ia and Ib. After three, four and seven months (T3, T4 and T7) damage to the cartilage appeared to extend deeper, albeit still involving $< 50\%$ of the thickness. Macroscopic changes correlated strongly both with time (perilesional cartilage: $r = 0.95$; defect: $r = -0.63$; both $p < 0.001$) and noticeably with each other ($r = -0.50$; $p < 0.001$) (Fig. 4).

In the histological analysis, the margins of the undamaged cartilage contained chondrocyte clusters and had lost metachromasia and Safranin-O staining. Further away from the lesion, the chondrocytes remained organised in columns, but the columns were tilted towards the lesion. The cellularity of the immediate neighbourhood of the lesion was reduced in two of the 23 cases (Figs 5 and 6).

The specimens gathered at T0 featured a mean Mankin score of 0.50 (SD 0.8). At one month, a tendency towards higher values could be detected in the structure, cellular abnormalities and matrix staining, with superficial fissures in nine knees and clusters of chondrocytes in two, but these were not statistically significant ($P_{\text{structure}} = 0.11$; $P_{\text{cell}} = 0.26$; $P_{\text{matrix}} = 0.81$, Mann-Whitney U test). Comparing the results of T3 with T1, a structural degradation was seen ($P_{\text{struc}} < 0.01$) which was accompanied by cell destruction ($P_{\text{cell}} < 0.01$) and a reduced matrix staining reaching into the interterritorial layers ($P_{\text{matrix}} < 0.01$, Mann-Whitney U test). Specimens at T4 and T7 showed no significant differences from the T3 analogue regarding the macroscopic findings (Table I).

The ICRS-VHS resulted in similar trends in evaluation according to the Mankin grading. There were significant signs of degeneration ($p = 0.03$, Mann-Whitney U test) for the ICRS parameter matrix after one month. The distinct degenerative progress from T1 to T3 with superficial discontinuities ($P_{\text{surface}} < 0.01$), alterations of the extracellular matrix ($P_{\text{matrix}} = 0.023$), clustering of chondrocytes ($P_{\text{cell distribution}} < 0.01$) and cell deterioration ($P_{\text{cell viability}} < 0.01$, Mann-Whitney U test) showed high levels of significance. Pathological calcifications of cartilage tended to occur three months after creation of the defect (T0 + T1: $n = 0$, T3: $n = 1$, T4: $n = 3$, T7: $n = 2$) but were not significant when the groups were compared statistically ($p = 0.22$, Kruskal-Wallis test).

The subchondral bone, which appeared intact in the beginning, showed clear signs of alteration ($p < 0.01$, Mann-Whitney U test), with increased bone remodelling in all cases and sporadic bone necrosis in two after only one month. Two months later (T3), bone necrosis was present in all specimens (8 of 8) with additional fracture callus at the base in one animal ($p < 0.01$, Mann-Whitney U test). The outcome of this is an accumulated histological ICRS score with a significant downward progress from T0 to T1 ($p < 0.01$) and from T1 to T3 ($p < 0.01$, Mann-Whitney U test). The

Fig. 4a

Fig. 4b

Histograms showing the results of the macroscopic evaluation of the perilesional cartilage (a) and the defect (b) using the International Cartilage Repair Society Cartilage Injury Classification (ICRS-CIC) (error bars represent two standard errors).

collected values at T4 and T7 showed no statistical differences from each other and from T3 (Table II).

The Mankin histological score and ICRS-VHS clearly correlated with time ($r_{\text{Mankin}} = 0.73$, $p < 0.01$; $r_{\text{ICRS-VHS}} = -0.86$, $p < 0.01$) as well as with each other ($r = -0.82$, $p < 0.01$). Both scores correlated with the macroscopic findings of the perilesional cartilage (Mankin: $r = 0.60$, $p < 0.01$; ICRS: $r = -0.67$, $p < 0.01$) and especially of the subchondral bone ($r = -0.64$, $p < 0.01$).

Human biopsies. Arthroscopic perilesional biopsies were evaluated from ten patients at a mean of 7.6 months (0.5 to 24) after injury to the knee (Table III, Figs 5 F, 6 E and F). None of the knees showed macroscopic signs of generalised osteoarthritis. No softening of cartilage was found further away from the defects. After biopsy, harvesting and subsequent debridement, the borders of the lesions were found to have stable tissue. Three patients required meniscal surgery and one had reconstruction of the anterior cruciate ligament. According to the Mankin score, all specimens except one showed superficial fissures in the radial layer. In one case superficial cartilage was absent, but in none of the biopsies was the calcified layer involved (Table I). All biopsies showed changes on the cellular level, eight with occurrence of chondrocyte clusters that were accompanied by initial hypocellularity in one case. In all but one specimen, mild to moderate reduction in the intensity of Safranin-O staining could be seen, especially in the superficial third. No correlation was found between size or time and the histological or macroscopic scores.

Discussion

We are aware of no data in the current literature concerning the evaluation of perilesional cartilage using established classification systems. Our results correspond with those of other studies in large animals, where staining and proteoglycan loss, hypercellularity and cartilage necrosis were observed in the edges of the defect.³⁸⁻⁴² However, because all the listed literature refers to the edges, it is difficult to determine to what extent these data apply to the perilesional cartilage investigated in the present study.

Although the site of the defect showed increased filling, the macroscopic appearance of the perilesional cartilage in the animal model was dominated by spreading degenerative changes. Similar findings were described for osteochondral defects with a diameter of 6 mm in goats.^{39,43} Histological evaluation of the perilesional cartilage indicated clear signs of degeneration incorporating the subchondral bone after only one month. These changes were progressive at three months. However, the Mankin histological scores showed values corresponding only to rather moderate cartilage damage. This is in accordance with histological results described for comparable macroscopic findings in the articular cartilage of sheep.⁴⁴ At four and seven months, no further significant progression of the degeneration could be seen at the microscopic level, but there was a tendency towards regression of matrix degeneration and a consistent excess of subchondral bone mineralisation. A possible explanation for this might be mechanical stabilisation of the edges of the defect due to its increasing filling.

Histological appearance of the perilesional cartilage (Safranin-O; $\times 4$; bar = 500 μm). In the animal model at the time of creation of the defect (a) there is normal hyaline cartilage with its characteristic columnar structure. At one month (b), initial hypercellular disorganisation, a slight interterritorial stain loss and subchondral bone remodelling can be seen. At three months (c), bone remodelling increases and chondrocyte clusters appear. Four months after creation of the defect (d) noticeable hypocellularity is visible accompanied by many cell clusters. These patterns persist unchanged at seven months (e). The human biopsy (f) is of greater thickness and shows hypercellularity, a loss of the columnar structure and a few chondrocyte clusters, very similar to the ovine biopsies at one and three months.

We do not know to what extent altered biomechanical stress patterns and the disturbed cytokine and transmitter balance contribute to the observed changes in the perilesional cartilage, and whether or not these are simply responses to the original defect. At a distance of 2 mm from the edge of the defect, all three factors can be of influence.⁴⁵ The macroscopic findings with fissures originating from the defect at T1 suggest direct expansion of the lesion. The early remodelling of subchondral bone can be explained either by the inflicted injury itself or by biomechanical overload.^{44,46,47} Finally, the quick cellular reaction and loss of proteoglycans indicate a disturbance of joint homeostasis.⁴⁷⁻⁴⁹ The observed macroscopic tibial changes opposite to the defects have been described previously.^{41,42,49,50} Mastbergen et al⁵⁰ concluded that these may be a reaction to increased proteinase and aggrecanase activity in the joint, rather than due to surgical damage. Previous studies attribute insufficient healing following intervention in older cartilage lesions to an imbalance of the intra-articular metabolic environment.¹⁵⁻¹⁷ Consequently, the results of this investigation suggest that this imbalance also leads to degeneration of the perilesional cartilage.

Remodelling of subchondral bone below the perilesional cartilage might be a reaction to elevated biomechanical loads, resulting in increased stiffness of the subchondral

bone and hence higher stresses on the cartilage matrix.⁴⁴⁻⁴⁶ This is another possible reason for the observed initial loss of proteoglycans in the perilesional cartilage.^{50,51} Irrespective of which process was the initial one, the parallel occurrence of cartilage degeneration and remodelling of subchondral bone after one month indicates that the defect became chronic.⁴⁸ Evidence for this is even more obvious after three months, when the imbalance of joint homeostasis may become manifest in the histological picture.

All elevated human biopsies presented with degenerative changes of the cartilage, particularly in the superficial third. At the cellular level, each of the samples showed at least a diffuse hypercellularity. These histological results are consistent with data published for similar macroscopic patterns in human knees.⁵¹⁻⁵³ In view of the documented size of the defects and the mean time between trauma and intervention of 7.6 months, it is apparent that most of the defects examined were of a chronic nature. Although there were no macroscopic signs of concurrent generalised osteoarthritis in the human knees, this could not be completely excluded at the histological level and must be considered in the comparison between ovine and human biopsies. The mean age of our patients of about 45 years is similar to that of patients who have been considered for cartilage repair.⁵³⁻⁵⁵

Ovine
1 month
(x 20 / x 10)

Fig. 6a

Fig. 6b

Ovine
3 months
(x 20 / x 10)

Fig. 6c

Fig. 6d

Human
(x 20 / x 10)

Fig. 6e

Fig. 6f

Histological appearance of the perilesional cartilage (Safranin-O $\times 20/\times 10$; bar = 100 μ m). The transitional zone shows hypercellularity in both the ovine (a + c) and the human (e) biopsies. Increasingly, subchondral bone remodelling and excessive mineralisation occur in the animal model (from b to d). Both could also be seen in the human biopsies (f).

Because of the different anatomical dimensions, the absolute sizes of the defects in human and ovine knees cannot be compared. Thus, in each joint a critical size for a lesion must be determined. A defect diameter of 10 mm (0.78 cm²) has been shown to be critical for increased perilesional contact stresses in the human knee.³⁷ Except in one patient, all of the human biopsies were obtained from knees with osteochondral lesions larger than this threshold size.

In the ovine knee, which has only about 60% of the contact area of a human knee, a defect of 7 mm in diameter might be reasonably assumed to be critical. In large animal models, osteochondral defects with a diameter of 7 mm were not able to heal completely on their own.^{15,16,29,38,39} This is analogous to our macroscopic findings for the defect zone.

The applied animal model is not suitable for every possible pattern of clinical defect. Purely chondral defects which do

Table I. Histological evaluation of the perilesional cartilage by the Mankin histological score³⁵ (all values mean, SD)

	Sheep					Human
	T0 (n = 8)	T1 (n = 23)	T3 (n = 8)	T4 (n = 8)	T7 (n = 7)	
Mankin (SD)	0.5 (0.8)	1.3 (1.4)	5.1 (1.6*) p < 0.001	5.1 (1.9)	4.4 (2.6)	4.2 (2.0)
Structure (SD)	0.0 (0.0)	0.4 (0.5)	2.4 (0.7*) p < 0.001	2.4 (0.7)	2.3 (1.8)	1.2 (1.0)
Cellular abnormalities (SD)	0.1 (0.4)	0.5 (0.7)	1.5 (0.8*) p = 0.003	1.5 (0.8)	1.4 (0.5)	1.9 (0.7)
Matrix staining (SD)	0.4 (0.5)	0.4 (0.5)	1.3 (0.5*) p = 0.004	1.3 (0.5)	0.7 (0.5)	1.1 (0.7)

* indicates a significant difference to T1, p < 0.05 (animal model only)

Table II. Animal model. Histological evaluation of the perilesional cartilage by the International Cartilage Repair Society Visual Histological Assessment Scale (ICRS-VHS)³¹ (all values mean, SD)

	Sheep				
	T0 (n = 8)	T1 (n = 23)	T3 (n = 8)	T4 (n = 8)	T7 (n = 7)
ICRS-VHS (SD)	17.9 (0.4)	15.7 (1.6*) p < 0.001	9.1 (3.4†) p < 0.001	8.5 (3.1)	9.7 (4.2)
Surface (SD)	3.0 (0.0)	2.5 (1.2)	0.4 (1.1†) p = 0.02	0.0 (0.0)	0.4 (1.1)
Matrix (SD)	3.0 (0.0)	2.5 (0.5*) p = 0.03	1.9 (0.4†) p = 0.023	2.0 (0.0)	1.7 (0.5)
Cell distribution (SD)	2.9 (0.4)	2.8 (0.4)	1.6 (0.7†) p < 0.001	1.5 (0.8)	1.7 (0.5)
Cell population viability (SD)	3.0 (0.0)	3.0 (0.0)	1.8 (1.0†) p = 0.007	2.3 (1.0)	2.4 (1.0)
Subchondral bone (SD)	3.0 (0.0)	1.9 (0.3*) p < 0.001	0.9 (0.4†) p < 0.001	0.9 (0.6)	1.3 (0.5)
Cartilage mineralisation (SD)	3.0 (0.0)	3.0 (0.0)	2.6 (1.1)	1.9 (1.6)	2.1 (1.5)

* indicates a significant difference to T0

† indicates a significant difference to T1, p < 0.05 (animal model only)

Table III. Data from human cartilage biopsies from the medial femoral condyle (n = 10)

Patient	Gender*	Age (yrs)	Time trauma to surgery (mths)	Defect size (cm ²)	ICRS-CIC†	Concurrent injuries‡
1	M	22	0.5	5	4	None
2	M	48	2	5.25	4	MM
3	F	51	2	3	4	None
4	M	49	3	4.5	4	None
5	M	44	5	1	4	None
6	F	49	5	1.5	4	MM
7	M	45	9	4.5	4	None
8	M	60	10	4	4	MM, LM
9	M	61	15	0.25	4	None
10	M	17	24	2.25	4	ACL, MM
Mean (range)		44.6 (17 to 61)	7.6 (0.5 to 24)	3.1 (0.5 to 53)	4.0 (4.0 to 4.0)	

* M, male; F, female

† ICRS-CIC, International cartilage repair society-cartilage injury classification

‡ MM, medial meniscus lesion; LM, lateral meniscus lesion; ACL, rupture of the anterior cruciate ligament

not affect the subchondral bone are difficult to standardise.^{7,33,43} With a depth of 2 mm, the described model resembles a severe osteochondral injury. In clinical practice, osteochondral lesions may not be the most frequent pattern of injury, but they account for over 9% of all cartilage lesions⁴⁰ and therefore pose a substantial problem.

In this study we investigated the perilesional cartilage at a distance limited to 2 mm from the defect, because for ethical reasons it was not acceptable to harvest biopsies further away in human knees. Another limitation was the short follow-up of seven months. For future studies it would be useful to address multiple sites in the joint and

include longer follow-up to see how quickly and how far degenerative changes spread with time.

The perilesional cartilage in the ovine defect model showed signs of chronic degeneration at one and three months. These may be comparable with those seen in cross-sectional human biopsies of chronic defects after trauma. Data are provided for future research in tissue-engineered cartilage substitutes and their regenerative potential in a chronic setting. Further research may focus on the question of whether the degeneration of the perilesional cartilage can be arrested or slowed by various techniques of repair. As a period of four weeks corresponds to

the usual duration for cell culture of ACI as well as bone marrow-based approaches,⁵⁶ future pre-clinical investigations on both cartilage and osteochondral repair may be performed on comparable chronic animal models.

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