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Matrix-associated autologous chondrocyte transplantation in a compartmentalized early stage of osteoarthritis

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SUMMARY

Objective: Cartilage restoration in joints with an early stage of osteoarthritis (OA) is an important clinical challenge. In this study, a compartmentalized, early-stage OA was generated surgically in sheep stifle joints, and this model was used to evaluate a matrix-associated cell transplantation approach for cartilage repair.

Method: Eighteen sheep were operated twice. During the first operation, a unicompartmental OA in a stable joint was induced by creating a critical-size defect. The second operation served as a regeneration procedure. The eighteen sheep were divided into three groups. One group was treated with spongialization (SPONGIO), while the two others had spongialization followed by implantation of a hyaluronan matrix with (MACT) or without chondrocytes (MATRIX). The follow-up took place 4 months after the second operation. Gross Assessment of Joint Changes score and Brittberg score were used for the macroscopic evaluation, Mankin score, O'Driscoll score, and immunohistochemistry for collagen type I and type II for histological evaluation.

Results: The MACT group achieved significantly better results in both macroscopic and histological examinations. In the regeneration area, a Mankin score of 7.88 (6.20; 9.55) [mean (upper 95% confidence interval; lower 95% confidence interval)] was reached in the MACT group, 10.38 (8.03; 12.72) in the MATRIX group, and 10.33 (8.80; 11.87) in the SPONGIO group. The O'Driscoll score revealed a highly significant difference in the degree of defect repair: 15.92 (14.58; 17.25) for the MACT group compared to the two other groups [5.04 (1.21; 8.87) MATRIX and 6.58 (5.17; 8.00) SPONGIO; $P < 0.0001$].

Conclusion: This study demonstrates promising results toward the development of a biological regeneration technique for early-stage OA.

Introduction

Cartilage defects are still a challenging clinical entity, especially in early osteoarthritic joints. Unicompartmental osteoarthritis (OA) can result from a cartilage defect[1] which is a common problem of young and active people. Cartilage defects in adults do not show any spontaneous healing response[2]. Only in osteochondral defects, when the defect reaches the underlying subchondral bone, a limited spontaneous repair toward a fibrocartilage scar can occur[3–5]. However, this repair tissue lacks the molecular composition, complex organization, stiffness and durability of native articular cartilage[3]. Much work has been done to follow up cartilage repair models clinically and *via* MRI[6–10]. It is remarkable that the presence of OA was a major risk factor for graft failure. Even in early stages of OA, cartilage defects could not be restored successfully[6]. However, data on biologic regeneration for osteoarthritic joints is still scarce[11–13].

The primary goal of this study was to determine whether cell-based cartilage repair, using a cartilage and bone preparation technique, is a possible solution for early OA joints. Evaluating the current OA animal models for a potential use in this study, we could not find an appropriate established model for cell-based regeneration. The existing options either destabilized the joint (meniscectomy models, anterior crucial ligament transection models)[14–17]

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or induced OA-like changes simultaneously throughout the whole joint (injection of quinolone or similar drugs)[18,19]. Because localized OA in a stable joint is needed to study cell-based regeneration, we developed a new model[1]. In this model we could demonstrate that a chondral defect with a diameter of 7 mm on the medial femoral condyle over a full weight bearing time of 12 weeks induced unicompartmental OA at the medial compartment in sheep. The current study is the first implementation of this sheep OA model. Our hypothesis was to achieve a cell-based regeneration in unicompartmental OA, which might be applied to the treatment of young patients with secondary OA resulting from a cartilage defect.

Methods

Study subjects

The study was approved by the ethics committee for animal studies at the Vienna Medical University and the Austrian Federal Ministry of Science and Research (No. 66.009/012BrGT/2006). Eighteen mature female Austrian Mountain sheep weighing an average of 71 kg (±11), with a physiological knee joint status were used for this investigation.

Index procedure to induce OA

OA on the medial femoral condyle was induced by creating a critical-size defect with a diameter of 7 mm and a weight bearing regime of 12 weeks as described[1]. Before the operation X-rays were performed in anterior–posterior and lateral view to assess the physis status and to ensure that there were no deformities or bone abnormalities.

During this first procedure, cartilage biopsy specimens for chondrocyte harvest were obtained from the created defect area. The biopsy material was processed in a specialized laboratory facility. After cell isolation, the autologous chondrocytes were expanded in a 2D culture. Subsequently, the cells were seeded onto hyaluronic acid scaffolds (Hyaff C plus, Fidia Advanced Biopolymers Laboratories, Padova, Italy) and cultured for 1 week. The cells adhered to the scaffold, continued to proliferate, and redifferentiated into mature chondrocytes capable of producing their own extracellular matrix[20]. This scaffold is entirely based on the benzyklic ester of hyaluronic acid and consists of a network of 20-μm-thick fibers with interstices of variable size[20].

Surgical procedure for regeneration

Twelve weeks following the initial procedure, the joint was opened using the same mini arthrothomy as during the index procedure. The 18 sheep were randomly divided into three different regeneration groups of six sheep each: bone preparation by spongialization alone (SPONGIO), spongialization followed by implantation of an unseeded Hyaff C plus hyaluronan matrix (MATRIX), and spongialization followed by implantation of a chondrocyte-seeded hyaluronan matrix (MACT) (Fig. 1).

SPONGIO group

An oval defect of 20 × 10 mm and a depth of 2.5 mm was created in the arthritic lesion of the medial condyle including an abrasion of the subchondral bone, as described by Ficat *et al.*[21] The osteoarthritic cartilage area was circumcised using a sharp standardized oval punch of 20 × 10 mm, carrying the incision down to the subchondral bone. The edges were maintained perpendicular to the cartilage surface in order to separate the diseased cartilage from the healthy cartilage. The cartilage inside the oval was then carefully detached from the subchondral bone using a sharp spoon and a curette, leaving the bone denuded. Next the subchondral bone plate was completely removed, exposing the living underlying cancellous bone. For this procedure we used a circular motorized dental drill. First we used a drill, which had a small central point to prevent lateral drifting, to reach the 2.5 mm of depth. Then a drill without a central point was used to make the surface smooth. The abrasion area was then dried with gauze and the bleeding from the bone was stopped with fibrin glue (Fibrin Sealant TISSEEL, Baxter, Austria). This group served as a control group.

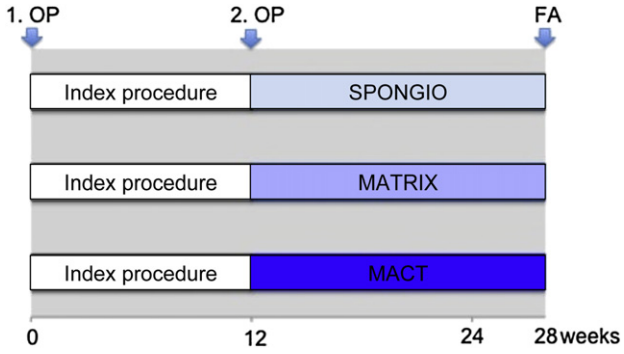

Fig. 1. Experimental design. The index procedure was done in all groups to induce OA. After the sheep had created unicompartmental OA after 12 weeks of weight bearing on the defect, they were divided in three treatment groups: MACT ($n = 6$), MATRIX ($n = 6$) and SPONGIO ($n = 6$). The follow-up took place after 28 weeks.

MATRIX group

The oval defect was created as described for the SPONGIO group. The scaffold was sized using the same punch used to circumcise the osteoarthritic area and was cut with a scalpel. After the abrasion area the condyle was dried with gauze, fibrin glue was used to stop the bleeding and to attach the scaffold to the defect. Six Monocryl 5-0 sutures (Ethicon, New Jersey, USA), three at the anterior part of the defect and three at the posterior part of the defect, were used to keep the scaffold in place. Before the joint closure the knee was flexed two times to confirm the stability of the implant.

MACT group

The oval defect was created as described for the SPONGIO group. The cultured chondrocytes seeded on the hyaluronan matrix (Fig. 2) were placed in the defect and fixed as described in the MATRIX group.

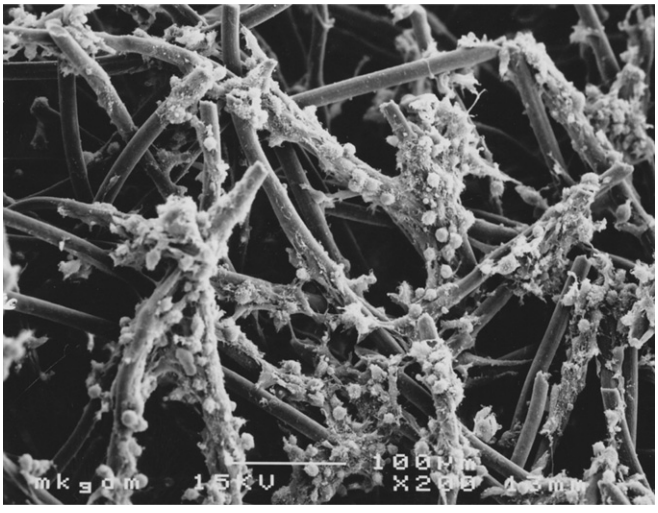

Fig. 2. Chondrocytes seeded onto the hyaluronic acid scaffold (electron microscopical picture).

After this step all groups underwent the same procedure. The wound was closed in layers. All sheep received an external plaster (Scotch/Soft Cast, 3 M Health Care, St Paul, MN, USA) to limit motion at the operated limb for 5 days, as described previously[7,22]. Free movement within the cage was allowed immediately after surgery. After 2 weeks the stitches were removed, and the sheep were returned to the fields for the remainder of the observation period.

Macroscopic grading

Four months after the regeneration procedure, the sheep were sacrificed and specimens were obtained under aseptic conditions. To document gross changes in the medial and lateral femoral condyles, the medial tibial plateau, and the meniscus, high-resolution photographs (Nikon D70S, AF-S VR Micro-NIKKOR 105 mm 1:2, 8G, Sigma Ringblitz EM_140 DG) were taken after removal of soft tissue.

Changes were rated according to the Gross Assessment of Joint Changes score[23], which grades macroscopic changes in cartilage and the meniscus on a scale of 0–4 in 12 different areas of the knee. The minimum score is zero points when no observable gross changes are seen, the maximum score is four points when more than 10% of bone is exposed and fragmentation of cartilage around the lesion can be detected. In between grade one stands for intact surface with color changes or surface irregularities or both, grade two for surface fibrillations or loss of cartilage with no bone exposed, and grade 3 for exposed bone less than 10% of surface area in the given region. The meniscus is scored zero for no apparent changes present, one for surface irregularity with color changes, two for partial-thickness tears with thinning of meniscal substance, three for full substance tears and four for no meniscal tissue present.

As a second macroscopic scoring system, the Brittberg score was used[24]. This score evaluates the quality of defect repair tissue and its integration with surrounding intact cartilage, and the macroscopic appearance. Each section can score from zero to four points. Therefore the overall score ranges from 0 to 12 points, with 12 points indicating the best result[24].

Cartilage damage and fibrillation were highlighted with Indian Ink Staining for photodocumentation according to Meachim's criteria[25]. The staining was performed as described before[1].

Tissue processing and histomorphometry

For microscopic evaluation, stifle joints first were fixed in 10% buffered formalin. Afterward all specimens were decalcified in a decalcification solution Titriplex-Tris-Solution (Gatt-Koller, Absam, Austria) 4–8 weeks at 37°C temperature. With a band saw the medial and lateral femoral condyles as well as the medial tibial plateau including the meniscus were cut along the sagittal plane into four pieces. On the medial femoral condyle two cuts were centered through the regeneration area. Additional cuts were made 2 mm medial and 2 mm lateral to the defect. Cuts on the tibial side were performed correspondingly. The specimens were embedded in paraffin, sectioned to 2.5-μm-thick slices using a microtome, and stained with hematoxylin/eosin (HE) for general morphology and Safranin O/fast green for glycosaminoglycans (GAG). Two independent readers (MS, NR) rated the cartilage according to the Mankin and the O'Driscoll scores[26,27]. The Mankin score ranges from 0 to 14 points, with zero indicating normal cartilage. The score includes evaluation of the cartilage surface, GAG content, cellular content, cloning, and tidemark integrity. The O'Driscoll score ranges from 0 to 24, with 24 indicating the best score. This defect score includes percentage of hyaline cartilage, surface regularity, structural integrity, thickness, bonding to the adjacent cartilage, cellular changes like normal or hypocellularity and cluster formations, and the freedom from degenerative changes in adjacent cartilage. Regenerated areas of the medial femoral condyle (two histo slices) were scored separately from the surrounding cartilage, including two histo slices beside the regeneration area and two slices made through the defect (scoring the cartilage anterior and posterior the regeneration area).

Immunohistochemisty with antibodies for type I and type II collagen (Southern Biotechnology Ass. Inc., AL, USA) was performed as previously described using a standard ABC protocol (Vector Laboratories INC, Burlingame, CA, USA)[28]. Immunhistochemical staining with antibodies to type I and type II collagen was graded 0 (no staining) to 3 (all tissue stained). Sections that could not be definitely assigned to 0 or 3 were graded as 1 or 2, depending on whether they were closer to 0 or 3[8].

Statistical methods

For descriptive statistics, mean value and 95% confidence intervals (CIs) were used except in case of skewed distributions, where the median (and quartile) values are given instead. In case of normally distributed data, *t*-tests and an analysis of variance model with *post hoc* testing (Tukey) were used to compare groups. Otherwise, the exact Wilcoxon Rank–Sum test and the exact Kruskal–Wallis test were used. A P value smaller than 0.05 was considered to indicate statistical significance.

Results

Surgeries were performed without any major complications. All animals remained healthy throughout the experiment and the post-operative cast for immobilization was well-tolerated by the animals. All sheep were fully weight bearing on the right hind leg and none was limping at the time of sacrifice. Clinical assessment showed neither instability nor intraarticular effusion. Manual palpation revealed mild subcutaneous fibrotic changes.

Creating the critical-size defects caused unicompartmental OA in all joints during the 12-week period following the first surgery (Fig. 3).

Gross assessment

Upon opening the joint, the synovial fluid was found to be clear in all animals at the time of the second operation as well as at the time of the follow-up. There was no sign of severe synovitis on gross examination. At the follow-up time point the cartilage surface in the MACT group seemed to be smooth and the regeneration area was well integrated. Only small osteophytes were present in some sheep. The surface of the regenerated area in the MATRIX group and especially in the SPONGIO group appeared irregular with bumpy formations. Small osteophytes were regularly present in these groups. The cartilage of the lateral condyle remained intact (Fig. 4).

The articular cartilage at the medial condyle showed color changes and surface irregularities. Some medial condyles even displayed areas with fibrillation, but bone was never exposed, except in one case within the regeneration area of the MATRIX group. Some condyles showed bumpy or soft surface instead of a hyaline cartilage, in particular in the MATRIX and SPONGIO groups. Interestingly, if fibrillations were present, they were more prevalent at the posterior femoral condyle (PFC) than at the anterior femoral condyle (AFC) [MACT group: AFC: 1 (1; 1), PFC: 1.0 (0.7; 1.6); MATRIX group: AFC 1.7 (0.8; 2.5), PFC 2.0 (1.3; 2.7); SPONGIO group: AFC: 1.3 (0.5; 2.2), PFC: 1.8 (1.4; 2.7)]. Compared to the degeneration before the regeneration operation, only the MACT group demonstrated improvement after 4 months. Evaluating the whole condyle with the Gross Assessment of Joint Changes score,

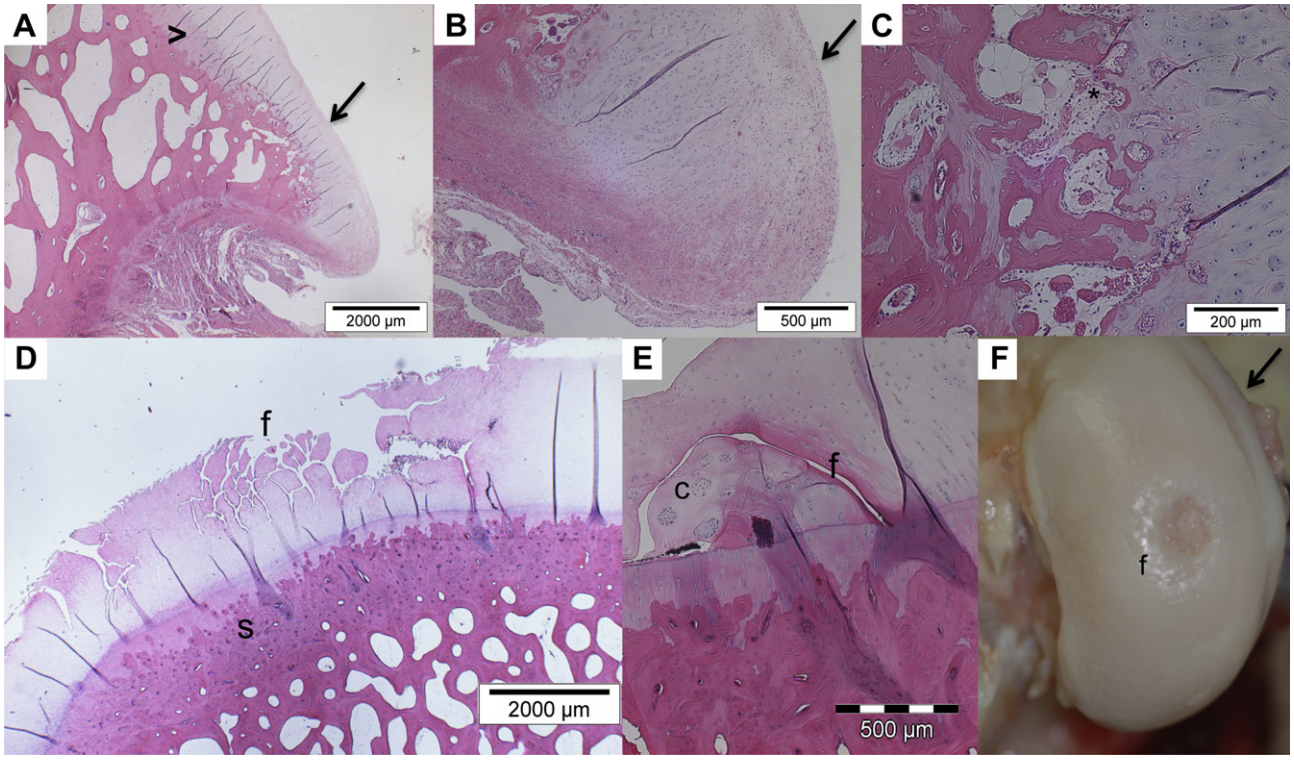

Fig. 3. After 12 weeks of weight bearing on a 7 mm cartilage defect on the medial femoral condyle unicompartmental OA was induced. All pictures shown in this figure represent the 12-week time point. Histological slides (A–E) were stained with HE. An osteophyte is marked with an arrow in A and B. The arrowhead indicates the calcified cartilage layer. C highlights the bone cartilage interface of the osteophyte in a bigger magnification. The tidemark is crossed by blood vessels as it is marked by * in C. In D fissures are flagged with "f" and the subchondral bone with "s". In E cloning (c) and fissures (f) are present. F is a picture of the medial femoral condyle. The cartilage defect area did not show any regeneration and fissures (f) are present in the surrounding cartilage. Osteophyte formation is present on the medial side (arrow).

a significant difference ($P = 0.02$) between the MACT and the MATRIX group could be detected (Table I).

Using the Brittberg score the MACT group reached 8.67 (5.88; 11.46) indicating superior results in comparison to the two other groups [MATRIX: 4.33 (2.07; 6.60) $P = 0.01$; SPONGIO: 3.33 (0.97; 5.70) $P = 0.004$] (Fig. 5).

Histology

Mankin score

In the regeneration area a Mankin score of 7.88 (6.20; 9.55) was reached in the MACT group. This was significantly better than the other two groups [MATRIX: 10.38 (8.03; 12.72) $P = 0.05$; SPONGIO: 10.33 (8.78; 1.87) $P = 0.02$]. In the area adjacent to the regeneration tissue, no significant differences could be detected (Fig. 6).

O'Driscoll score

The O'Driscoll score demonstrates a clearly superior outcome for the MACT group 15.92 (14.58; 17.25) in comparison to both, the MATRIX group 5.04 (1.21; 8.87) and the SPONGIO group 6.58 (5.67; 8.00), with P values <0.0001 for both comparisons (Figs. 6 and 7).

Immunhisto

The immunohistochemical staining for collagen type II revealed a significant difference ($P = 0.006$) between the MACT group and

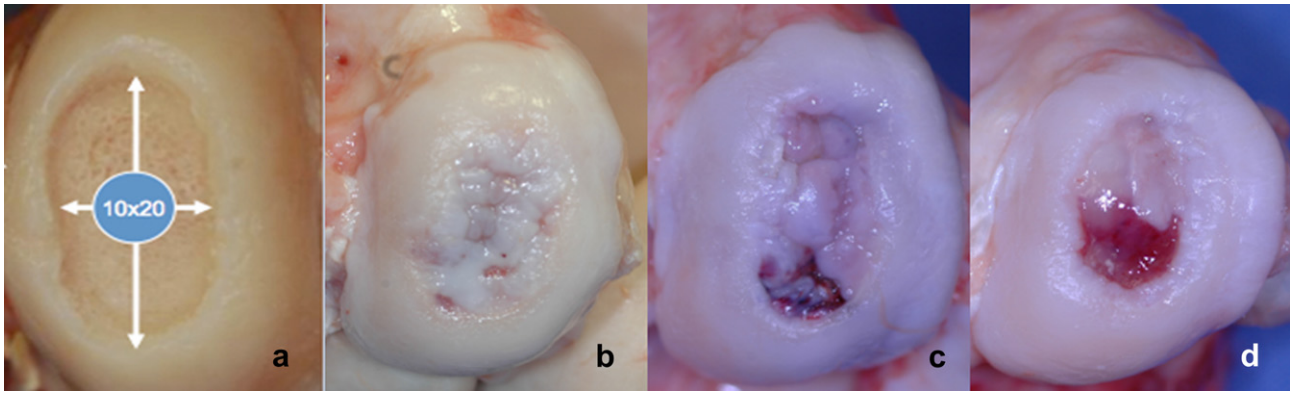

Fig. 4. Macroscopic appearance of representative condyles immediately following the spongialization procedure (12 weeks time point) (a) or after treatment with (MACT; b), with scaffold alone (MATRIX; c), or without treatment following spongialization (SPONGIO; d). Pictures b, c, and d were taken at the follow-up time point at 28 weeks. As shown in (a), the dimensions of the spongialization were 10 × 20 mm on the surface and 2.5 mm in depth.

Table I
Gross Assessment of Joint Changes score

Anatomic site	Time point*	MACT group			MATRIX group			SPONGIO group		
		Mean grade	Lower 95% CI	Upper 95% CI	Mean grade	Lower 95% CI	Upper 95% CI	Mean grade	Lower 95% CI	Upper 95% CI
Anterior medial femoral condyle	First OP	**0.0**	0.0	0.0	**0.0**	0.0	0.0	**0.3**	−0.5	1.2
	Second OP	**1.7**	1.1	2.2	**1.5**	0.9	2.1	**1.2**	0.7	1.6
	FA	**1.0**	1.0	1.0	**1.7**	0.8	2.5	**1.3**	0.5	2.2
Posterior medial femoral condyle	First OP	**0.0**	0.0	0.0	**0.0**	0.0	0.0	**0.2**	−0.3	0.6
	Second OP	**2.0**	2.0	2.0	**1.8**	1.4	2.3	**1.7**	1.1	2.2
	FA	**1.0**	0.7	1.6	**2.0**	1.3	2.7	**1.8**	1.4	2.7
Mean summative score	First OP	**0.0**			**0.0**			**0.3**		
	Second OP	**1.8**			**1.7**			**1.5**		
	FA	**1.0**			**1.8**			**1.6**		

Values are presented as mean values, lower and upper 95% CIs.
The mean grad was highlighted with bold numbers.
* First OP: time point at the first operation; Second OP: time point at the second operation; FA: follow-up time point.

the MATRIX and the SPONGIO groups, in agreement with the other assessments described above (Fig. 8).

Discussion

Biological joint reconstruction in patients with early-stage OA remains challenging and is an issue of interest in the orthopedic community[11–13]. Cartilage regeneration has been attempted in knee joints with early osteoarthritic degeneration, but the graft failure was common in such patients[6].

To determine if cell-based cartilage repair is a possible solution for early OA joints, we developed a research model involving induction of localized, early-stage OA in a stable joint, which is suitable for cartilage repair experiments. In our model a unicompartmental OA was achieved by creating a critical-size defect in an ovine model, which is comparable to posttraumatic OA in patiens[1]. The current regeneration study represents the first implementation of this OA model and demonstrated the compatibility of the cell implantation technique.

Three treatment strategies were used in this model. One group received only the bone preparation, or spongialization, as it was described by Ficat *et al.*[21] The rationale behind using spongialization instead of microfracture, was the complete removal of subchondral bone plate changes in the repair area. We believe this is crucial in joints with early osteoarthritic changes. The other two groups received the spongialization followed by the implantation of a hyaluronan scaffold with chondrocytes (MACT group) or without chondrocytes (MATRIX group). We found that the defects receiving cell-seeded scaffolds had significantly better results in the regeneration area than the other groups, regardless of whether the repair tissue was evaluated by histological or macroscopic criteria. This is a noteworthy finding, considering that matrix-associated cell transplantation is only recommended when there are no OA changes present. The bone preparation technique may contribute to the improved performance of MACT in our study. It is well known that OA also includes the bone not only the cartilage[29]. In preparation for the implant, most regeneration models only debride the defect to the calcified cartilage layer, leaving the underlying bone untouched. We prepared a defect of 2.5 mm depth, which always reached the spongiosa.

The MATRIX group might be expected to score better than the SPONGIO group, where no scaffold was implanted, but in fact scored worse. This was an interesting observation, since there is still uncertainty as to whether or not culture-expanded cells should be used for regeneration. Several prior studies have suggested that regeneration is significantly improved with cultured cells[30–36]. Fewer studies were able to achieve good results without cells[37,38]. However, one can think that fibrin glue itself also represents a kind of matrix[39], and could have contributed to the non-inferior results of the SPONGIO group in comparison to the MATRIX group.

In our study, the results in the MACT group after 4 months are promising. However, the regeneration still appears to be in progress

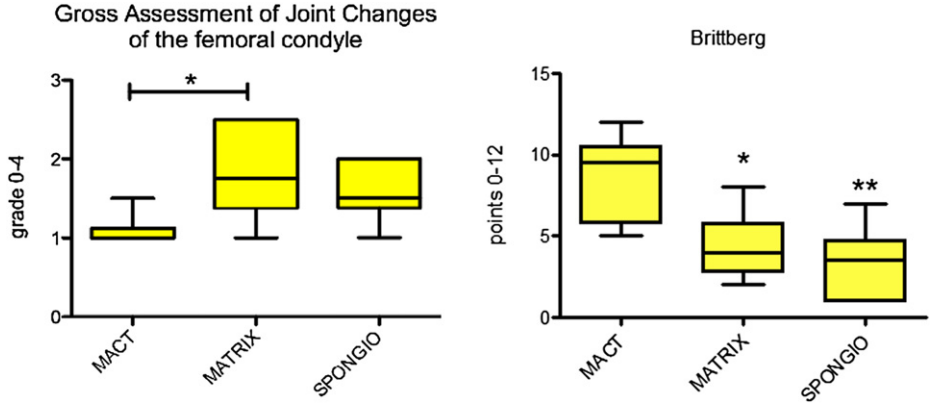

Fig. 5. Two different macroscopic scoring systems were used for assessing the femoral condyle at the follow-up: the Gross Assessment of Joint Changes score of the whole medial condyle (left) and the Brittberg score (right). Both scores found significant differences between the MACT ($n = 6$) and the MATRIX ($n = 6$) group ($P = 0.002$ for the Gross Assessment and $P = 0.01$ using the Brittberg score), whereas the more precise Brittberg score also found a difference between the MATRIX and the SPONGIO ($n = 6$) group ($P = 0.004$). Each column defines a group. The boxes represent the values between the 25th and 75th percentile. The error bars (whiskers) represent all values within 1.5 times the interquartile range (IQR). No outliers were present in our dataset.

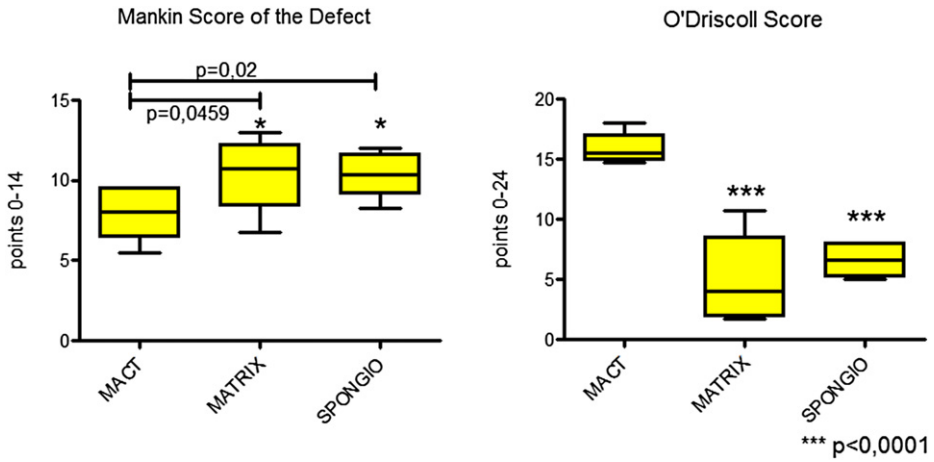

Fig. 6. The two scores that were used for histological assessment at the follow-up were the Mankin score (left), which is a well known OA score, and the O'Driscoll score (right), which is often used for assessing defect regenerations. With both scores the MACT group has the best results significantly different from the other groups. Each column defines a group. The boxes represent the values between the 25th and 75th percentile. The error bars (whiskers) represent all values within 1.5 times the IQR.

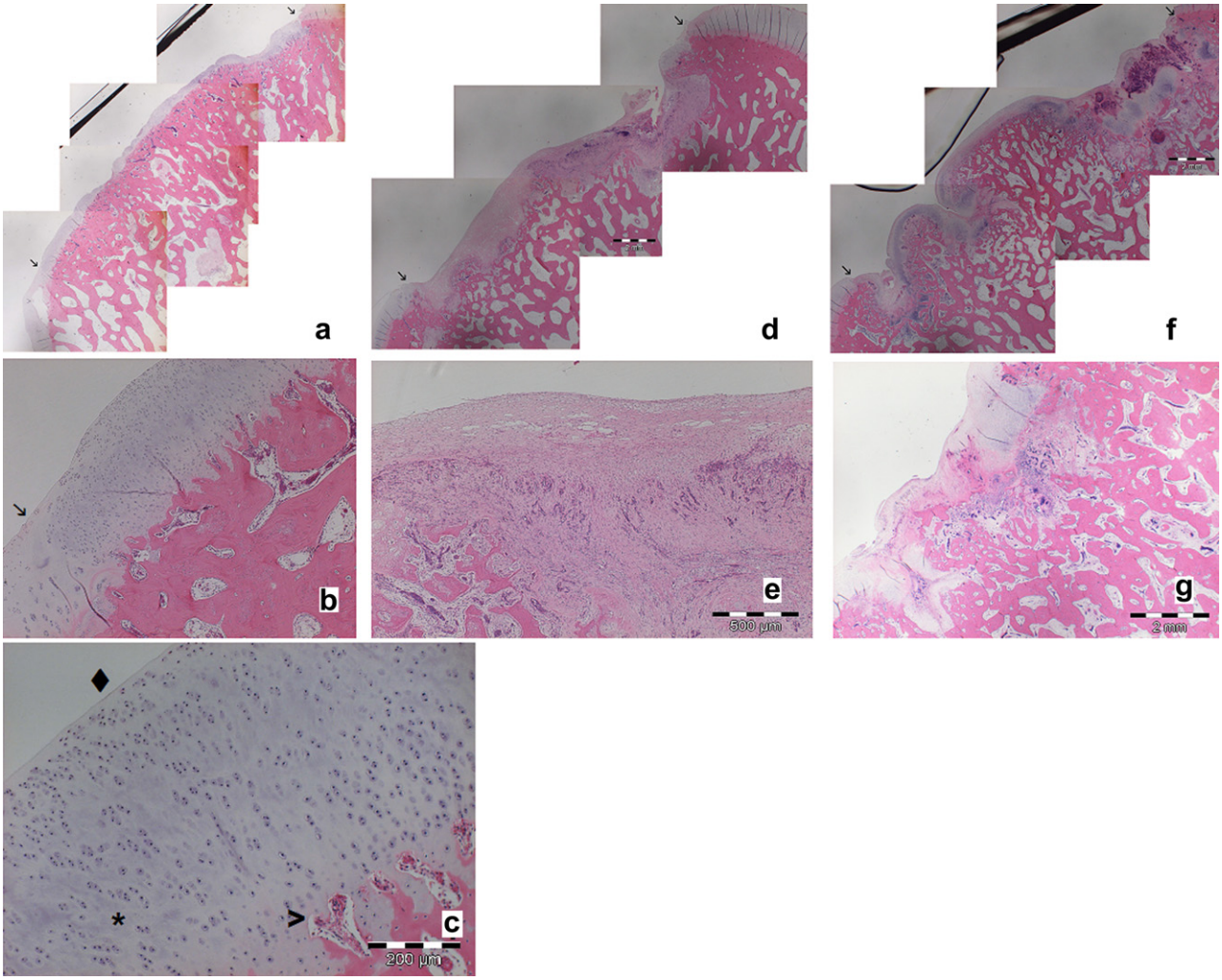

Fig. 7. Histological analysis of representative specimens from the MACT (a, b, c), the MATRIX (d, e), and the SPONGIO (f, g) groups. The arrows (a, d, f) mark the interface between the regeneration area and the original cartilage. In panel c, the arrowhead marks blood vessels that are still penetrating the tidemark between cartilage and bone. The asterisk was put in the middle of the regenerated cartilage, where cartilage cells already begin to form column like structures. The surface of the regenerate (diamond) was notably smooth for these specimens.

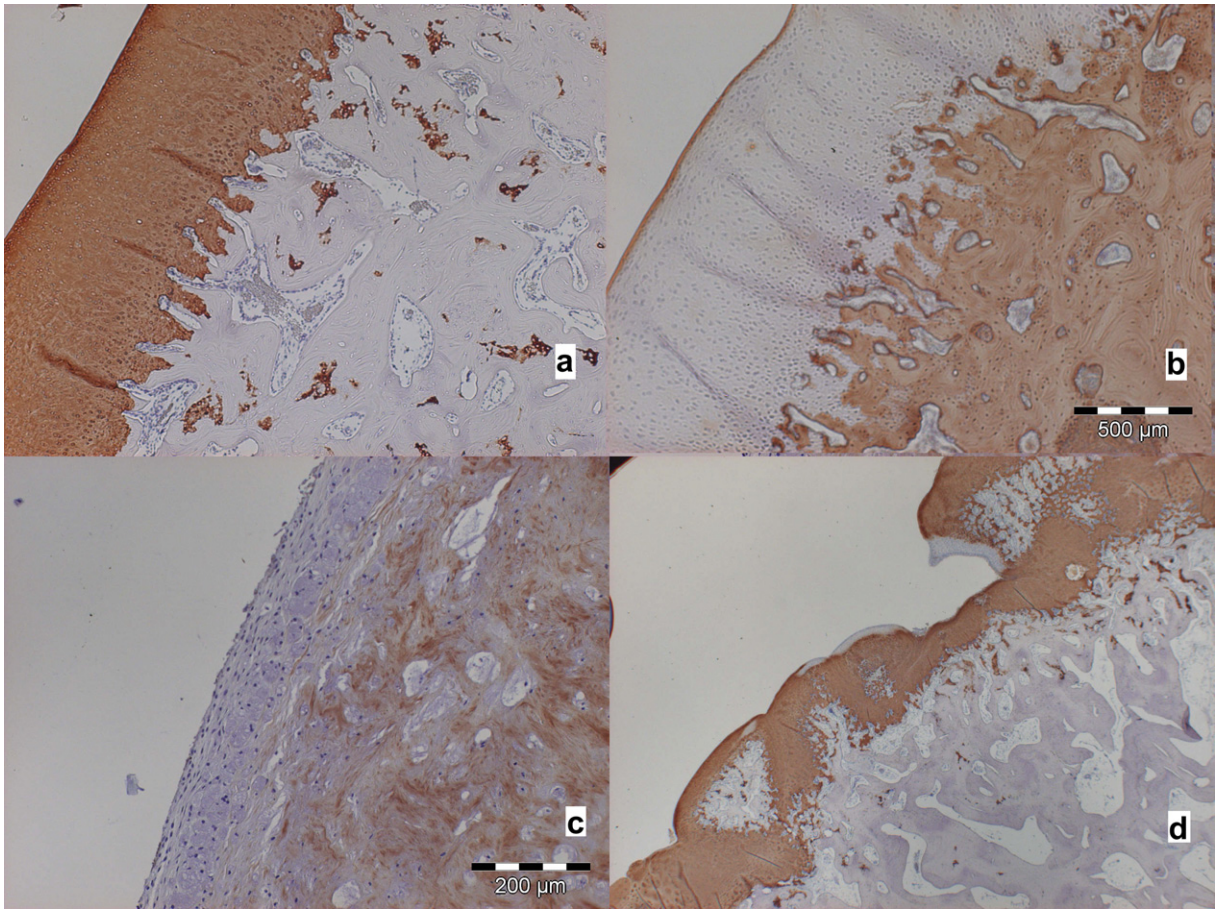

Fig. 8. Immunhistochemical staining for collagen type II (a, c, d) and collagen I (b) for the MACT (a, b), MATRIX (c), and SPONGIO (d) groups. For the MACT group, collagen II staining is specific to the cartilage layer of the regenerate (a), whereas collagen I is found only in the subchondral bone and at the cartilage surface. The higher magnification image in panel (c) shows less intense and homogenous distribution of collagen II in the regeneration area of the MATRIX group compared to the MACT group (a), and panel (d) highlights irregular collagen II staining within the rough surface that was present in the SPONGIO group.

after 4 months, because there was no calcified cartilage layer formed and vessels were crossing the bone cartilage interface.

The animal model used in this study has both advantages and limitations. The sheep used have large enough joints for surgical procedures that better simulate clinical cases, and the cartilage is closer to that of humans compared to other commonly used animals. However, post-operative rehabilitation has limitations. Sheep start full weight bearing on the first day after the operation, as there is no equivalent to the function of crutches that they would tolerate. Also, the condyle is smaller than a human condyle, so that the area where we did the spongialization was approximately two-thirds of the medial femoral condyle, a relatively large surface fraction.

It can be concluded that a biological repair of an early stage of OA is feasible in a sheep model. At 4-month follow-up we could see particularly promising results in the cell-based MACT group.

Role of founding source

This research was supported by the European Commission (FP6-500465).

Author contributions

Schinhan M: conception of the study, sheep operations, histology, data collection, data analyses, stats, manuscript writing; Gruber M: conception of the study, sheep operations, histology; Dorotka R: conception of the study, editing; Pilz M: histology, data collection, data analyses; Stelzeneder D: data analyses, stats, manuscript writing; Chiari C: conception of the study, analysis and interpretation of data, Rössler N: histology, data collection, data analyses; Windhager R: editing, data analysis; Nehrer S: conception of the study, data analysis, editing.

Conflict of interest

The authors have no conflict of interest.

Acknowledgments

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