Treatment of osteochondral defects of the talus

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Chapter 13

Osteochondral defects of the talus: a novel animal model in the goat

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Tissue Engineering Part C: Methods
Abstract

Osteochondral defects of the talus pose a difficult therapeutic challenge. An experimental animal model of the ankle joint is not available. The aim of this study was to test a newly developed animal model for osteochondral defects of the ankle in vivo. Osteochondral defects were created in the talus of goat hind legs using a posterolateral surgical approach. The defects were filled with either autologous cancellous bone or donor demineralized bone matrix, or left empty as control. After 12 weeks of healing, the specimens were analyzed with radiography, macroscopy, micro-computed tomography, histology, histomorphometry, and fluorescence microscopy. It was possible to create a standardized defect in each talus. The implanted material remained in place. The analyses showed that most bony tissue was generated in the defects filled with autologous bone and least in the control defects. Our findings show that a standard osteochondral defect can be created in the talus by a relatively simple procedure in a large animal that allows qualitative and quantitative evaluation. The model can be used in future experiments to investigate alternative treatment methods before they are introduced into clinical practice.

Introduction

Osteochondral defects of the talus (OCDs) pose a challenging problem to orthopaedic surgeons. Patients with an OCD are typically young adults who experience deep ankle pain on weight bearing, often long after a supination trauma. Nonoperative treatment yields unsatisfactory results in the majority of patients. The primary surgical treatment of most defects is arthroscopic debridement combined with bone marrow stimulation. If results are unsatisfactory – which happens more often in large defects – more invasive surgical options are indicated. Current treatment options for these secondary OCDs include autologous cancellous bone grafting, osteochondral autograft transfer and autologous chondrocyte implantation. Each of these options has specific disadvantages, for example, an additional operative site with possible donor-site pain, limited availability of material, impaired graft integration, and the necessity of a medial malleolar osteotomy for exposure. Therefore, experimental studies focus on improvement of these methods and development of alternatives. However, almost all studies investigate articular defects of the knee, while the ankle joint is rarely investigated. These knee studies cannot reliably be extrapolated to patients with ankle defects, because biomechanical and biochemical properties of the knee and ankle are clearly different. The ankle is a congruent joint with thin cartilage that is less susceptible to osteoarthritis than the knee.

The use of animal models is often an essential step in the testing of orthopaedic procedures before clinical use in humans. To our knowledge, an animal model for ankle OCDs is not described in the literature. The aim of this study was to test a newly developed caprine model for OCDs of the talus, by evaluating an established treatment (autologous cancellous bone graft), an alternative treatment (demineralized bone matrix [DBM]), and no treatment (control). We defined the following criteria for a successful model: 1) the animal’s joint anatomy and body weight are comparable to humans, 2) the surgical technique is reproducible (regarding the operative approach as well as size and location of the OCD), 3) the morbidity of the animals is acceptable (defined as full weight bearing and free of pain within 1 week), 4) the
repair is best after applying an established treatment and minimal in control defects, and 5) the method must be accurate enough to detect small differences between groups.

Materials and methods

Animals, experimental design, and operative technique

The study was approved by the Animal Care and Use Committee of the University of Amsterdam, the Netherlands. Three adult female Dutch milk goats (Capra Hircus Sana) were used with an approximate age of 4 years and a weight of 50, 80, and 89 kg, respectively. All goats were healthy, according to physical examination and blood tests by a veterinarian. They were kept in group housing starting 2 weeks before surgery.

Surgery was performed on both ankles in a sterile fashion with the goat in the lateral decubitus position under general anesthesia with endotracheal intubation. Before anesthesia, intramuscular injections with prophylactic antibiotic (Pen & Strep, Fendigo sa/nv, Brussels, Belgium), ketamine 10 mg/kg (Alfasan International BV, Woerden, the Netherlands) and atropine 1.5 mg (Centrafarm Services BV, Etten-Leur, the Netherlands) were administered. Intravenous etomidate (B.Braun Melsungen AG, Melsungen, Germany) was injected to induce

Figure 1. Digital pictures showing key aspects of the surgical technique. The talar dome was exposed through a posterolateral approach (A). A cylindrical defect with a diameter and a depth of 6 mm was created (B) and filled up to the level of the cartilage (C). DBM = demineralized bone matrix.
general anesthesia. Fentanyl 250 µg (Hameln pharmaceuticals gmbh, Hameln, Germany) and midazolam 15 – 20 mg (Dormicum, Roche Nederland BV, Woerden, the Netherlands) were injected intravenously, and repeated, when necessary. Isoflurane 1.0% - 2.5% (Nicholas Piramal Limited, London, UK) was administered by inhalation.

A curvilinear skin incision was made just posterior to the lateral malleolus, parallel to the Achilles tendon (proximal) and the calcaneus (distal). After mobilization of the lateral saphenous vein, an arthrotomy was performed in the plane between the peroneal tendon (anterior) and the lateral saphenous vein (posterior). A Hohmann retractor was placed behind the medial talus to optimize exposure of the talus and protect the tendons and neurovascular structures. Full dorsiflexion of the ankle allowed maximal exposure of the articular surface of the talus (Figure 1A). In the center of the talar trochlea, a custom-made drill socket with a diameter of 6 mm was placed, which ensured drilling to a precise depth of 6 mm. Under continuous cooling with saline, the defect was drilled perpendicularly to the talar surface, starting with a sharp drill and finishing with a milling cutter to achieve a flat base of the defect (Figure 1B). A goat study investigating the natural course of knee defects showed that 6-mm defects do not heal spontaneously within 1 year.\textsuperscript{198} Each defect was treated according to a predefined randomization scheme with autologous cancellous bone (harvested from the iliac crest) or commercially available cortical demineralized bone matrix (Bonust\textsuperscript{TM} DBM, Biomet BV, Dordrecht, the Netherlands) hydrated with normal saline, or left empty (untreated control). Filling up to the level of the articular cartilage surface (Figure 1C) was ensured by inserting ample material press-fit and moving the ankle through a full range of motion (removing any abundant material above the surface of the defect due to the congruency of the ankle joint). The joint capsule and subcutaneous tissue were closed with interrupted 2-0 absorbable sutures, and the skin was closed with absorbable sutures placed in a continuous intracutaneous pattern.

Postoperatively, analgesic medication consisted of a single subcutaneous injection of 0.005 mg/kg buprenorphine (Temgesic\textregistered, Schering-Plough BV, Utrecht, the Netherlands), twice daily, as pain demanded (indicated by limping). After wound healing was completed, which took ~1 week, the animals were kept outdoors in a large natural environment with food ad libitum and without activity restrictions. Eating habits, ambulatory activities, and health status were monitored daily.

After 12 weeks, the goats – back in the experimental animal center – were sacrificed by injecting a lethal dose of pentobarbital (Euthasol 20%, ASTfarma BV, Oudewater, the Netherlands) in the jugular vein, after they had been sedated with an intramuscular injection of ketamine 10 mg/kg (Alfasan International BV, Woerden, the Netherlands), xylazine 2 ml (Sedazine\textregistered, ASTfarma, Oudewater, the Netherlands) and atropine 1 mg (Centrafarm Services BV, Etten-Leur, the Netherlands).

**Analyses**

**Radiography**

Lateral radiographs of the ankles were taken before surgery and directly after surgery. Anteroposterior and lateral radiographs were taken after autopsy.

**Macroscopy**

The tali were excised directly after terminating the goats, and digital high-resolution photographs were taken of the talar and opposite articular surfaces. Macroscopic grading was
performed by two observers. A general macroscopic articular evaluation system was used that awards 0 to 2 points to each of five categories: range of motion, intra-articular fibrosis, restoration of contour, cartilage erosion, and appearance. Ten points indicate a completely normal appearance, and 0 points indicate a severely damaged joint surface. Furthermore, the talar articular surface was assessed according to the validated International Cartilage Repair Society (ICRS) cartilage repair assessment. This score consists of three items (i.e., degree of defect repair, integration to border zone, and macroscopic appearance) that are each assigned a maximum of 4 points, with a total of 12 indicating a normal appearance. The scores of the two observers were averaged and outliers with a difference of more than 1 point were scored again, until consensus was reached.

Micro-computed tomography

The talar heads were sawn off with a water-cooled band saw to optimize penetration of fixative for histological evaluation and allow placement of the tali in the micro-computed tomography (μCT) scanner. Specimens were fixed in 4% phosphate-buffered formaldehyde for 1 week. After they were submerged in 70% ethanol and subjected to a vacuum to remove all the air from the cancellous bone, they were scanned in a μCT scanner (μCT 40, Scanco Medical AG, Brütisellen, Switzerland). With the defects facing down, the specimens were mounted in cylindrical specimen holders and secured with synthetic foam. This setup ensured consistent scanning of the defects parallel to the axis of the scan tube. The resolution of the scans was 18 μm, the voltage 70 kV, the current 114 μA, and the integration time 1000 ms. To discriminate between bone and background, the reconstructions were segmented using an adaptive threshold procedure, which determines the minimum between the two peaks in the gray values histogram, representing bone and background, respectively.

For quantitative analysis, two cylindrical volumes of interest were defined: one representing the complete OCD, measuring 6 mm in diameter and 6 mm in depth, and one representing the central OCD, measuring 3 mm in diameter and 5 mm in depth. The latter volume of interest was defined at 5 mm in depth to avoid analyzing original bone at the base of the OCD. The ratio between bone volume and tissue volume (BV/TV) was determined for all volumes of interest using morphometric software (Scanco Medical AG, Brütisellen, Switzerland).

In addition to the six operated specimens, five unaffected goat tali from a spinal fusion study (unpublished data) were μCT scanned and analyzed in the same manner to obtain normative data.

Histology

After fixation, the specimens were dehydrated using ascending grades of ethanol (70% to 100%) in two steps a week, and embedded in methylmethacrylate (BDH Laboratory Supplies, Poole, England). After cold polymerization, the undecalcified specimens were cut into coronal 5-μm sections with a Jung-K microtome (R. Jung, Heidelberg, Germany). Seventy-five central sections were obtained from each specimen. Every third section (n = 25) was stained with Goldner’s trichrome method, giving the following staining pattern: green for mineralized tissue, red for osteoid, blue or black for cell nuclei, and light orange or red for cytoplasm. The Goldner stained sections were used for evaluation of mineralized bone tissue, osteoid, fat cells, osteocytes, osteoblasts, and osteoclasts. Every second out of three sections was stained with toluidine blue for examination of structural details and cell appearance of the articular surface of the defects. Every third out of three sections was
left unstained for fluorescence microscopy. Two observers, blinded to the treatment modality, qualitatively assessed the stained sections using transmitted light microscopy.

Histomorphometry

One representative Goldner-stained mid-section of each talus was analyzed using a Leica DMRA microscope that was connected to Leica Qwin computer software (Leica Microsystems Imaging Solutions, Cambridge, UK). A special routine was written for quantitative measurements of bone parameters.

Three areas of interest were defined: (1) the center of the OCD, (2) close to the borders of the defect (lateral, medial, and bottom), and (3) close to the articular surface of the defect. Six representative measurement fields of 307,000 µm² were selected and digitized at a magnification of ×200, resulting in one to three measurement fields for each area of interest (Figure 2). The mineralized bone area (bone%) and osteoid area (osteoid%) were measured. The number of osteocytes, osteoblasts, and osteoclasts was counted at a magnification of ×400. All measurements were performed twice by one observer and once by a second observer, both blinded to treatment allocation. The mean of both observers is reported, and intraobserver and interobserver reliability were calculated.

Fluorescence microscopy

Fluorochrome labels were injected at 3 (oxytetracycline 20 mg/kg), 6 (calcein green 10 mg/kg), and 9 weeks (alizarin red 25 mg/kg) after surgery. The dosages were based on the recommendations by van Gaalen et al.435 One unstained section that was adjacent to the Goldner stained section was used for histomorphometry. Six measurement fields (magnification, ×400), similar to the areas of interest of histomorphometry, were digitized using fluorescent filters. According to the manufacturer's guidelines, the D-filter (excitation 355 – 425 nm, emission 470 nm) was best used for oxytetracycline and alizarin, and the I3-filter (excitation 450 – 490 nm, emission 515 nm) was best used for calcein, although each label was visible with both filters. One independent observer, blinded to treatment allocation, measured the distance between two consecutive lines at ~10 locations within a measurement field. The mineral apposition rate (MAR, µm/day) of the bone was calculated as the average distance between the corresponding edges of two consecutive fluorescent bone labels, divided by the number of days between start and end of the administration period.

Statistical analysis

Individual quantitative results are presented. The treatment pairs were not statistically compared because of the small sample size. Mean and standard deviation (SD) were calculated of the µCT scans of five unaffected goat tali. To assess intra- and interobserver reliability of the
histomorphometric measurements, intraclass correlation coefficients (ICCs) were calculated using SPSS version 18.0 (SPSS, Chicago, IL). ICCs of more than 0.75 are considered excellent.134

Results

With the operative technique described, it was possible to create a standardized OCD in the talus. After surgery, the first two animals recovered well without complications. The goats were able to walk on their hind legs within 24 h. The third goat had persistent pain of the right ankle.

Figure 3. Histological analysis of a defect filled with DBM, 2 weeks after implantation, shows that the material remained in situ.

Figure 4. Macroscopic view of three specimens. (A) Autologous bone. (B) DBM. (C) Control. The arrows indicate the osteochondral defects.
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despite treatment with subcutaneous analgesics for 2 weeks and intramuscular antibiotics (Pen & Strep, Fendigo sa/nv, Brussels, Belgium) for 1 week. There were no signs of infection. Surgical exploration of the painful ankle after two weeks revealed no pathology. Cultures showed no pathologic micro-organisms. An exact cause could not be determined. The goat was terminated for ethical reasons 2 weeks following initial surgery. Obduction revealed no pathologic conditions. Histology of the right talus showed that the original DBM was still in situ (Figure 3). In consultation with the Animal Care and Use Committee, the sacrificed animal was replaced by another goat, which recovered well from surgery and completed the follow-up period.

**Radiography**

Preoperative radiographs showed normal ankle joints without signs of osteoarthritis. Immediate postoperative radiographs showed the OCDs in three cases (one of each treatment); the defects were not visible in the other three. On the final radiographs after the 12-week period, none of the defects was visible.

**Macroscopy**

Macroscopic inspection of the ankle joints revealed no signs of inflammation. All defects were covered with tissue resembling fibrocartilage (Figure 4). The surrounding talar cartilage and opposite joint surfaces appeared completely normal. The highest macroscopic assessment scores were achieved in an autologous bone specimen and the lowest in a DBM specimen (Table 1).

**Micro-CT**

In the complete 6-mm OCDs, BV/TV ranged from 0.10 to 0.45 (Table 2). In comparison, mean

<table>
<thead>
<tr>
<th>Table 1. Results of macroscopy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment (goat)</td>
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<tr>
<td>------------------</td>
</tr>
<tr>
<td>AB (1L)</td>
</tr>
<tr>
<td>AB (2R)</td>
</tr>
<tr>
<td>DBM (2L)</td>
</tr>
<tr>
<td>DBM (3R)</td>
</tr>
<tr>
<td>Control (1R)</td>
</tr>
<tr>
<td>Control (3L)</td>
</tr>
</tbody>
</table>

AB = autologous bone, DBM = demineralized bone matrix, ICRS = International Cartilage Repair Society cartilage repair assessment,62 L = left, and R = right.

<table>
<thead>
<tr>
<th>Table 2. Results of μCT, showing the ratio between bone volume and tissue volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment (goat)</td>
</tr>
<tr>
<td>------------------</td>
</tr>
<tr>
<td>AB (1L)</td>
</tr>
<tr>
<td>AB (2R)</td>
</tr>
<tr>
<td>DBM (2L)</td>
</tr>
<tr>
<td>DBM (3R)</td>
</tr>
<tr>
<td>Control (1R)</td>
</tr>
<tr>
<td>Control (3L)</td>
</tr>
<tr>
<td>Unaffected (mean ± standard deviation)</td>
</tr>
</tbody>
</table>
BV/TV of the five unaffected tali was 0.68 (SD, 0.06) in the complete cylinder and 0.70 (SD, 0.05) in the central 3-mm cylinder. The 3-mm central cylinders can be considered indicative for the regenerative capacity of the treatment applied because bone had regenerated from the borders and bottoms of the defects. In these 3-mm cylinders, the tali treated with autologous bone showed the most bone tissue, and the control group the least (Figure 5).

**Histology**

In the OCDs treated with autologous bone, diffuse bone formation and near-complete filling was observed (Figure 6A). There was continuing bone remodeling, as indicated by large areas of osteoid tissue and the presence of abundant osteoblasts and a rare osteoclast. At the articular surface, the defect was mainly composed of (mineralizing) fibrocartilage tissue with some chondrocytes embedded. Underneath the surface there were areas of woven and lamellar bone.

In the defects treated with DBM, some bone formation was observed – both lamellar and woven – with abundant osteoid in one specimen (Figure 6B). Most bone was formed at the borders but also some in the center of the defects. The original DBM was not visible. In one specimen the surface of the defect was composed of a thick layer of fibrocartilage. In the other, the surface was mainly composed of connective tissue, fibroblasts, and leukocytes (lymphocytes and granulocytes).

The control defects did not heal (Figure 6C). Some woven bone originated from the borders of the defects. Occasional mineralization of cartilaginous tissue at the surface suggested endochondral ossification. The greatest part of the defects was filled with fat cells and connective tissue.

**Histomorphometry**

Histomorphometry followed the same pattern as the μCT analysis (Table 3). Most bone formation was observed in the autologous bone pair; most...
Figure 6. Histologic sections with Goldner's trichome staining; magnification, ×25. (A) Autologous bone. (B) DBM. (C) Control. Scale bar = 1 mm.
osteoid formation was found in DBM. There was only one specimen (autologous bone) with bony and osteoid tissue in the measurement field of the surface. Osteoclasts were rarely found: only a single osteoclast was seen in one measurement field (center) in one specimen (autologous bone).

The intra- and interobserver reliability of the histomorphometric measurements were excellent; the lowest ICC was 0.80 (interobserver reliability of osteoblasts in the center; \( p = 0.016 \)), and the highest was 1.00 (intra- and interobserver reliability of bone\% in all areas of interest; \( p < 0.001 \)).

**Fluorescence microscopy**

MAR varied from 0 μm/d in the areas where no bone growth was observed to 11 μm/d in the center of the defect in an autologous bone specimen (Table 4). The calcein green labels were best visible, followed by oxytetracyclin and alizarin, respectively (Figure 7). The MAR could not be analyzed in two occasions because alizarin red labels could not be detected (see Table 4).

**Discussion**

A new caprine model was developed for the comparison of treatments for osteochondral ankle lesions. The model is presented in this study and seems feasible for the evaluation of new treatment options. Similar to the human ankle joint, the caprine ankle is congruent and able to bear high loads. The goat's body weight and metabolic and remodeling rates correspond to those of humans. Furthermore, the proportion of cartilage to subchondral bone and the subchondral bone consistency are reportedly close to humans. The operative technique described is reproducible, a standardized OCD can be created in each talus, and the implanted material remains in situ. Most importantly, there

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**Table 3. Results of histomorphometry**

<table>
<thead>
<tr>
<th>Treatment (goat)</th>
<th>Bone% Center</th>
<th>Bone% Borders</th>
<th>Bone% Surface</th>
<th>Osteoid% Center</th>
<th>Osteoid% Borders</th>
<th>Osteoid% Surface</th>
<th>Osteocytes (no.) Center</th>
<th>Osteocytes (no.) Borders</th>
<th>Osteocytes (no.) Surface</th>
<th>Osteoblasts (no.) Center</th>
<th>Osteoblasts (no.) Borders</th>
<th>Osteoblasts (no.) Surface</th>
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</thead>
<tbody>
<tr>
<td>AB (1L)</td>
<td>62.1</td>
<td>67.8</td>
<td>19.8</td>
<td>3.2</td>
<td>1.4</td>
<td>0.2</td>
<td>16</td>
<td>24</td>
<td>4</td>
<td>15</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>AB (2R)</td>
<td>16.7</td>
<td>38.2</td>
<td>0.0</td>
<td>1.7</td>
<td>3.1</td>
<td>0.0</td>
<td>4</td>
<td>17</td>
<td>0</td>
<td>3</td>
<td>9</td>
<td>0</td>
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<tr>
<td>DBM (2L)</td>
<td>5.0</td>
<td>15.3</td>
<td>0.0</td>
<td>0.2</td>
<td>1.2</td>
<td>0.0</td>
<td>0</td>
<td>11</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>DBM (3R)</td>
<td>26.0</td>
<td>35.7</td>
<td>0.0</td>
<td>9.8</td>
<td>9.8</td>
<td>0.0</td>
<td>4</td>
<td>16</td>
<td>0</td>
<td>8</td>
<td>21</td>
<td>0</td>
</tr>
<tr>
<td>Control (1R)</td>
<td>0.4</td>
<td>14.8</td>
<td>0.0</td>
<td>2.8</td>
<td>5.1</td>
<td>0.0</td>
<td>1</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Control (3L)</td>
<td>0.0</td>
<td>83.7</td>
<td>0.0</td>
<td>0.0</td>
<td>1.0</td>
<td>0.0</td>
<td>0</td>
<td>30</td>
<td>0</td>
<td>0</td>
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**Table 4. Results of fluorescence microscopy (mineral apposition rate, μm/d)**

<table>
<thead>
<tr>
<th>Treatment (goat)</th>
<th>Week 3-6 Center</th>
<th>Week 3-6 Borders</th>
<th>Week 3-6 Surface</th>
<th>Week 6-9 Center</th>
<th>Week 6-9 Borders</th>
<th>Week 6-9 Surface</th>
</tr>
</thead>
<tbody>
<tr>
<td>AB (1L)</td>
<td>6.1</td>
<td>2.8</td>
<td>2.7</td>
<td>8.3</td>
<td>5</td>
<td>No label</td>
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<tr>
<td>AB (2R)</td>
<td>11.0</td>
<td>5.7</td>
<td>0.0</td>
<td>6.4</td>
<td>9</td>
<td>0.0</td>
</tr>
<tr>
<td>DBM (2L)</td>
<td>6.4</td>
<td>7.1</td>
<td>0.0</td>
<td>No label</td>
<td>6.5</td>
<td>0.0</td>
</tr>
<tr>
<td>DBM (3R)</td>
<td>0.0</td>
<td>3.7</td>
<td>0.0</td>
<td>0.0</td>
<td>6.7</td>
<td>0.0</td>
</tr>
<tr>
<td>Control (1R)</td>
<td>0.0</td>
<td>5.6</td>
<td>0.0</td>
<td>0.0</td>
<td>5.8</td>
<td>0.0</td>
</tr>
<tr>
<td>Control (3L)</td>
<td>0.0</td>
<td>5.9</td>
<td>0.0</td>
<td>0.0</td>
<td>4.7</td>
<td>0.0</td>
</tr>
</tbody>
</table>
is a clear difference in outcome between defects treated with autologous cancellous bone and control defects. This was particularly shown by the quantitative analyses with μCT and histomorphometry. The mean BV/TV of the central OCDs was 0.25 in the autologous bone pair, 0.07 in the DBM pair, and 0.02 in the control pair (see Table 2). Similarly, mean central bone% according to histomorphometry was 39.4 in the autologous bone pair, 15.5 in the DBM pair, and 0.2 in the control pair (see Table 3). However, no definite conclusions can be drawn on the effectiveness of the treatment because of the low number of animals studied.

Previous animal studies have been performed predominantly on the stifle joint.8,31 These studies may be inadequate for assessing treatment modalities for ankle OCDs, as there are some specific differences between the knee and ankle. First, physiological cartilage properties differ. Congruent joints, such as the ankle, have a thin layer of cartilage to distribute the load and maintain an acceptable level of stress, whereas incongruent joints, such as the knee, have thicker areas of cartilage to increase the contact area between joint surfaces.354 The cartilage of the ankle has a greater compressive modulus compared with the knee.354,399 Second, the knee and ankle joints seem to respond differently to cartilage injury. There appears to be a net anabolic response to injury in the ankle cartilage and a net catabolic response in the knee.26,181,311 Ankle cartilage may be more resistant to osteoarthritis development for a number of reasons, including higher equilibrium and compressive moduli, better intercellular communications, increased resistance to inflammatory molecules, increased
metabolic activity, and higher congruency of the articular surfaces. Because of these differences, an animal model specifically developed for the ankle joint is required.

Although most animal models are designed for unilateral surgery, a bilateral defect was created in the present model. This method reduces the number of animals needed and allows for paired treatment comparison. A paired analysis may be a better strategy than unilateral evaluation or random assignment of treatment in bilateral models, since the regenerative capacities may differ from animal to animal (see Tables 2 and 3).

Clinically, plain radiographs are obtained for the initial diagnosis of OCD and for follow-up. However, in the present animal model, radiographic evaluation as an outcome measure proved to be useless because the defects were not visible in most instances. In contrast, histomorphometry is traditionally used to measure bone formation and is considered the gold standard. It is common practice to analyze representative areas of interest to approximate the total defect. Disadvantages of this method are a possible modification of the structure of the tissue by slice preparation and the long time required for all processing steps. MicroCT provides a fast and nondestructive technique to characterize and measure the 3-dimensional geometric and density properties of a bone specimen. Gielkens et al. compared the intraobserver reliability of μCT and histomorphometry. They concluded that both analyses are reliable but are used preferably in combination.

Fluorescence microscopy is a useful supplement to plain histology to detect the speed of regeneration during the follow-up period. When using fluorescence microscopy, there is no need to sacrifice additional animals at a shorter follow-up period. The success of fluorochrome detection depends on various aspects, for example, the type, concentration, route of administration and methods of visualization. In the present study, the recommendations of van Gaalen et al. were used. Unfortunately, the alizarin red labels were not always visible, without an obvious cause. In contrast, calcein green was clearly visible in all cases. No adverse events related to the injections of the fluorochrome labels were seen in this study.

The follow-up period was 12 weeks. Revascularization and conversion of a bone graft into a vital trabecular structure have been reported to occur at approximately 3 months in the goat, which corresponds to 8 months in humans. However, the regeneration process was ongoing, observed by large areas of osteoid tissue and the presence of abundant osteoblasts. The final situation may thus not be achieved after 12 weeks. In studies with multiple follow-up periods, no substantial regeneration of OCDs in the knee joint was observed after 24 weeks, suggesting that a follow-up period of 24 weeks may show the final outcome.

The principal limitation of the present study is the limited number of animals used. In consultation with the Animal Care and Use Committee, only three animals were studied because the morbidity of the animals could not be anticipated, as this was the first time the ankle model was used. Out of the initial six ankles, one showed persistent pain. This goat was sacrificed, and the substitute goat recovered uneventfully. Thus, one out of eight ankles had unacceptable morbidity. This morbidity corresponds to that of other goat studies investigating cartilage repair. We therefore believe that following studies with the presented model are justified. Because of the small number of animals and the variability in reparative capacity of the goats, we cannot reliably draw conclusions on the investigated repair procedures. Larger studies with paired analyses are indicated.
Conclusions

The caprine model described in the current study seems suitable for the study of osteochondral ankle defect treatment in vivo. The operative technique is fairly simple and allows the creation of a standardized OCD for qualitative and quantitative evaluation. The goat model can be used in future experiments investigating alternative treatment methods for this challenging condition before introduction into clinical practice.

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