Treatment of osteochondral defects of the talus
van Bergen, C.J.A.

Citation for published version (APA):

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Chapter 14

Demineralized bone matrix and platelet-rich plasma do not improve healing of osteochondral defects of the talus: an experimental goat study

Christiaan J.A. van Bergen
Gino M.M.J. Kerkhoffs
Murat Özdemir
Clara M. Korstjens
Vincent Everts
Leo J. van Ruijven
C. Niek van Dijk
Leendert Blankevoort

Osteoarthritis and Cartilage
Abstract

Objective

The purpose of this study was to evaluate the effectiveness of demineralized bone matrix (DBM) with and without platelet-rich plasma (PRP) in the treatment of osteochondral defects (OCDs) of the talus. We hypothesized that treatment with DBM would result in more bone formation than no treatment in control OCDs, and that PRP would further enhance the regenerative capacity of DBM.

Method

A standardized 6-mm OCD was created in each talus of 16 adult goats. According to a randomization scheme, one OCD of each goat was treated with allogeneic DBM hydrated with normal saline (n = 8) or hydrated with autologous PRP (n = 8). The contralateral OCD (n = 16) served as control. After 24 weeks, the animals were euthanized and the tali excised. Various outcome parameters were analyzed with use of macroscopic evaluation, micro-computed tomography, histology, histomorphometry, and fluorescence microscopy.

Results

None of the analyses revealed statistically significant differences between the groups for any of the parameters analyzed in any volume of interest. For example, the mean bone volume fraction of the defect, as measured by micro-computed tomography, was 0.56 (95% confidence interval [CI], 0.44 – 0.68) for DBM hydrated with normal saline and 0.52 (95% CI, 0.40 – 0.65) for DBM hydrated with PRP, compared to 0.53 (95% CI, 0.45 – 0.61) and 0.54 (95% CI, 0.44 – 0.64) for the internal controls, respectively (p >0.05).

Conclusion

In contrast to our hypotheses, no beneficial treatment effect of DBM with or without PRP was found for OCDs of the caprine talus.

Introduction

In the treatment of talar osteochondral defects (OCDs), repair of the subchondral bone is an important aim of the procedure. The presence of subchondral bone is essential for survival of chondrocytes. The affected subchondral bone is thought to cause the pain. Restoration of the subchondral bone may improve the weight-bearing capacity of the ankle and prevent further cyst formation.

Urist pioneered the use of demineralized bone matrix (DBM) for bone defects. Since his work, there has been increasing experience with DBM in both animals and humans. The sequence of events after implantation of DBM mirrors that of endochondral ossification. Bone morphogenetic proteins
(BMP-2, -4, and -7) seem to be responsible for the formation of bone and possibly cartilage that are induced by DBM. The BMPs present in DBM attract mesenchymal stem cells through chemotaxis and act as morphogens that may direct the differentiation of these cells into an osteochondrogenic lineage. Different fluids can be used for rehydration of the DBM before application, including normal saline, bone marrow aspirate, antibiotics solution, whole blood, or platelet concentrate.

Platelet-rich plasma (PRP) is a promising biomaterial that contains concentrated growth factors, including transforming growth factor-β (TGF-β) and platelet-derived growth factor (PDGF). TGF-β in PRP may stimulate chemotaxis and mitogenesis of osteoblast and chondroblast precursors and inhibit osteoclast formation and bone resorption. PDGF may promote mitogenesis, angiogenesis, and chondrocyte proliferation. Although, in theory, PRP may enhance the biologic activity of DBM, the combination of DBM and PRP has had contradictory results.

The purpose of the present study was to evaluate the effectiveness of DBM with or without PRP in the treatment of ankle OCDs in goats. We hypothesized that (1) treatment with DBM would repair more bone than control OCDs, and that (2) PRP would further enhance the regenerative capacity of DBM.

Materials and methods

Animals and experimental design

The study was approved by the Animal Care and Use Committee of the University of Amsterdam. A caprine model was used, specifically designed for ankle OCDs. Sixteen adult female Dutch milk goats (Capra Hircus Sana) were included with an approximate age of 4 years. All goats were healthy, according to physical examination and blood tests performed by a veterinarian. The goats were weighed on a digital scale before surgery and at final follow-up. Surgery was performed in a sterile manner on both ankles, with the goat under general anesthesia with endotracheal intubation. A single intramuscular dose with prophylactic antibiotics (Pen & Strep, Fendigo sa/nv, Brussels, Belgium) was injected preoperatively. The ankle joint was exposed through a posteromedial approach. Normal articular surfaces were confirmed by visual inspection. Standard OCDs of 6 mm in diameter and depth were created with specially developed instruments. According to a predefined randomization scheme, one defect of each goat was treated with DBM hydrated either with normal saline (0.9% NaCl solution) (“DBM treatment”; n = 8) or with PRP (“DBM+PRP treatment”; n = 8), and the other served as a control (“DBM control” or “DBM+PRP control”). In each case, the material was inserted press-fit up to the level of the adjacent cartilage surface. The joint capsule and skin were closed in a standard fashion.

During recovery, the animals were kept outdoors in a large natural environment, without activity restrictions, and with food ad libitum. Eating habits, ambulatory activities, and health status were monitored daily.

Since previous studies showed no substantial change in repair of knee OCDs after 24 weeks, the goats were euthanized 24 weeks after surgery by injecting a lethal intravenous dose of pentobarbital. All analyses were performed by observers blinded to the treatment provided.

Demineralized bone matrix

Commercially available cortical DBM (Bonus™ DBM, Biomet BV, the Netherlands) was used. This DBM was obtained from human donors.
from qualified tissue banks that were registered with the FDA and accredited by the American Association of Tissue Banks. It was granulated, demineralized with organic solvents, freeze-dried (i.e., lyophilized) and processed aseptically. This process resulted in calcium levels of less than 0.1%.\(^{318}\) It was combined with a collagen-derived carrier (gelatin) from the same donor, packaged in a rehydration syringe, and sterilized by gamma irradiation.

**Platelet-rich plasma**

Autologous PRP was used for rehydration of the DBM. After induction of anesthesia and before surgery, 27 ml venous goat blood was aspirated into a 30-ml syringe that contained 3 ml of anticoagulant citrate dextrose A. PRP was isolated by centrifugation at 3200 rpm for 15 min using the gravitational platelet system II (GPS II, Biomet BV, the Netherlands). This preparation system produces 3 ml of PRP with a reported eightfold increase in platelet concentration and a fourfold to sevenfold increase in growth factor concentration compared with whole blood.\(^{120,173,454}\) The concentration of platelets in the PRP of each subject in the present study was measured using an automated hematology analyzer (XE-5000, Sysmex, Japan) after 5 min of resuspension on a rocker, as recommended by Woodell-May et al.\(^{454}\) The median platelet concentration of the PRP was 1511 × 10^9/l (range, 82 – 2090 × 10^9/l).

**Macrosopy**

After the goats were euthanized, the tali were excised and digital high-resolution photographs were taken of the talar articular surfaces. Two independent observers macroscopically graded the photographs with use of the validated International Cartilage Repair Society (ICRS) cartilage repair assessment.\(^{62,420}\) This score ranges from 0 to 12 points and is subdivided into degree of defect repair, integration to border zone, and macroscopic appearance (4 points each), with a score of 12 indicating a completely normal appearance. The scores of the two observers were averaged, and outliers with a difference of more than 1 point were scored by consensus.\(^{91}\)

![Micro-CT analysis. Transverse cross-section of the talus (left) and the two cylindrical volumes of interest representing the complete defect (middle) and the central 3×5 mm (right).](image-url)
Micro-computed tomography

The anterior part of the talus, at safe distance from the OCD, was sawn off with a water-cooled band saw to reduce the size of the specimen, allowing it to be placed in the micro-computed tomography (μCT) scanner, and to optimize penetration of fixative into the specimen. After 1 week in fixative (4% phosphate-buffered formaldehyde), the specimens were submerged in 70% ethanol and temporarily subjected to a vacuum. In the 70% ethanol solution, they were placed in a μCT scanner (μCT 40, Scanco Medical AG, Bassersdorf, Switzerland) and scanned with a resolution of 18 μm. To minimize the noise in the reconstructions, an integration time of 1000 ms was used.

Micro-CT reconstructions were segmented with a threshold level of 467 mg HA/cm³. Two 3-dimensional cylindrical volumes of interest were defined: one representing the complete OCD (6 mm in diameter and depth), and one representing the central OCD (3 mm in diameter and 5 mm in depth) (Figure 1). Using morphometric software (Scanco Medical AG, Bassersdorf, Switzerland), the following parameters were analyzed: bone volume fraction (bone volume [BV]/tissue volume [TV]), tissue mineral density (TMD) of BV and of TV, and trabecular number, thickness, and separation. The bone volume fraction was the primary outcome of the study.

Histology

After fixation, the specimens were dehydrated using ascending grades of alcohol and embedded in methyl-methacrylate. After cold polymerization, the undecalcified specimens were cut into 5-μm sections with a Jung-K microtome (R. Jung, Heidelberg, Germany). Thirty central sections were obtained of each specimen to overcome sampling error. Every third section was stained with Goldner's trichrome method or toluidine blue for light microscopy, or left unstained for fluorescence microscopy. Two observers simultaneously assessed the stained sections and identified the type of healing.

Histomorphometry

A 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) with a custom-made routine for quantitative measurements of bone parameters. Two observers simultaneously made the choice for the representative section, based on technical quality and overall appearance. Three areas of interest were defined: (1) the center of the OCD, (2) sides and bottom of the defect (close to the lateral, medial, and deep borders), and (3) the surface (close to the articular surface of the defect). For each area of interest, one (surface), two (center) or three (sides and bottom) representative measurement fields of 3.07 × 10² μm² were digitized at a magnification of ×200. Mineralized bone surface area (bone%) and osteoid surface area (osteoid%) were assessed. The numbers of osteocytes, osteoblasts, and osteoclasts were counted at a magnification of ×400 and expressed per area of mineralized bone tissue. These histomorphometric measurements have been shown excellent intraobserver and interobserver reliability.

Fluorescence microscopy

Fluorochrome labels were administered at week 1 (Oxytetracyclin yellow, 32 mg/kg intramuscularly) and weeks 6, 12, 18, and 23 (Calcein green, 20 mg/kg subcutaneously). Fluorescence microscopy was used with fluorescence filters and unstained sections to analyze the speed of bone regeneration (mineral apposition rate...
Six measurement fields (magnification, ×400) were digitized, similar to the areas of interest of histomorphometry. The distance between two consecutive fluorescent bone labels was measured at approximately 10 locations within a measurement field by an independent observer blinded to treatment allocation. The MAR (μm/day) was calculated as the average distance divided by the number of days between two injections.

**Statistical analysis**

The sample size was calculated before the start of the study and was based on the primary outcome BV/TV. Prior data from a pilot study indicated that the standard deviation of BV/TV in the central 3 mm of the OCDs treated with DBM was 0.086. With an intended difference in the mean BV/TV of matched pairs of 0.10, a sample size of eight ankle pairs was able to reject the null hypothesis that the response difference was zero with probability (power) 0.80. The Type I error probability associated with this test of this null hypothesis was 0.05. Because two pairs of treatment were investigated, the total sample size was 16 goats.

Statistical analyses were performed with use of SPSS software (version 18.0; Chicago, Illinois). Variables with normal distribution are reported as mean and 95% confidence intervals (CIs). Data with skewed distribution are reported as median and range. The Wilcoxon signed-rank test was used to calculate p-values of matched pairs (DBM vs. control and DBM+PRP vs. control). The Mann-Whitney U test was used to compare the treatment effect of DBM (i.e., difference of DBM and internal control) with the treatment effect of DBM+PRP (i.e., difference of DBM+PRP and internal control). MARs of different administration periods were compared using the Wilcoxon signed-rank test.

The results were considered statistically significant if the p-value was less than 0.05 for the primary outcome (i.e., BV/TV). To correct for multiple testing, Holm's method was used.

![Figure 2. Examples of the best (A; ICRS cartilage repair assessment, 10) and worst (B; ICRS cartilage repair assessment, 3.5) macroscopic appearance of the osteochondral defects (arrows) after 24 weeks of healing. Both are control specimens.](image-url)
Figure 3. Bar chart showing the mean and 95% confidence interval of the bone volume fraction (BV/TV), according to µCT analysis, of (A) the complete defect and (B) the central 3×5 mm. The differences were not statistically significant (p >0.05).

Results

General results

The surgical procedures and functional recovery were uneventful in 15 goats. After 24 h, no signs of limping or abnormal motion were observed. In one goat, there was a technical failure of the instruments, resulting in an OCD drilled completely through the talus. In consultation with the Animal Care and Use Committee, this animal was terminated and replaced by another, which had no complications. The mean body weight was 73.5 kg (95% CI, 67.1 – 79.8 kg) before surgery and 69.3 kg (95% CI, 63.7 – 75.0 kg) at 24 weeks follow-up.

Macroscopy

All OCDs were macroscopically covered with fibrocartilaginous tissue. The mean ICRS cartilage repair assessment was 8.0 (95% CI, 7.3 – 8.7) for DBM treatment, 8.4 (95% CI, 7.4 – 9.5) for DBM control, 6.9 (95% CI, 5.3 – 8.6) for DBM+PRP treatment, and 7.4 (95% CI, 6.0 – 8.9) for DBM+PRP control (differences not statistically significant) (Figure 2). The surrounding talar cartilage and opposite joint surfaces appeared unaffected.

Micro-CT

There were no significant differences between groups on the primary outcome measure BV/TV or any of the other µCT parameters in either volume of interest (Figure 3 and Table 1). In the DBM+PRP treatment group, there were no statistically significant differences between goats with high concentrations of PRP and those with low concentrations. For example, BV/TV in the complete OCDs of the four goats with the highest PRP concentrations was 0.49 (95% CI, 0.39
Table 1. Outcome of μCT

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>BV/TV</th>
<th>TMD of BV (mg HA/cm³)</th>
<th>Complete defect</th>
<th>TMD of TV (mg HA/cm³)</th>
<th>Tb.N (mm⁻¹)</th>
<th>Tb.Sp (mm)</th>
<th>BV/TV</th>
<th>TMD of BV (mg HA/cm³)</th>
<th>TMD of TV (mg HA/cm³)</th>
<th>Tb.N</th>
<th>Tb.Th (mm)</th>
<th>Tb.Sp (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DBM Treatment</td>
<td>0.56</td>
<td>709 (682 - 735)</td>
<td>454 (373 - 536)</td>
<td>1.9 (1.4 - 2.4)</td>
<td>0.46 (0.35 - 0.57)</td>
<td>1.1 (0.7 - 1.5)</td>
<td>0.37</td>
<td>680 (653 - 707)</td>
<td>333 (240 - 426)</td>
<td>1.4</td>
<td>0.33 (0.23 - 0.50)</td>
<td>1.2 (0.9 - 1.5)</td>
</tr>
<tr>
<td>Control</td>
<td>0.53</td>
<td>700 (680 - 721)</td>
<td>431 (377 - 486)</td>
<td>2.0 (1.3 - 2.6)</td>
<td>0.41 (0.32 - 0.49)</td>
<td>1.2 (0.7 - 1.6)</td>
<td>0.28</td>
<td>661 (633 - 689)</td>
<td>276 (180 - 373)</td>
<td>1.7</td>
<td>0.26 (0.14 - 0.43)</td>
<td>1.2 (0.7 - 1.8)</td>
</tr>
<tr>
<td>DBM+PRP Treatment</td>
<td>0.52</td>
<td>695 (681 - 710)</td>
<td>429 (348 - 509)</td>
<td>1.6 (0.9 - 2.3)</td>
<td>0.45 (0.34 - 0.55)</td>
<td>1.6 (0.8 - 2.4)</td>
<td>0.23</td>
<td>657 (632 - 682)</td>
<td>244 (129 - 358)</td>
<td>4.2</td>
<td>0.24 (0.07 - 0.39)</td>
<td>1.1 (0.5 - 1.6)</td>
</tr>
<tr>
<td>Control</td>
<td>0.54</td>
<td>684 (666 - 701)</td>
<td>432 (380 - 485)</td>
<td>1.8 (1.1 - 2.5)</td>
<td>0.37 (0.30 - 0.43)</td>
<td>1.3 (0.9 - 1.7)</td>
<td>0.20</td>
<td>650 (639 - 662)</td>
<td>221 (150 - 292)</td>
<td>0.9</td>
<td>0.23 (0.09 - 0.31)</td>
<td>1.8 (1.3 - 2.3)</td>
</tr>
</tbody>
</table>

The values are presented as the mean, with the 95% confidence interval in parentheses. There were no statistically significant differences between the groups.

DBM and PRP for goat OCDs

- 0.58), compared to 0.55 (95% CI, 0.30 – 0.80) in the four goats with the lowest PRP concentrations (p = 1.00).

Histology

Four types of healing patterns were recognized (Figure 4). Type 1 was almost completely healed (>75% repair). In type 2, the subchondral bone was (almost) restored but a cystic lesion underneath the restored bone remained. Type 3 was characterized by regeneration from the margins and bottoms of the defects but a superficial defect remained (25% - 75% repair). In type 4, no or only minimal healing was observed (<25% repair); the original defects were filled with fatty and connective tissue, and the surface was covered with a thin layer of cells. There was high variation in healing patterns between animals (Figure 5), while the left and right tali within a single animal showed more consistency. There were remnants of DBM in two cases: one DBM treatment and one DBM+PRP treatment. Additionally, macrophages were observed in five defects: one DBM treatment, one DBM control, and three DBM+PRP control. Osteoclasts were not found.

Histomorphometry

There were no statistically significant differences in histomorphometry between the groups for any of the parameters analyzed in any of the areas of interest (Table 2).

Fluorescence microscopy

There were no statistically significant differences between the groups (Table 3). Some data were missing due to either no bone or no detectable label in the measurement field, especially in the fourth administration period (week 18 – 23). The MAR of the second administration period (week 6 – 12) of each group was significantly higher than that of the first administration period (week 1 – 6). The MAR did not change significantly in subsequent periods.

Discussion

This study aimed to evaluate the effectiveness of DBM and PRP in the treatment of ankle OCDs in goats. A previously established goat model was utilized, enabling evaluation of treatment of a standard OCD of the talus in a large animal with qualitative and quantitative analyses.517 The control OCDs in this study can be regarded as bone marrow stimulation of the defect without additional filling.

In contrast to our hypotheses, DBM did not result in improved repair compared to the control defects, nor did PRP enhance the regenerative capacity of DBM. There were no statistically significant differences between the groups on the primary outcome BV/TV, as measured by µCT, or the secondary outcomes, as measured by macroscopy, histology, histomorphometry, and fluorescence microscopy. However, some generalization uncertainty remains because of the dispersion of the outcome data, as demonstrated by the width of the 95% CIs (see Figure 3 and Tables 1 and 3). There were no observations of abnormal motion or limping after 24 h. Although a significantly higher MAR was shown after the first 6 weeks, this finding was similar in each treatment group (see Table 3).

Gao et al. investigated the effect of allogeneic rabbit demineralized cortical and trabecular bone matrix for the treatment of 3-mm OCDs in 10 young rabbit medial femoral condyles.140 At 6 and 12 weeks postoperatively, the defects were repaired up to 95% of their depth but there was a clear difference between cortical and trabecular DBM. In most of the specimens...
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Figure 4. Goldner-stained sections showing four types of healing. Type 1 (A) was almost completely healed. In type 2 (B), the subchondral bone was (almost) restored but a cystic lesion underneath the restored bone remained. Type 3 (C) was characterized by regeneration from the margins and bottoms of the defects but a superficial defect remained. In type 4 (D), no or only minimal healing was observed. The original defects are indicated with black lines. Subtle differences in size of the defects may be due to slice preparation.

Figure 5. Distribution of histologic healing types.
Table 2. Outcome of histomorphometry

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Bone%</th>
<th>Osteoid%</th>
<th>N.Oc (×10⁻⁴/µm²)</th>
<th>N.Ob (×10⁻⁴/µm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Center</td>
<td>Sides and bottom</td>
<td>Surface</td>
<td>Center</td>
</tr>
<tr>
<td>DBM Treatment</td>
<td>7.7</td>
<td>55.1</td>
<td>0.0</td>
<td>0.1</td>
</tr>
<tr>
<td>DBM Control</td>
<td>33.8</td>
<td>58.8</td>
<td>5.5</td>
<td>0.1</td>
</tr>
<tr>
<td>DBM+ Treatment</td>
<td>0.0</td>
<td>44.8</td>
<td>9.0</td>
<td>0.0</td>
</tr>
<tr>
<td>DBM+ Control</td>
<td>2.6</td>
<td>58.0</td>
<td>0.0</td>
<td>0.1</td>
</tr>
</tbody>
</table>

The values are presented as the median, with the range in parentheses. There were no statistically significant differences between the groups. N.Ob = number of osteoblasts and N.Oc = number of osteocytes.
Table 3. Outcome of fluorescence microscopy

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>MAR week 1 - 6 (µm/d)</th>
<th>MAR week 6 - 12</th>
<th>MAR week 12 - 18</th>
<th>MAR week 18 - 23</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Center</td>
<td>Sides and bottom</td>
<td>Surface</td>
<td>Center</td>
</tr>
<tr>
<td>DBM Treatment</td>
<td>0.29</td>
<td>0.35</td>
<td>0.31</td>
<td>1.02</td>
</tr>
<tr>
<td></td>
<td>(0.19 - 0.39)</td>
<td>(0.26 - 0.45)</td>
<td>(0.20 - 0.42)</td>
<td>(0.39 - 1.66)</td>
</tr>
<tr>
<td>Control</td>
<td>0.47</td>
<td>0.51</td>
<td>0.53</td>
<td>1.13</td>
</tr>
<tr>
<td></td>
<td>(0.22 - 0.71)</td>
<td>(0.36 - 0.66)</td>
<td>(0.36 - 0.68)</td>
<td>(0.99 - 1.26)</td>
</tr>
<tr>
<td>DBM+ PRP Treat-</td>
<td>0.45</td>
<td>0.52</td>
<td>0.36</td>
<td>1.18</td>
</tr>
<tr>
<td></td>
<td>(0.14 - 0.75)</td>
<td>(0.40 - 0.63)</td>
<td>(0.28 - 0.43)</td>
<td>(0.83 - 1.54)</td>
</tr>
<tr>
<td>Control</td>
<td>0.41</td>
<td>0.43</td>
<td>0.31</td>
<td>1.11</td>
</tr>
<tr>
<td></td>
<td>(0.25 - 0.56)</td>
<td>(0.32 - 0.54)</td>
<td>(0.19 - 0.42)</td>
<td>(0.95 - 1.27)</td>
</tr>
</tbody>
</table>

The values are presented as the mean, with the 95% confidence interval in parentheses.

The MAR of the second administration period (week 6 – 12) of each group was significantly higher than that of the first administration period (week 1 – 6). The MAR did not change significantly in subsequent periods.

MAR = mineral apposition rate and NA = not applicable.
treated with cortical DBM, the repair tissue was composed of subchondral bone and a top layer of cartilage that was smooth and integrated with the adjacent cartilage. In contrast, trabecular DBM resulted in a fibrillated surface without integration with the adjacent cartilage. Gurevitch et al. studied 1.5-mm OCDs in the intercondylar region of rat knees after implantation of cortical DBM particles. In contrast to Gao et al., the former authors observed no proper healing of the defects after a follow-up of up to 24 weeks. Likewise, Dahlberg and Kreicbergs investigated allogeneic DBM in intercondylar groove OCDs of rabbit distal femora, and found absence of bone differentiation toward the joint surface. They concluded that the synovial environment seemed to prevent bone formation otherwise induced by DBM. The present study investigated cortical DBM for talar OCDs and found no beneficial effect. The differences in outcome between our study and the former studies might be due to the fact that we studied larger and older animals with larger OCDs. Additionally, an important difference between the studies is the joint investigated; the knee and ankle joints differ in congruency, cartilage thickness, and loading characteristics. These joints might thus not be reliably compared in osteochondral defect healing models.

In general, the effectiveness of DBM depends on numerous variables, including the age of the donor, the tissue bank and the lot, prolonged heat treatment, the size or shape of the graft particles, the nature of the carrier material, the extent of residual bone mineral content, and the method of sterilization. However, most of these variables are not applicable to the commercially available DBM used in the current study. This type of DBM has shown consistent composition and proven effectiveness in previous studies using a heterotopic bone formation model and an in vitro model. Possibly, the natural circumstances of the joint (i.e., high loading forces and possible intrusion of fluid into the OCD) might preclude the effectiveness of the DBM in the present study. Remnants of the DBM were found in two specimens, suggesting not all the material had remodeled. Furthermore, DBM was used as a xenograft; that is, human DBM was implanted in caprine OCDs. This might theoretically influence the effectiveness of DBM. However, the use of human DBM in animals did not seem to affect positive results in other studies. Hence, this aspect is probably not a good explanation for the lack of effectiveness. The immune response of the animals was not clearly related to the implantation of DBM; macrophages were observed in one DBM specimen and in four control specimens.

The addition of PRP to DBM has led to equivocal results in the literature. Butcher et al. found a significant increase in DNA content and mineralization level by adding PRP to DBM in vitro. Likewise, a viable osteochondral construct has been created with DBM and PRP ex vivo. In contrast, Ranly et al. found a neutral or even inhibitory effect of PRP to the ectopic formation of bone induced by DBM in a nude mouse model. This negative effect of PRP to DBM may have been caused by the activation with thrombin. Han et al. demonstrated that PRP can increase the osteoinductivity of DBM, but only when it has not been activated by thrombin. Even though in the present study the PRP was not activated with thrombin either, a beneficial effect of PRP could not be demonstrated.

Strengths of this study include the use of an established model with a large animal; a power analysis to identify the minimal number of animals; an internal control in each goat; and numerous qualitative and quantitative analyses after relatively long follow-up.

The study also has limitations. There was a single follow-up assessment of 24 weeks.
This way, the number of goats required for the study could be limited to 16. Instead of using additional animals for shorter follow-up assessments, fluorochrome labels were injected at various moments during the follow-up period. This allowed analysis of the speed of repair during the recovery period and made the inclusion of additional animals unnecessary. Another limitation was the variable concentration of platelets in the PRP. The mean concentration was approximately four times that of unprepared caprine whole blood, but the dispersion was high. This variation was also observed in previous studies and might be due to either the preparation or the analysis of PRP. Although both systems – GPS II for preparation and XE-5000 for analysis – have been developed for human blood, others have successfully used them in animals; e.g., a GPS II system for bovine blood (increasing the platelet concentration fourfold), and an XE-2100 analyzer for rat PRP. We therefore believe the systems can be used in other species. We also believe the observed variation in platelet concentration does not affect the conclusions, as there was no statistically significant difference (p = 1.0) in outcome between specimens with high concentrations and those with low concentrations of PRP. Besides, the ideal concentration of PRP remains unknown. Further studies are indicated to determine the optimal concentration of PRP as well as the ideal preparation of DBM before they can be uniformly investigated in (pre)clinical studies.

**Conclusion**

DBM with or without PRP was not beneficial in the treatment of OCDs of the talus in goats.

**Acknowledgment**

Biomet BV, Dordrecht, the Netherlands, is gratefully acknowledged for financial support of the study and supply of the demineralized bone matrix and gravitational platelet system. The company was not involved in the study design; in the collection, analysis and interpretation of data; in the writing of the report; or in the decision to submit the paper for publication.