# Reaming debris as a novel source of autologous bone to enhance healing of bone defects

**Astrid D. Bakker,[1] Robert Jan Kroeze,[1,2] Clara Korstjens,[1] Ruben H. de Kleine,[3] Jan Paul M. Frölke,[4] Jenneke Klein-Nulend[1]**

[1]Department of Oral Cell Biology, ACTA-University of Amsterdam and VU University Amsterdam, Research Institute MOVE, Amsterdam, The Netherlands
[2]Department of Orthopaedic Surgery, VU University Medical Center, Research Institute MOVE, Amsterdam, The Netherlands
[3]Department of Surgery, University Medical Center Groningen, Groningen, The Netherlands
[4]Department of Surgery, Radboud University Nijmegen Medical Center Nijmegen, The Netherlands

Received 31 August 2010; revised 11 January 2011; accepted 19 January 2011
DOI: 10.1002/jbm.a.33080

**Abstract:** Reaming debris is formed when bone defects are stabilized with an intramedullary nail, and contains viable osteoblast-like cells and growth factors, and might thus act as a natural osteoinductive scaffold. The advantage of using reaming debris over stem cells or autologous bone for healing bone defects is that no extra surgery is needed to obtain the material. To assess the clinical feasibility of using reaming debris to enhance bone healing, we investigated whether reaming debris enhances the healing rate of a bone defect in sheep tibia, compared to an empty gap. As golden standard the defect was filled with iliac crest bone. Bones treated with iliac crest bone and reaming debris showed larger callus volume, increased bone volume, and decreased cartilage volume in the fracture gap, and increased torsional toughness compared to the empty gap group at 3 weeks postoperative. In addition, bones treated with reaming debris showed increased torsional stiffness at 6 weeks postoperatively compared to the empty defect group, while bending stiffness was marginally increased. These results indicate that reaming debris could serve as an excellent alternative to iliac crest bone for speeding up the healing process in bone defects that are treated with an intramedullary nail. © 2011 Wiley Periodicals, Inc. J Biomed Mater Res Part A: 97A: 457–465, 2011.

**Key Words:** bone tissue engineering, autologous bone graft, intramedullary reaming, long bone, sheep

## INTRODUCTION

Large bone defects caused by trauma or tumor resection often result in long lasting morbidity due to their limited healing capacity. Standard treatment options consist of the use of autologous or allogenic bone grafts, or distraction osteogenesis.[1,2] The clinical outcome of these treatment modalities often seems satisfactory, although they have major drawbacks such as donor site morbidity and/or host versus graft disease, as well as long healing times.[3–5] The healing rate of large bone defects might be enhanced by a tissue engineering approach whereby autologous stem cells or osteoprogenitor cells on a suitable carrier are introduced at the site of injury. Currently several sources of stem cells are used for bone healing applications such as bone marrow-derived mesenchymal stem cells, periosteal cells, and adipose tissue-derived mesenchymal stem cells.[6–9] All these cell sources have their own advantages and drawbacks, but one common drawback shared by these sources of stem cells is that surgical intervention is required to obtain the cells. In addition, introduction of the cells in the defect requires a suitable carrier, but so far no ideal carrier or consistent cell seeding method has been identified, making the clinical outcome of stem cell-based solutions somewhat unpredictable.

A seemingly simple solution may exist that circumvents all these drawbacks, i.e., the use of reaming debris. Large long bone defects require stable fixation of the bone parts, which is routinely obtained by the placement of intramedullary nails.[10–13] The diameter of the nail is exponentially correlated to the increased rigidity of the stabilization. The placement of such a large caliber intramedullary nail usually requires widening of the narrow medullary canal with a reamer, resulting in the formation of relatively large quantities of reaming debris.[14] We have shown previously that reaming debris is a source of viable osteoblast-like cells that readily proliferate and respond to 1,25-dihydroxyvitamin $D_3$ with increased alkaline phosphatase activity in a manner that is very similar to osteoblasts obtained as outgrowth from collagenase-treated bone pieces from the iliac crest.[15] Osteoblasts obtained as outgrowth from bone pieces have been shown to form bone tissue *in vitro* when seeded on an appropriate carrier.[16–19] These cells also form bone like tissue already at 2 weeks after implantation on a collagen sponge in SCID mice.[20] In addition, the fatty layer of aspirate obtained by reaming the femoral shaft proliferate on ceramic bone void filler and have the potential to differentiate along an osteogenic pathway.[21] This suggests that

**Correspondence to:** A. D. Bakker; e-mail: a.bakker@acta.nl
Contract grant sponsor: Research Institute MOVE of the VU University Amsterdam

the viable cells in reaming are able to contribute to bone formation *in vivo*. The small pieces of bone matrix in reaming do not only contain viable cells, but also growth factors such as bone morphogenetic proteins,[22] which further facilitates rapid bone healing and stimulates blood vessel ingrowth. The reaming debris itself might thus act as a natural osteoinductive scaffold for the newly formed bone, whereby the matrix serves as a natural source of bone growth-enhancing factors, while the viable bone cells offer an intrinsic healing capacity. To assess the clinical feasibility of this novel approach towards healing of large bone defects, we investigated whether reaming debris enhances the healing rate in a large bone defect in sheep tibia. Cancellous bone from the iliac crest was used as the golden standard to fill the defect.

## MATERIALS AND METHODS

### Animals

All procedures were performed with approval of the animal care and use committee of the VU University Amsterdam, and all procedures were in accordance with Dutch legislation on animal experiments. A total of 36 adult female sheep were included in the study. Maturity was confirmed by radiographic examination for closure of the proximal tibial and distal femoral growth plate. Sheep were housed in groups of 6–8 animals in stables of $5 \times 10$ m$^2$ and fed hay, sheep chow, and water *ad libitum*. Sheep were randomly assigned to 1 out of 3 study groups before the start of the experiment.

### Surgery

All sheep were administered a single dose [10 mg/kg body weight (b.w.)] amoxycillin immediately before surgery. Ketamine (10 mg/kg b.w.) and atropine (1.5 mg) were injected intramuscularly as preanaesthetic agents. Animals were anesthetized by intravenous injection of fentanyl (2 µg/kg b.w.) and midazolam (0.1 mg/kg b.w.). Anaesthesia was maintained by a mixture of environmental air and 50% $O_2$, to which 1–2 vol % isoflorane was added. The animals were placed in right lateral recumbence, and the left hind leg was aseptically prepared and draped for surgery. A drill template was placed over the tibia, and a unilateral external fixator (Mathys Medical Nederland BV, Zeist, The Netherlands) was mounted with two screws proximally and two screws distally on the tibia. A small longitudinal incision was made over the midshaft of the tibia and the bone was exposed. A transverse osteotomy was performed with an oscillating saw equidistant of the fixator screws. The two bone pieces, including the periosteum, were distracted for 5 mm.

A small incision was made, and the contra-lateral greater trochanter exposed. An entry hole was made with a 9-mm drill, and a guide wire was inserted along the full tract of the intramedullary cavity. The femoral medullary cavity was reamed stepwise with flexible reamers of increasing diameter (Stryker BV, Waardenburg, The Netherlands), starting with a reamer diameter of 10 mm. The reamer diameter was increased in steps of 0.5 mm until the reamer head was touching the inner cortical wall. Thereafter the reamer diameter was increased by 0.5 mm twice more. The reaming debris were harvested from the medullar cavity with a suction curette connected to a syringe. The reaming debris was also removed from the flutes of the two largest reamer heads.

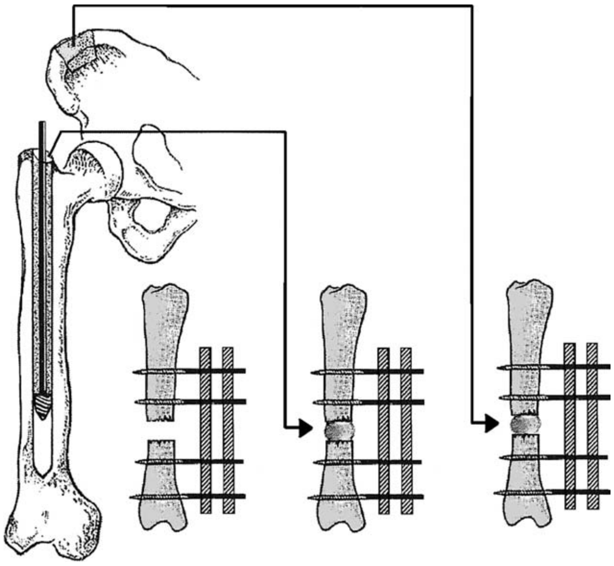

FIGURE 1. Schematic representation of the bone defect model used to test the bone healing efficacy of reaming debris. A transverse osteotomy was performed in the tibia of adult sheep, and the two bone pieces were distracted for 5 mm. The defects were left empty, filled with minced bone, or with reaming debris. Reaming debris was obtained from the contra-lateral femur, while bone pieces were harvested from the iliac crest.

A small incision was also made over the ipsilateral iliac crest to expose the iliac crest. The outer cortical wall was removed with a chisel. Cancellous bone was scraped from the crest. In 12 sheep the osteotomy gaps were left untreated (empty defect group), in 10 sheep, the osteotomy gaps were filled with autologous compacted cancellous bone from the iliac crest (iliac crest group), and in 14 sheep, the osteotomy gaps in the tibia were densely packed with autologous reaming debris (reaming group; Fig. 1).

All sheep received 0.5 mg buprenorfine subcutaneously twice daily during the first 2 days postoperatively. After 3 weeks, five sheep from each group were anesthetized by intramuscular injection of a mixture of ketamine (10 mg/kg b.w.) and xylazine (40 mg), followed by intravenous administration of 1200 mg sodiumpentobarbital and 1.4 mg potassium chloride. The remaining animals were sacrificed 6 weeks postoperatively.

### X-ray analysis

X-rays were taken at 1 week postoperatively, and before sacrifice at 3 or 6 weeks postoperatively, for quantification of callus formation. Callus volume was measured in two directions and the mean was calculated according to the method described by Perkins et al.[23]

### Mechanical testing

After sacrifice both the experimental and control tibiae were explanted, soft tissues removed, and placed in 70%

alcohol for 14 days. Subsequently, a standard 4-point non-destructive bending test was performed to measure the stiffness of experimental and control tibiae in 24 directions. The 24 stiffness values of each animal were plotted in polar coordinates, followed by regression analysis resulting in two ellipses, from which the area ratio and stiffness index were calculated. The area ratio is the ratio of the ellipses of the experimental and control tibia, providing a parameter for the total stiffness of the defect tibia in comparison with the intact tibia. The stiffness index is the ratio of the stiffness in the defect leg compared to the intact tibia, in the direction where the ratio is minimal, thereby providing the weakest possible comparison. After a non-destructive bending test, a torsion test-to-failure was performed to determine torsional flexibility and strength. The values are expressed as percent of the intact tibiae.

### Histology and histomorphometry

After the mechanical tests were performed, from the medial, lateral, dorsal, and ventral cortex a 2-mm thick longitudinal sample of the fracture gap was harvested using a diamond band exact saw (Exakt Apparatenbau, Norderstedt, Germany). After dehydration in ascending alcohol series, samples were embedded in methyl metracylate (BDH Laboratory supplies, UK). Undecalcified longitudinal sections of 5 μm thickness were cut with a Jung-K microtome (Reichert Jung, Heidelberg, Germany). The sections were marked with pen to delineate the margin between the existing bone and newly formed bone within the callus and fracture gap. This delineation was made after carefully studying the pre and postsurgery radiographs in combination with the macroscopic and microscopic appearance of the bone. Sections were stained with Goldner's Trichrome method,[24] which stains osteoid red, mineralized bone matrix green, and nuclei blue/black. Parallel sections were stained with toluidine blue for determination of cartilage volume. At least four sections per animal were used for histomorphometric measurements. The measurements of bone volume (BV) and cartilage volume (CV) were carried out at 50× magnification. Measurements of osteoid volume (OV) and resorption surface (RS) were carried out at 200× magnification for easier identification of osteoclasts and Howship's lacunae. A Leica DMRA microscope connected to a computer using an electronic stage table and a Leica DC 200 digital camera was used for histomorphometrical measurements. Leica Qwin computer software was used to process and measure the digitized image (Leica Microsystems Imaging Solutions, Rijswijk, The Netherlands). The total bone volume (BV/TV) was calculated as the percent of mineralized bone tissue (BV) of the total tissue volume (TV) according to Parfitt et al.[25] CV/TV and OV/TV were also calculated to express both CV and OV as percent of the total tissue volume. RS was defined as the surface of Howship's lacunae divided by the total bone surface.

### Statistical analysis

All data was normally distributed. For comparison of histomorphometrical parameters between the reaming group or the iliac-crest bone group with the empty defect group, 95% confidence intervals were calculated. Values were considered different from the empty defect group if the 95% confidence interval did not include the value "100." For comparison of mechanical parameters between the reaming group or the iliac-crest bone group with the empty defect group, a student *t*-test was performed. Since the material was obtained from large animal experiments, only a limited number of samples was available for mechanical testing. Therefore the cut-off for the *p*-value for these tests was set at 0.1. All values are expressed as mean ± SEM.

## RESULTS

### Animals, general

In three sheep, two from the reaming group and one from the empty defect group, a Schantz screw broke from the bone. In one sheep from the reaming group, the screws failed. One sheep from the empty defect group had to be terminated due to spontaneous proximal femoral fracture at the reamed side. Finally, one animal from the reaming group died due to bowel obstruction on the 10th day postoperatively. Bones of above-mentioned sheep were not analyzed. As a result, the bones of a total of 10 animals per treatment group were analyzed in this study. Systemic infections did not occur, yet six sheep experienced pin-tract infections at the most proximal screw. In one sheep of the empty defect group and in one sheep of the iliac crest group, the pin tract infection resulted in osteomyelitis, leading to loosening of the screws and minor displacement of the osteotomy. As the x-rays showed no apparent effect on the healing of the osteotomy, these animals were not excluded from the study.

### Callus formation

Callus formation was visible on the x-rays of all animals at 3 weeks postoperatively. At 3 weeks postoperatively, the mean callus volume was significantly higher in the groups containing reaming debris (9489 ± 2075 $\text{mm}^3$, $p = 0.03$, $n = 10$) or iliac crest bone (10,183 ± 1357 $\text{mm}^3$, $p = 0.04$, $n = 10$) compared to the empty defect (3176 ± 988 $\text{mm}^3$, $n = 10$). After 6 weeks, callus formation in the empty gap group seemed to have gained on the other two groups, since there were no statistical differences in callus volume between the groups at this time point (Fig. 2).

### Histology

At 3 weeks postoperatively, cartilage tissue presumably emanating from the periosteum was clearly visible in the fracture callus at the periphery of the defect in animals of all 3 groups [Fig. 3(A–C)]. After 6 weeks, cartilage tissue could still be detected within the fracture gap in all groups, suggesting that the healing process was still ongoing and occurred via enchondral bone formation [Fig. 3(D–F)]. As early as 3 weeks after creation of the defect, new bone formation was already visible around the fracture gap, forming a stable fracture callus in the empty defect group [Fig. 4(A)]. No new bone formation was apparent inside the fracture gap at this time point in any of the groups [Fig. 4(A–C)]. Individual pieces of iliac crest bone or reaming

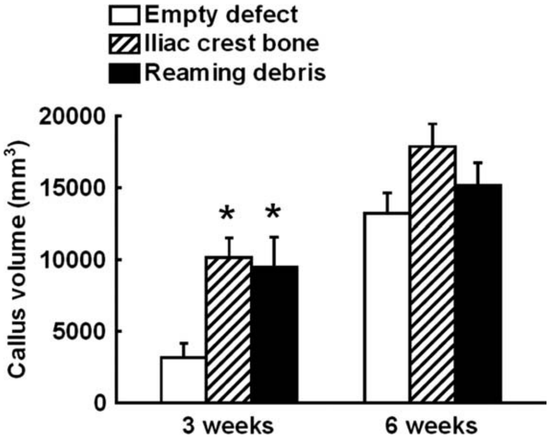

**FIGURE 2.** Effect of reaming debris on callus volume in a defect in a sheep tibia. Both iliac crest bone and reaming debris significantly increased callus volume at 3 weeks postoperatively, as measured in X-ray photographs. At 6 weeks postoperatively, no difference in callus volume could be observed between the groups. *Significantly different from the empty gap group $p < 0.01$.

debris were still clearly visible within the fracture gap at this time point [Fig. 4(B,C)]. Three weeks later, at 6 weeks postoperatively, new bone had formed within the fracture gap in sheep of all three groups, bridging the original bone pieces in some cases [Fig. 4(D–F)]. The pieces of iliac crest bone were likely resorbed or integrated within the newly formed bone at this time, as they could not be identified in the histological pictures. Bone and cartilage volumes were quantified using histomorphometry, and the results are provided below.

### Histomorphometry

In the empty defects, CV/TV within the fracture gap was 0.050 ± 0.043 at 3 weeks postoperatively, and 0.050 ± 0.016 at 6 weeks postoperatively ($n = 4$), showing that enchondral bone formation was still occurring at a steady pace after 6 weeks. CV/TV was significantly lower in sheep receiving reaming debris and iliac crest bone than in sheep with an empty defect at 3 weeks postoperatively [Fig. 5(A)], cartilage tissue being nearly absent in the iliac crest bone group. After 6 weeks of healing, CV/TV in the iliac crest group was still only 35.8% of that in the empty defect (95% confidence interval 0–92.1%, $n = 4$), while in defects containing reaming debris the CV/TV seemed to have increased, but was not statistically different from the empty defects [Fig. 5(A)].

OV/TV was 0.012 ± 0.004 in the empty defect group at 3 weeks postoperatively, and 0.012 ± 0.010 at 6 weeks

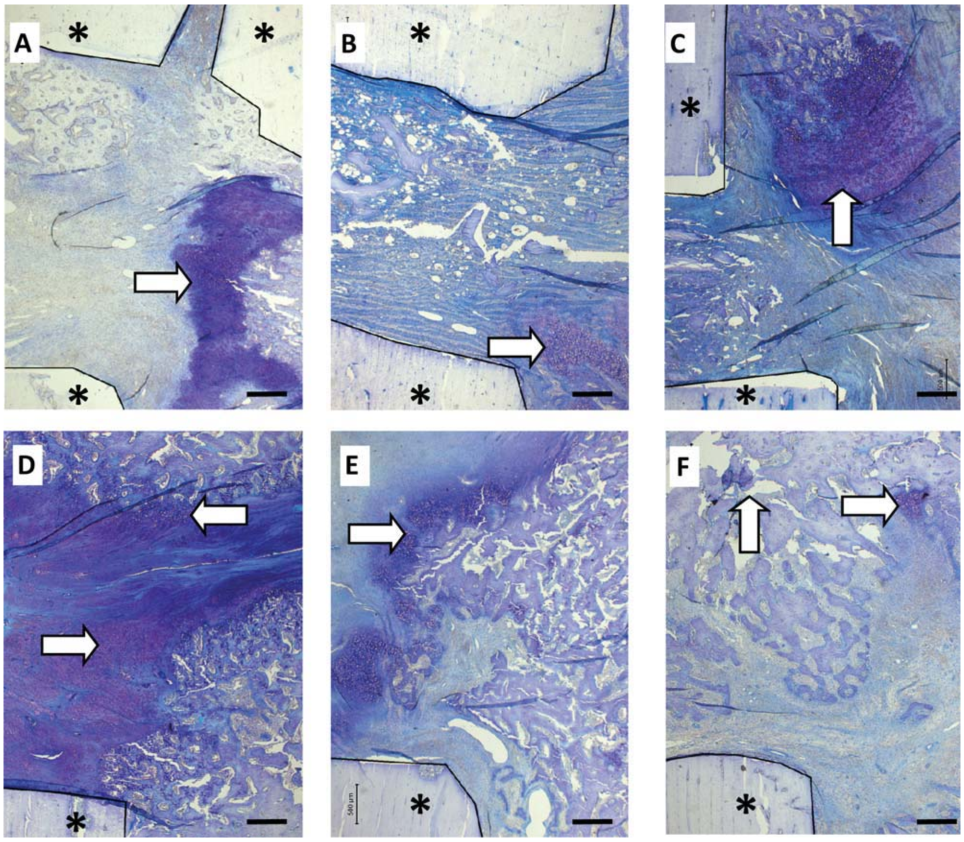

**FIGURE 3.** Effect of reaming debris on enchondral bone formation in a defect in a sheep tibia. (A) Cartilage tissue in the empty defect at 3 weeks postoperatively; (B) cartilage tissue in the iliac crest bone filled defect at 3 weeks postoperatively; (C) cartilage tissue in the reaming group at 3 weeks postoperatively; (D) cartilage tissue in the empty defect at 6 weeks postoperatively; (E) cartilage tissue in the Iliac crest group at 6 weeks postoperatively; (F) cartilage tissue in the reaming group at 6 weeks postoperatively. Open arrows, cartilage tissue (purple); *Native sheep tibia. Scale bar, 500 μm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

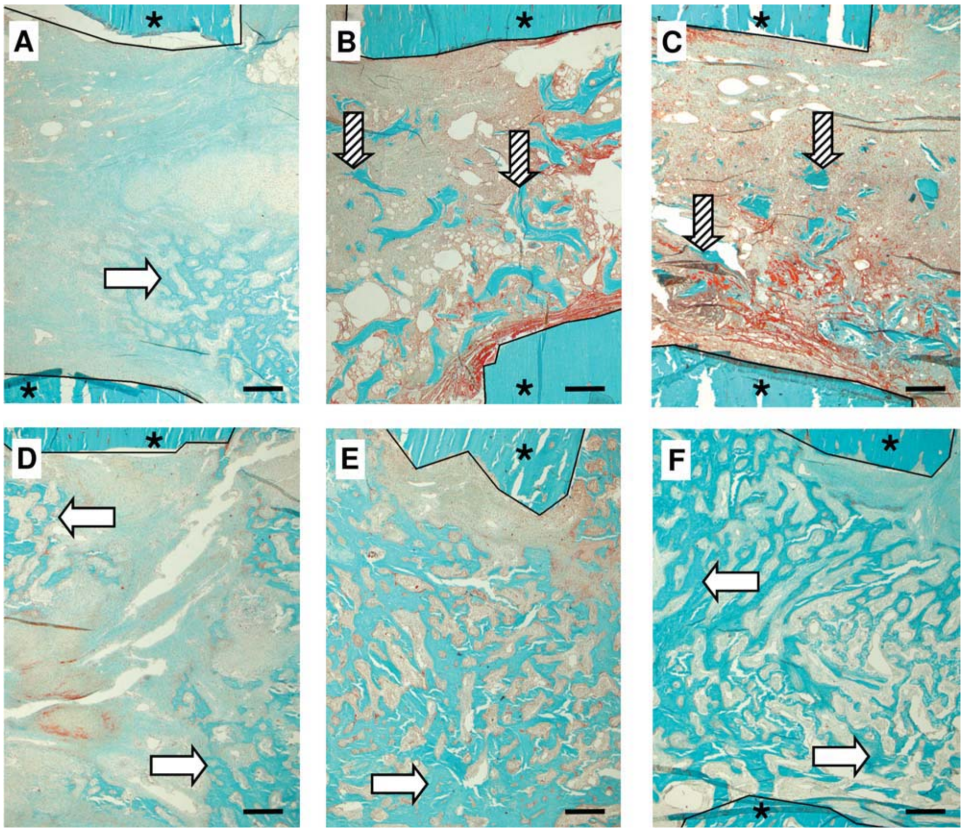

**FIGURE 4.** Effect of reaming debris on bone formation in a defect in a sheep tibia. (A) Bone tissue in the empty defect at 3 weeks postoperatively; (B) bone tissue in the iliac crest bone filled defect at 3 weeks postoperatively; (C) bone tissue in the reaming group at 3 weeks postoperatively; (D) bone tissue in the empty defect at 6 weeks postoperatively; (E) bone tissue in the Iliac crest group at 6 weeks postoperatively; (F) bone tissue in the reaming group at 6 weeks postoperatively. Open arrows, newly formed bone tissue (green); Striped arrows, grafted bone tissue. *Native sheep tibia. Scale bar, 500 μm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

postoperatively ($n = 5$). The OV/TV was significantly lower in sheep receiving reaming debris and iliac crest bone than in sheep with an empty defect at 3 weeks postoperatively [Fig. 5(B)]. After 6 weeks of healing, OV/TV in the iliac crest group was more similar to fractures receiving no graft material than at 3 weeks, although OV/TV was still significantly lower than in the empty gap group (68.9% of that in the empty defect, 95% Confidence interval 52.7–85.1, $n = 5$). In defects containing reaming debris the osteoid volume also seemed to have increased compared to 3 weeks postoperatively, but this was not statistically different from the empty defects [Fig. 5(B)].

As expected from the histologic pictures, bone volume in the fracture gap (BV/TV) showed a sharp increase over time, from 0.078 ± 0.016 at 3 weeks to 0.224 ± 0.019 at 6 weeks in the empty gap group [$n = 5$, Fig. 5(C)]. At 6 weeks postoperatively, bone volumes were similar between all three groups, but at 3 weeks differences were apparent. At this time point, bone volume in defects filled with Iliac crest bone was 137% of the empty defect group (95% confidence interval 103.3–171.4, $n = 5$), while in the defects containing reaming debris, the bone volume was 119% of that in the empty defect group (95% confidence interval 104.2–134.4), both differences being statistically significant.

In the group where the bone defect was left empty, the percentage of the bone surface that was actively being resorbed was 0.67 ± 0.07% at 3 weeks postoperatively, and 0.77 ± 0.18% at 6 weeks postoperatively ($n = 5$). The percentage of bone undergoing resorption was significantly higher in the sheep with reaming debris (95% confidence interval 105.7–398.7, $n = 5$) at 3 weeks postoperatively, showing that remodeling of the grafted bone was already taking place at this early time point. There was a trend, although not statistically significant, towards increased bone resorption in the reaming debris group compared to the empty gap group at 6 weeks as well. Taken together, there appeared to be a difference in histomorphometric parameters between the two grafted groups versus the empty gap group of sheep, especially at early time points. However, neither the appearance of new tissue formation, nor the amount of new tissue determines the success of bone healing. The primary task of bone is to withstand forces, and therefore we performed mechanical tests to assess the function of the newly formed bone in the different groups.

## Mechanical properties

Mechanical test results are all expressed as percentage, i.e., the mechanical properties of the bone on the operated side

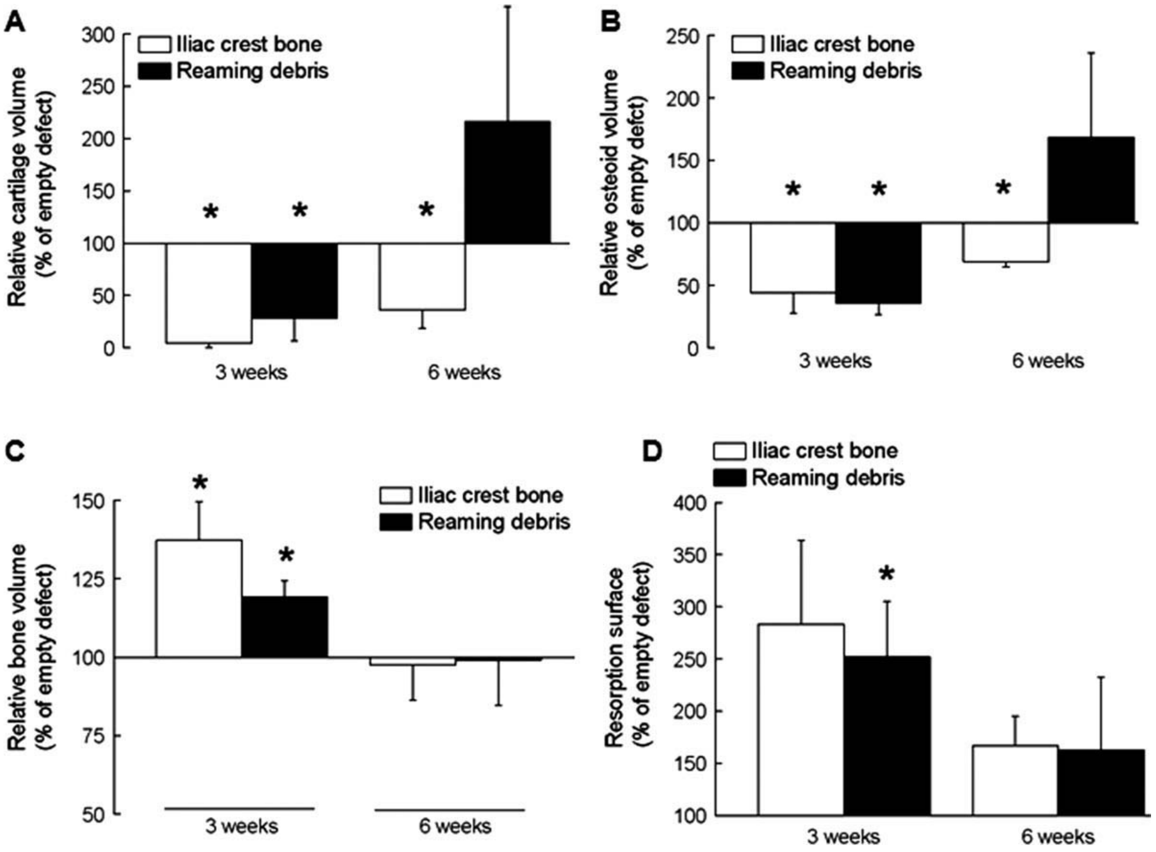

**FIGURE 5.** Effect of reaming debris on the amount of cartilage, osteoid, newly formed bone, and resorbed bone, in a defect in a sheep tibia. (A) CV/TV was lower in sheep receiving reaming debris and iliac crest bone than in sheep with an empty defect at 3 weeks postoperatively. After 6 weeks of healing, CV/TV seemed increased in defects containing reaming debris. (B) OV/TV was significantly lower in sheep with reaming debris and iliac crest bone than in sheep with an empty defect at 3 weeks postoperatively. After 6 weeks of healing, OV/TV in the iliac crest group was still lower than in the empty gap group. (C) At 3 weeks postoperatively, BV/TV in defects filled with Iliac crest bone was 137% of the empty defect group, while in the defects containing reaming debris, BV/TV was 119% of that in the empty defect group. At 6 weeks postoperatively, bone volumes are similar between all three groups. (D) The percentage of bone undergoing resorption was significantly higher in sheep with reaming debris at 3 weeks postoperatively. No differences were observed after 6 weeks postoperatively. *Significantly different from the empty gap group.

are expressed as percent of the mechanical properties of the contra lateral intact bone. A percentage of 100% indicates that the mechanical properties of the defect side are identical to the properties of intact bone. At 3 weeks postoperatively, stiffness in bending was still extremely low when compared to the untreated bone in all of the 3 groups [Fig. 6(A,B)], and the area ratio was not even reaching 1% of the intact side in either of the groups. At 6 weeks postsurgery, however, both stiffness index and area ratio were increased in all groups. Although statistical significance was not reached, the bones treated with reaming debris appeared to have higher stiffness in bending than the bones with empty gaps, i.e., the stiffness index was 38% ± 7% in the reaming group versus 24% ± 8% in the empty gap group ($p = 0.21$, $n = 5$), and the area ratio was 24% ± 8% in the reaming group versus 10% ± 5% in the empty gap group ($p = 0.19$, $n = 5$) [Fig. 6(A,B)]. Torsional stiffness showed a similar trend as bending stiffness, i.e., the group treated with reaming debris displayed a significantly higher stiffness after 6 weeks of healing than the empty gap group [reaming group, 67% ± 11% versus empty gap group, 41% ± 3%, $p = 0.09$, $n = 5$, Fig. 6(C)]. Torsional strength did not differ between the groups at 3 weeks or 6 weeks postoperatively [Fig. 6(D)]. At 3 weeks postoperatively torsional toughness was significantly different between bones of sheep that received reaming debris (20% ± 3% of the intact side, $p = 0.04$, $n = 5$) or iliac crest bone (24% ± 4% of the intact side, $p = 0.04$, $n = 5$) compared to the empty gap group (only 13% ± 1% of the intact side, $n = 5$) [Fig. 6(D)]. In the following 3 weeks, the torsional toughness in the empty defect group increased, reaching 53% ± 15% of that of the intact leg at 6 weeks postoperatively. This torsional toughness value was not significantly different for the other two treatment groups [Fig. 6(D)].

## DISCUSSION

The aim of our study was to test whether reaming debris enhances healing of a large defect in a load bearing bone in a sheep model, while cancellous bone from the Iliac crest was used as a golden standard. When compared to the empty gap group, iliac crest bone-treated defects showed larger callus volume, increased bone volume within the fracture gap, less cartilage within the fracture gap, and higher torsional toughness at 3 weeks postoperatively, as can be

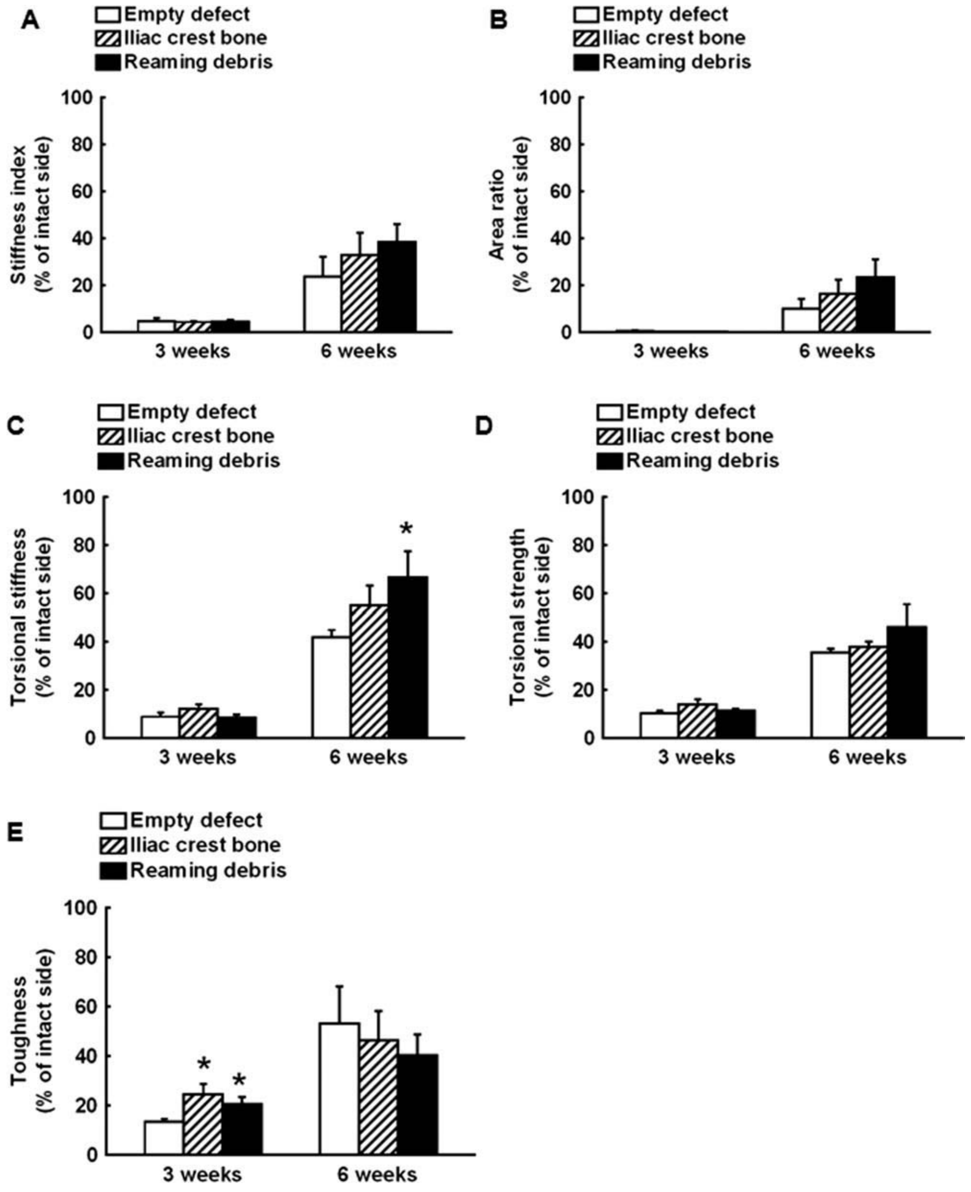

**FIGURE 6.** Effect of reaming debris on mechanical properties of sheep tibia after 3 and 6 weeks of recovery from a large bone defect. (A) At 3 weeks postoperatively, stiffness in bending was low compared to untreated bone in all 3 groups. At 6 weeks postoperatively, stiffness index was increased for all groups. (B) At 3 weeks postoperatively, the area ratio did not reach 1% of the intact side in any of the groups. At 6 weeks postoperatively, area ratio was increased, and was similar for all groups. (C) Torsional stiffness showed a similar trend to bending stiffness, i.e., the reaming debris group displayed significantly higher stiffness after 6 weeks of healing than the empty gap group. (D) Torsional strength did not differ between the groups at 3 weeks or 6 weeks postoperatively. (E) At 3 weeks postoperatively torsional toughness was significantly different between bones of sheep with reaming debris or iliac crest bone compared to the empty gap group. *Significantly different from the empty gap group.

expected from a golden standard. At 6 weeks postsurgery these differences mostly disappeared, indicating that iliac crest bone mainly served to speed up the healing process. Bones treated with reaming debris showed similar characteristics as the iliac crest group, with larger callus volume, increased bone volume, less cartilage within the fracture gap, and higher toughness at 3 weeks postoperatively compared to the empty gap group. In addition, bones treated with reaming debris showed higher torsional stiffness at 6 weeks postoperatively compared to the empty defect group, while bending stiffness showed a trend towards being higher as well. This indicates that reaming debris could serve as an excellent alternative to iliac crest bone for speeding up the healing process in large bone defects that are treated with an intramedullary nail. In general, sufficient quantities of reaming debris can be obtained to fill fracture gaps when reaming the medulla, without the need of harvesting additional donor material.[14] Harvesting of iliac crest bone on the other hand can lead to comorbidity, and up to 16.5% of the patients who underwent bone grafting procedure for spinal surgery claim that they suffer more pain from the donor site than from the primary surgery site, even at 12 months after the harvesting procedure.[3,4] Therefore, the important advantage of using reaming debris over iliac crest bone is that reaming debris is surgical waste material which otherwise would be discarded, and no additional intervention is needed to obtain the material.

It is likely that reaming debris aids bone healing because it is a source of growth factors and viable osteoblast-like cells.[15] Osteoblasts obtained from bone pieces have been shown to form bone tissue *in vitro* and *in vivo* when seeded on an appropriate carrier.[16–20] With this regard, it is important to realize that the reaming speed and the sharpness of cutting blades of a reamer will influence the amount of heat that is generated during the reaming process. Heat generation can negatively affect the viability of the cells in the reaming debris, and thus the outcome of the healing process. In extreme circumstances, when the medullary cavity is narrow or scarred, the heat production may develop extreme values of more than 70°C, which will damage the cortical wall and produce dead reaming particles.[26] However, no harmful heat production is measured when using average surgical-grade reamers (50–250 r/min, 100 in/lb torque), as long as they have sharp, deep cutting flutes.[27]

If one considers that healing occurs emanating from the periosteum via endochondral ossification, then the order of tissues formation in the fracture gap would be cartilage, osteoid, and bone, eventually followed by bone remodeling to change trabecular bone into neat cortices.[28] Bone resorption was more present in the reaming debris and iliac crest groups than the empty gap group, suggesting that the healing time was shortened by the grafting of bone. These observations are supported by our data obtained from the x-rays, which mainly provide information about the fracture callus, but not about the actual healing within the fracture gap, and yet they show very similar dynamics as the mechanical tests and the histomorphometric parameters. At 3 weeks postoperatively, there was more extensive callus formation in the iliac crest and reaming groups than in the empty defect group. At 6 weeks postoperatively the empty defect had gained callus on the other groups, and there was no significant difference in fracture callus volume anymore. Interestingly, in the empty gap group the callus volume was clearly increased between week 3 and week 6 postoperatively, suggesting that the periosteum was still increasing cartilage and bone volume to stabilize the defect.

The segmental fracture model was created to reproduce the injury patterns seen in an open human tibia fracture.[29] The creation of a segmental pattern of bone injury and extensive periosteal stripping ensured devascularization of a large segment of diaphyseal bone. However, there were some differences between our surgical model and a high-energy tibia fracture, as the overlying soft tissue envelope was left intact, and there were no complex fracture patterns and/or bacterial contamination. It is important to note that the defect was not, and was not intended to be, a critical size defect. The empty defect showed good healing over time, whereby the healing mainly emanated from the periosteum. As a matter of fact, the healing seemed to have originated from the periosteum in all groups, suggesting that the advantageous effect of reaming debris was mainly targeted at the periosteum. It appears that reaming debris can speed up healing of a large defect; whether the osteogenic properties of reaming debris can induce healing in a critical size defect remains to be elucidated.

We have used sheep as a model for defects in human load-bearing bones. The tibiae of sheep are somewhat smaller than human tibiae and contain fewer Haversian channels than human bone.[30] Besides these differences, comparative studies have shown that sheep tibiae match better with human tibiae than the bones of other commonly used laboratory animals.[30] The long bones of adult sheep are quite comparable to human long bones with regard to size, shape, structure, mechanical load, and vascularization, but they are known to heal faster than human bones. The good intrinsic healing capacity of sheep bone could potentially obscure significant differences in treatment modality compared to an untreated bone defect. Yet we found, at least at 3 weeks postoperatively, that both iliac crest bone and reaming debris significantly enhanced bone healing in these animals.

At 3 weeks postoperatively, we found that torsional toughness was higher in the iliac crest and reaming debris group, when compared to the empty gap group. The difference in torsional toughness between the groups is highly important, as this parameter represents the total amount of energy that a bone can absorb before it breaks, and is has been shown to be a good predictor for fracture risk.[31] At 6 weeks postoperatively none of the mechanical parameters at the defect side reached values similar to those at the intact side, in any of the experimental groups. From the histological pictures at 6 weeks postoperatively, it is clear that the newly formed bone mainly consisted of trabecular-like bone within the fracture gap, rather than compact bone at the periphery of the tibia. This distribution of bone mass does not favor mechanical properties, especially in torsion. However it is likely that the mechanical properties will further increase over time, as cartilage tissue and osteoid were still present in the fracture gap, which indicates that the healing process was still ongoing. This suggests that we have terminated our experiment too early. On the other hand, we found that most advantageous effects of using bone as a graft material were visible in the first 3 weeks after creation of the defect. At 6 weeks, all of the groups showed similar mechanical characteristics. Thus, it is unlikely that we would have gained additional information by investigating fractures at later time points postoperatively.

Taken together, reaming debris and iliac crest bone both seem the speed up healing of a large segmental defect in sheep tibiae. Reaming debris and iliac crest bone showed no differences in efficiency for bone healing, suggesting that reaming debris works just as well as the current golden standard, and simultaneously causes less morbidity. In conclusion, reaming debris could serve to speed up the healing process of large segmental bone defects, and could provide an attractive source of filler material in the clinical setting.

## REFERENCES

1. Veitch SW, Stroud RM, Toms AD. Compaction bone grafting in tibial plateau fracture fixation. J Trauma 2010;68:980–983.
2. Ai-Aql ZS, Alagl AS, Graves DT, Gerstenfeld LC, Einhorn TA. Molecular mechanisms controlling bone formation during fracture healing and distraction osteogenesis. J Dent Res 2008;87:107–118.

3. Nocini PF, Bedogni A, Valsecchi S, Trevisiol L, Ferrari F, Fior A, Saia G. Fractures of the iliac crest following anterior and posterior bone graft harvesting. Review of the literature and case presentation. Minerva Stomatol 2003;52:441–452.
4. Kim DH, Rhim R, Li L, Martha J, Swaim BH, Banco RJ, Jenis LG, Tromanhauser SG. Prospective study of iliac crest bone graft harvest site pain and morbidity. Spine J 2009;9:886–892.
5. Schaaf H, Lendeckel S, Howaldt HP, Streckbein P. Donor site morbidity after bone harvesting from the anterior iliac crest. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2010;109:52–58.
6. Hanada K, Dennis JE, Caplan AI. Stimulatory effects of basic fibroblast growth factor and bone morphogenetic protein-2 on osteogenic differentiation of rat bone marrow-derived mesenchymal stem cells. J Bone Miner Res 1997;12:1606–1614.
7. Eyckmans J, Roberts SJ, Schrooten J, Luyten FP. A clinically relevant model of osteoinduction: A process requiring calcium phosphate and BMP/Wnt signaling. J Cell Mol Med 2010;14:1845–1856.
8. Helder MN, Knippenberg M, Klein-Nulend J, Wuisman PIJM. Stem cells from adipose tissue allow challenging new concepts for regenerative medicine. Tissue Eng 2007;13:1799–1808.
9. Farré-Guasch E, Martí-Pagès C, Hernández-Alfaro F, Klein-Nulend J, Casals N. Buccal fat pad, an oral access source of human adipose stem cells with potential for osteochondral tissue engineering: An in vitro study. Tissue Eng Part C Methods 2010;16:1083–1094.
10. Bong MR, Kummer FJ, Koval KJ, Egol KA. Intramedullary nailing of the lower extremity: Biomechanics and biology. J Am Acad Orthop Surg 2007;15:97–106.
11. Kessler SB, Hallfeldt KKJ, Perren SM, Schweiberer L. The effects of reaming and intramedullary nailing on fracture healing. Clin Orthop 1986;212:18–25.
12. Stegemann P, Lorio M, Soriano R, Bone L. Management protocol for unreamed interlocking tibial nails for open tibial fractures. J Orthop Trauma 1995;9:117–120.
13. Keating JF, O'Brien P, Blachut P, Meek RN, Broekhuyse HM. Locked intramedullary nailing with and without reaming for open fractures of the tibial shaft: A prospective, randomized study. J Bone Joint Surg Am 1997;79:334–341.
14. Frölke JP, Bakker FC, Patka P, Haarman HJ. Intramedullary reamings in osteotomized sheep tibiae. J Trauma 2001;50:65–70.
15. Frölke JP, Klein-Nulend J, Semeins CM, Bakker FC, Patka P, Haarman HJ. Viable osteoblastic potential of cortical reamings from intramedullary nailing. J Orthop Res 2004;22:1271–1275.
16. Hofmann A, Konrad L, Gotzen L, Printz H, Ramaswamy A, Hofmann C. Bioengineered human bone tissue using autogenous osteoblasts cultured on different biomatrices. J Biomed Mater Res A 2003;67:191–199.
17. Gugala Z, Gogolewski S. The in vitro growth and activity of sheep osteoblasts on three-dimensional scaffolds from poly(L/DL-lactide) 80/20%. J Biomed Mater Res A 2005;75:702–709.
18. Gugala Z, Gogolewski S. Attachment, growth, and activity of rat osteoblasts on polylactide membranes treated with various low-temperature radiofrequency plasmas. J Biomed Mater Res A 2006;76:288–299.
19. Yoshii T, Sotome S, Torigoe I, Maehara H, Sugata Y, Yamada T, Shinomiya K, Okawa A. Isolating osteogenic progenitor cells from trabecular bone for bone tissue engineering. Tissue Eng Part A 2010;16:933–942.
20. Yamanouchi K, Satomura K, Gotoh Y, Kitaoka E, Tobiume S, Kume K, Nagayama M. Bone formation by transplanted human osteoblasts cultured within collagen sponge with dexamethasone in vitro. J Bone Miner Res 2001;16:857–867.
21. Kay Sinclair SS, Jeray KJ, Tanner SL, Burg KJ. Evaluation of the lipid-rich layer of reamer aspirate. J Tissue Eng Regen Med 2010; 4:491–495.
22. Urist MR, Mikulski A, Lietze A. Solubilized and insolubilized bone morphogenetic protein. Proc Natl Acad Sci USA 1979;76: 1828–1832.
23. Perkins R, Skirving AP. Callus formation and the rate of healing of femoral fractures in patients with head injuries. J Bone Joint Surg Br 1987;69:521–524.
24. Romeis B. Mikroskopische Technik, 17th ed, Trichromfärbung nach Goldner. Munchen: Urban & Scharzenberg; 1989. p 558.
25. Parfitt AM, Drezner MK, Glorieux FH. Bone histomorphometry: Standardization of nomenclature, symbols, and units. Report of the ASBMR histomorphometry nomenclature committee. J Bone Miner Res 1987;6:595–610.
26. Pfeifer R, Sellei R, Pape HC. The biology of intramedullary reaming. Injury 2010;41:S4–S8.
27. Agnew SG. Intramedullary reamer redesign: The effect on pressure and temperature. Tech Orthop 1998;13:9–14.
28. Schindeler A, McDonald MM, Bokko P, Little DG. Bone remodeling during fracture repair: The cellular picture. Semin Cell Dev Biol 2008;19:459–466.
29. Clancey GJ, Hansen ST Jr. Open fractures of the tibia: A review of one hundred and two cases. J Bone Joint Surg Am 1978;60: 118–122.
30. Wissing H. Die Wertigkeit verschiedener versuchstierspecies fur experimentelle untersuchungen am knochen. Hefte Unfallheilkunde 1990;212:479–488.
31. Ritchie RO, Buehler MJ, Hansma P. Plasticity and toughness in bone. Phys Today 2009;62:41–47.