

# Nanocrystalline Hydroxyapatite Facilitates Bone Apposition to Polymethylmethacrylate: Histological Investigation Using a Sheep Model

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**ABSTRACT:** Polymethylmethacrylate (PMMA) is the most commonly used bone void filler for vertebral augmentation in osteoporotic fracture. It provides mechanical stability and immediate pain relief; however, PMMA is not osteointegrated and is separated from the surrounding bone tissue by a thin fibrous layer. The aim of this study was to investigate the effect of nanocrystalline hydroxyapatite (HA) on osteointegration of PMMA in a sheep model. A composite material, consisting of PMMA and nanocrystalline HA (70:30, v/v), was implanted in one distal femur, with pure PMMA in the other femur as a control. Three and 6 months after implantation, the distal femora were histologically investigated. All composite implants exhibited a tight junction to the surrounding bone tissue, with minimal bone ingrowth into the outer surface of the implant. In comparison, with use of the control implants, we observed an overall bone resorption around pure PMMA, with fibrous connective tissue encapsulating the implant. These results suggest that nanocrystalline HA enables osteointegration of PMMA in bone tissue, which might alter the biomechanical characteristics of the osteoporotic vertebral body after augmentation. © 2012 Orthopaedic Research Society. Published by Wiley Periodicals, Inc. *J Orthop Res* 30:1290–1295, 2012

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Since the late 1980s, vertebral augmentation has been widely used to treat vertebral body compression fractures caused by different pathologies. At present and with increasing incidence, osteoporotic compression fractures of the spine represent the most frequent indication for vertebroplasty or kyphoplasty.<sup>1,2</sup> Polymethylmethacrylate (PMMA) is the material most frequently used for vertebral augmentation. Despite good clinical results, several shortcomings of PMMA prompt a continuing search for alternative filler material. One problem is that PMMA is a bioinert material that is not involved in the bone remodeling process and is almost always capsulated by fibrous tissue without tight contact to the surrounding bone.<sup>3,4</sup> Additionally, the heat generated by the exothermic curing process of PMMA is theorized to damage the surrounding tissue, as is the possible release of toxic MMA monomers. Furthermore, there is evidence that a PMMA implant increases the incidence of new adjacent vertebral fracture, due to discrepancies between the mechanical properties of PMMA and vertebral cancellous bone.<sup>5,6</sup> Over the few past years, calcium phosphate cement (CPC) has been considered as filler material for vertebral augmentation.<sup>7–9</sup> The main advantages of CPC are its osteoconductive property and promotion of creeping substitution by surrounding bone tissue after implantation. However, due to its brittleness and insufficient mechanical stability, the clinical use of CPC for vertebral augmentation is limited.<sup>7,10</sup> Consequently, some research has focused on composite materials to combine the advantages of

different materials.<sup>11,12</sup> In an animal study, we investigated the histological effects of using a composite material consisting of PMMA and nanocrystalline hydroxyapatite (HA) for implantation into the distal femur of sheep. The objective of the present study was to analyze whether addition of a nanocrystalline HA material to PMMA could induce bone apposition and ingrowth into the composite material after implantation in a bone defect.

## METHODS

### Study Design

After receiving approval from the local institutional animal use and care committee (approval No. D20/Anz.01), 18 adult female sheep, each 18 months of age with body weights ranging from 40 to 50 kg, were purchased through the animal resource center at the university hospital of Frankfurt, Germany. The animals were divided in two groups, one group for a follow-up period of 3 months and the other group for a follow-up period of 6 months. Each animal underwent surgery on both medial femoral condyles for implantation of PMMA in one side and of composite material consisting of PMMA and nanocrystalline HA (Ostim<sup>®</sup>35, aap Biomaterials, Dieburg, Germany) in the other side. After the follow-up period, femora of three animals in each group were assigned for histological investigation and femora of six animals were assigned for biomechanical tests. The results of biomechanical examination will be reported elsewhere.

### Implanted Materials

The PMMA (BonOs<sup>®</sup> Inject, aap Biomaterials, Dieburg, Germany) used in this study, was manufactured for vertebral body augmentation (vertebroplasty). As recommended by the manufacturer, the preparation of PMMA was performed at the time of surgery. In a vacuum tube, we combined 24 g powder containing 12.7 g PMMA, 10.8 g zirconium dioxide (a radiopaque agent), and 0.5 g benzoyl peroxide (to initiate

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polymerization) with 10 ml liquid containing 9.93 ml methylmethacrylate monomer, 0.07 ml *N,N*-dimethyl-*p*-toluidine and 60 ppm hydroquinone (a polymerization stabilizer). The polymerization process of BonOs<sup>®</sup> Inject generates a peak temperature of up to 71°C and the setting time is 20 min, as measured prior to start the study. The compressive strength of BonOs<sup>®</sup> Inject is  $84.6 \pm 5.3$  MPa with a Young's modulus of  $2,040 \pm 148$  MPa and the bending strength is  $58.2 \pm 2.9$  MPa, as specified prior to start the study. The composite material for implantation into the femoral condyle of the other side consisted, by volume, of 70% PMMA (BonOs<sup>®</sup> Inject) and 30% nanocrystalline HA (Ostim<sup>®</sup> 35, aap Biomaterials). Ostim<sup>®</sup> 35 was synthesized by a wet chemical precipitation reaction of CaO and H<sub>3</sub>PO<sub>4</sub> in water, which led to the formation of nanosize HA crystals, as a suspension in water with a HA volume fraction of 35%. The atomic ratio of calcium:phosphorus was 1.67, which was close to that of the mineral phase of bone. The needle-shaped HA crystals had an average size of 100 nm and a large bioactive specific surface area of 106 m<sup>2</sup>/g.<sup>13</sup> For preparing the composite material, Ostim<sup>®</sup> 35 was added to the prepared PMMA and was mixed again. The addition of Ostim<sup>®</sup> 35 to PMMA reduced the peak temperature generated during the polymerization process to 51°C without altering the setting temperature. Further, the compressive strength, Young's modulus and bending strength of the composite material were reduced to  $28.0 \pm 1.9$  MPa,  $1,048 \pm 106$  MPa, and  $19.6 \pm 1.5$  MPa, respectively. Prior to starting our animal study, we performed scanning electron microscopy (SEM) of the composite material for structural analysis.

#### Surgical Procedure

The animals were intubated and anesthetized with propofol (2%). The operative field of the sheep were sheared and shaved, and the skin was sterilized with betadine scrub. Right and left medial femoral condyles were exposed by an approximately 2-cm incision through the skin and facial layers. A 10-mm diameter hole that penetrated 15 mm into the area of trabecular bone was made using a diamond bone cutting system (DBCS, Biomet Merck, Darmstadt, Germany) in the medial condyle of both sides. Right and left femoral defects were randomly assigned for injection with PMMA or composite material. Ten minutes after mixing, PMMA was injected from the preparation tube into the femoral defect of one side and composite material into the femoral defect of another side. The injection continued until the material completely filled the defect and was contiguous with the peristal surface. After defects in femoral condyles were completely filled with materials, the tissues were closed in layers. After surgery, all animals were allowed to walk freely.

#### Sample Processing

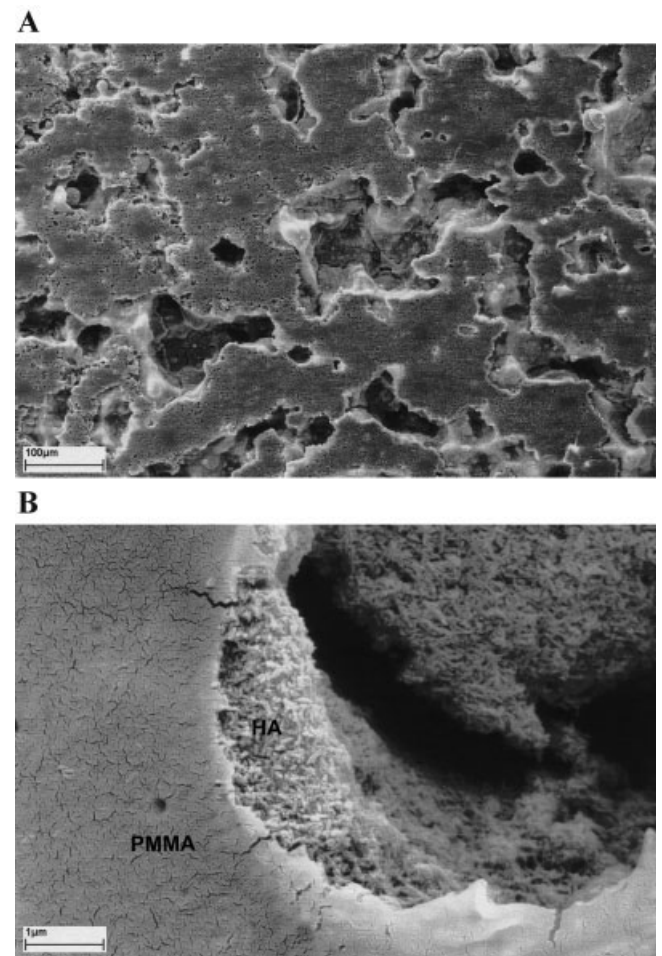
Three and 6 months after surgery, respectively, the animals in groups one and two were killed by intravenous anesthesia and administration of T61<sup>®</sup> (Intervet, Unterschleissheim, Germany). Distal femora were cut out and detached from soft tissue. Femora for histological investigation were fixed in 4% paraformaldehyde fixative. The femur condyles were cut in slices, perpendicular to the implant axis, with an electric band saw (Exact<sup>®</sup>, Exact Vertriebs, Norderstedt, Germany), so that the each section included both the implanted material and the surrounding bone. After stepwise ethanol dehydration, the sections were embedded in Technovit<sup>®</sup> 7200

(Heraeus Kulzer, Hanau, Germany) and were grinded and polished to a thickness of <50 µm. For evaluation with light microscopy, the sections were stained with hematoxylin and eosin as well as with toluidine blue.

#### RESULTS

Structural analysis of composite materials with SEM revealed that addition of Ostim<sup>®</sup> 35 to PMMA produced pores in PMMA, which were up to 100 µm in size and made up to 40% of the surface area (Fig. 1A). This was accomplished by the hydrophilic action of Ostim<sup>®</sup> 35 to hydrophobic PMMA and by vaporization of the trapped water of Ostim<sup>®</sup> 35 in PMMA during the polymerization process. Furthermore, SEM analysis revealed that the wall of created void spaces in the composite material were predominantly covered with nanocrystalline HA with tight contact to PMMA (Fig. 1B).

All surgeries were performed without complications; wound healing was uneventful, without infection or wound dehiscence. All animals recovered well and fast



**Figure 1.** Scanning electron microscopy (SEM) of cross-section surface of the composite material. (A) The porosity of the composite material created by addition of nanocrystalline hydroxyapatite (HA) Ostim<sup>®</sup> 35 to polymethylmethacrylate (PMMA). The pore wall is mainly covered by HA. (B) The tight connection of HA to PMMA in pore.

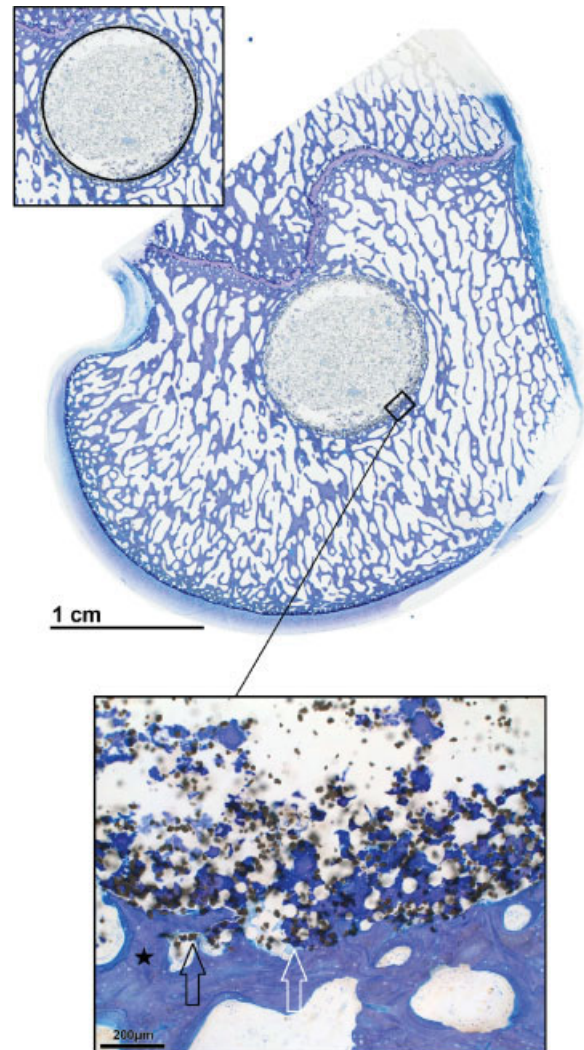
and returned to normal eating and movement activities in a short time. In all implantation studies, the macroscopic view of the sections revealed no cracking of either PMMA or composite implants.

Three months after implantation, bone trabeculae surrounding both implant materials were more compact than other areas of the femoral condyle and were organized in concentric circles. Bone trabeculae had increased in length and diameter and contained well-mineralized, newly formed woven bone covered by osteoid. Composite implants exhibited direct surface contact with the bone tissue throughout the whole circumference, whereas PMMA implants were completely covered by a layer of fibrous connective tissue and thus separated from surrounding bone. In some areas, the composite material penetrated the bone trabeculae surrounding the defect, and was tightly enclosed by newly formed woven bone. We did not observe bone ingrowth into the composite material in the defect cavity. The sizes of the bone defects containing the two implant materials were conspicuously different; all bone defects containing composite material were 10 mm in diameter, the same as originally created at the time of implantation, whereas all bone defects containing PMMA were larger than 10 mm. Higher microscopic magnification revealed no inflammatory reaction in all of viewed sections. The bone surface area in the region of interface with PMMA material showed scattered resorption lacuna beside new bone formation. Few multinucleated giant cells were observed to be dispersed in the interface tissue surrounding PMMA as well as composite implants.

Six months after implantation, the same microscopic changes were observed as after 3 months; bone trabeculae around both implant materials were more compact than other areas of femoral condyle, and the intertrabecular space was filled with newly formed woven bone covered by osteoid. Composite material had a tight contact with the surrounding bone and the size of bone defect was the same as that created during surgery. PMMA implants were covered completely by a layer of fibrous connective tissue and the size of the bone defect was greater than the drill hole created during surgery (Fig. 2). All but one showed no inflammatory reaction; one animal showed minimal monocytic cellular infiltration in some areas around PMMA material. Compared with in the 3-month samples, we observed some slight bone ingrowth into the composite material (Fig. 3). In all sections, scattered multinucleated giant cells were detected.

## DISCUSSION

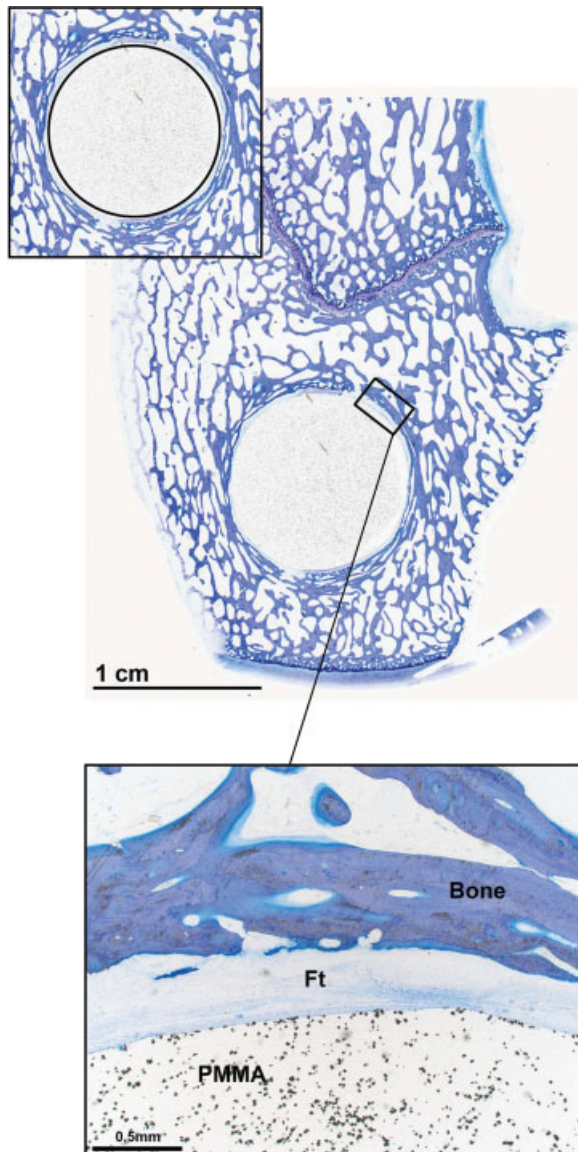
In our study, we observed distinct effects of the implanted materials in the region of interface with the surrounding bone. There was a tight apposition of bone tissue to the composite material, in contrast to separation of pure PMMA by fibrous tissue from surrounding bone. This reaction of bone tissue to implanted PMMA has been known since the use of



**Figure 2.** Histological section of distal femur containing composite material, 6 months after implantation, stained with toluidine blue. The circle plotted on the cut-out image measures 10 mm in diameter, indicating the size of the bone cutting system used during surgery for bone defect creation. The size of the bone defect containing the composite material is exactly the same size as the cutting system, without any bone resorption around the composite material. The magnified image shows tight contact between bone tissue and the composite material, with newly built woven bone (\*) enclosing the nanocrystalline hydroxyapatite (HA) and polymethylmethacrylate (PMMA) particles of composite material. Black spots (•) are zirconium dioxide in PMMA, dark blue-stained particles (•) are nanocrystalline HA.

PMMA in joint replacement surgery. The bone at the PMMA interface undergoes resorption with subsequent fibrosis in the resultant gap, which is frequently visible as a radiolucent line at the bone–PMMA interface after arthroplasty.<sup>3</sup> This has also been observed in histological evaluations of vertebral bodies treated with PMMA-augmentation. Togawa et al.<sup>4</sup> performed histological evaluation of four vertebral bodies retrieved from human patients after augmentation with PMMA. They found that PMMA was separated from the bone by a thin fibrous tissue. In other animal studies where vertebral bodies were augmented with





**Figure 3.** Histological section of distal femur containing polymethylmethacrylate (PMMA), 6 months after implantation, stained with toluidine blue. Significant bone resorption is observed around the bone defect, indicated by the larger size of the bone defect compared to the 10-mm circle. The gap between bone tissue and PMMA is filled with fibrotic connective tissue. Ft = fibrotic tissue.

PMMA, histological evaluation 6 months after surgery revealed that in all animals, the PMMA cement was also covered by thin fibrous tissue, with few regions of necrotic bone in some animals.<sup>14,15</sup> In another study, the immediate effect of PMMA on surrounding bone was investigated after vertebroplasty of rabbit lumbar spine.<sup>16</sup> Twenty-four hours after PMMA injection, 12 of 24 vertebrae had clear evidence of bone necrosis along the cement–bone interface.

The mechanism leading to the formation of fibrous tissue in the bone–PMMA interface is unknown. One hypothesis is that bone resorption with subsequent formation of fibrous tissue seen at the bone–PMMA interface is the result of thermal injury from the

exothermic polymerization reaction of PMMA.<sup>17,18</sup> Another hypothesis involves macrophage-mediated bone resorption generated by the presence of unpolymerized MMA monomer or beads of PMMA shed from the surface as major factor resulting in the fibrotic bone–PMMA interface. As we observed, the size of bone defects that were filled with PMMA were larger than those originally created at the time of surgery. According to the above-mentioned hypothesis, we can suggest that the bone surrounding the PMMA implants underwent a resorption process and the resultant gap in the interface region was filled with fibrous connective tissue. This process did not take place around the composite material containing nanocrystalline HA. Both implanted materials in our study contained PMMA, but the temperature release by the composite material was much lower than that of pure PMMA, suggesting that the formation of a fibrotic bone–PMMA interface was induced by thermal injury generated by the exothermic reaction of PMMA polymerization, and not by toxic monomers or small particles of PMMA.

Another factor leading to differences in the effects of the two implant materials is that the nanocrystalline HA content in our composite material has an osteoconductive property; this might explain the tight apposition of the surrounding bone to the implanted composite material. As bioresorption of HA in the body is influenced by the size of crystals,<sup>19</sup> the small size and the high surface area of HA crystals in Ostim<sup>®</sup> 35 may facilitate its resorption by osteoclasts, thus enabling it to be involved in the bone remodeling process with subsequent new bone formation. The efficacy of Ostim<sup>®</sup> 35 as a bone substitute with osteoconductive effect was first investigated in the field of odontofacial surgery. Two clinical studies confirmed, with 6 months of postoperative X-ray follow-ups, that bone regeneration and reconstitution occurred after filling bone defects in the jaw with Ostim<sup>®</sup> 35.<sup>20,21</sup> In an animal study, Schnettler et al.<sup>22</sup> histologically evaluated the osteointegration, biodegradation, and biocompatibility of Ostim<sup>®</sup> 35 after implantation in bone defects in various animal models (pig, sheep, and rabbit). They observed fragmentation of Ostim<sup>®</sup> 35 immediately after implantation, which allowed cellular infiltration and vascularization of the implant. Two months after implantation the defect was completely filled with newly formed bone, and implant particles were enclosed and covered tightly with ramified trabecular bone, without formation of fibrous tissue in interface region. In another study, Huber et al.<sup>23</sup> performed histological analysis of bone biopsies of distal radius, tibial head, and calcaneus that were obtained 3–14 months after fracture fixation and implantation of Ostim<sup>®</sup> 35 as a void filler for defects of cancellous bone. They found residues of implanted material dispersed in the retrieved bone biopsies that were covered by new bone without fibrous interface. Bone defects that were filled with nanocrystalline HA were bridged by newly formed trabecles, as seen in normal cancellous bone.

Despite the good osteoconductive activity of Ostim<sup>®</sup> 35, we did not observe significant ingrowth of bone tissue into the composite material. Merely a minimal amount of bone ingrowth into the margin of the composite material was seen 6 months after surgery. It is known that several requirements must be met for bone ingrowth into implant material. One crucial requirement is the porosity of the material, that is, pore size, pore volume, and interconnection of pores.<sup>24,25</sup> The pore size must be adequate to accommodate cells, and the interconnection of pores should be sufficient for vascular supply. Based on the work of Hulbert et al.,<sup>26</sup> the minimum recommended pore size is 100  $\mu\text{m}$ . Later studies demonstrated better osteointegration of implants with pore sizes of about 300  $\mu\text{m}$ .<sup>27</sup> The lack of bone ingrowth into the composite material in our study might be the result of insufficient interconnection of pores of the material. The SEM analysis in our study illustrated shape and size of the pores in the cross-section area of the composite material, but the interconnection of pores was not investigated. On the other hand, it is questionable whether PMMA can serve as a scaffold for bone remodelling at all. A review of the literature did not reveal any data about the use of PMMA as scaffold in bone remodeling process.

In summary, the histological results in this study suggest that the addition of nanocrystalline HA to PMMA enabled bone apposition to the implant material, whereas PMMA alone became encapsulated by fibrotic connective tissue. However, we did not observe significant bone ingrowth into the porous structure of the PMMA made by the addition of nanocrystalline HA. The osteointegration of PMMA with the support of nanocrystalline HA is likely of interest for use in vertebral augmentation. Biomechanical investigations should be conducted to investigate if this material composition is superior to plain PMMA for vertebral body augmentation.

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