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Nanocrystalline hydroxyapatite for bone repair: an animal study

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Abstract Hydroxyapatite has become the most common material to replace bone or to guide its regeneration. Nanocrystalline hydroxyapatite suspension had been introduced in the clinical use recently under the assumption that small dimension of crystals could improve resorption. We studied the resorption and osteointegration of the nanocrystalline hydroxyapatite Ostim® in a rabbit model. The material was implanted either alone or in combination with autogenic or allogenic bone into distal rabbit femora. After survival time of 2, 4, 6, 8 and 12 weeks the implants had been evaluated by light and electron microscopy. We observed a direct bone contact as well as inclusion into soft tissue. But we could observe no or only marginal decay and no remarkable resorption in the vast majority of implants. In situ the nanocrystalline material mostly formed densely packed agglomerates which were preserved once included in bone or connective tissue. A serious side effect was the initiation of osteolysis in the femora far from the implantation site causing extended defects in the cortical bone.

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1 Introduction

The progress in contemporary skeletal surgery enlarged the possibilities of joint replacement as well as skeletal repair in bone tumors and trauma management. But the reconstruction of traumatized bone often needs to bridge large size bone defects. Autogenic bone grafts are estimated as the "gold standard" for skeletal repair today but many problems related with their harvest: to get sufficient quantity is often limited by morbidity and constitution of the patient and the harvest needs an additional surgical procedure causing local injury, risk of infection and very often chronic or long-lasting pain. From allogenic bone grafts taken from femoral heads and necks or cadaver bone arise other problems as the danger of viral infections, cost-intensive manufacturing and storage, the limited preservation time and not least the problems of antigenicity and limited availability. For this reason treatment approaches with biomimetic ceramic materials seem to be the most suitable alternative and became an interesting and widely used substitute to fill bone defects. An optimal substitute should replace the lacking tissue for a limited period, should favour bone apposition and gradually disappear with the formation of new bone without any remnants when the bone got sufficient stability and structure. The in vivo performance of ceramic materials depends on a variety of factors such as chemical composition, crystallinity, phase purity, stability in body fluids and resorption behaviour, respectively. Biocompatibility, the complete absence of mutagenic and cancerogenic components and the possibility of sterilization are absolute necessities for

clinical use. However, the most bioceramic implants used today outlast in the bone, hamper its complete regeneration and may become a biomechanical risk for fractures later on. Their degradation behaviour in vivo is discussed controversially. Hydroxyapatite (HA) as the most frequently used ceramic implant in orthopaedic surgery shows a very good biocompatibility, no specific adverse or inflammatory reactions and its direct, physico-chemical bonding to bone was shown by Jarcho first [17]. A partial solubility of the surface enables this bonding with direct deposition of calcified bone matrix. Synthetic HA for medical use as implant material resembles in its chemical composition the natural bone mineral and consists of sintered, polygonal shaped crystals. The porosity of the ceramic (in micro- and macrorange), phase impurities and the size of the crystals influence degradation behaviour in vitro but not the binding of bone tissue [9, 18]. The dimension of HA crystals seem to determine the mechanisms of active resorption: while microcrystals may be resorbed by osteoclasts [6, 22] a crystal size above 0.1–0.3 μm reduce the resorption by cells [21]. Crystal dimensions larger than 1–3 μm are considered to prevent resorbability unless the description of multinucleated giant cells at the surface of implants and changes in the material density at the surfaces, which could be caused by three dimensionality [44]. There is accumulating evidence that osteoclasts may resorb calcium phosphate ceramics in vivo [12, 45] as well as in vitro [22]. Wenisch et al. [43] could show by ultrastructural investigation in female sheep using transmission electron microscopy that osteoclasts contributes to degradation of calcium phosphate ceramics by means of resorption and phagocytosis. They found osteoclasts in close contact with the materials surface showing characteristic phenotypic features. Calcium phosphate crystals from the surface, modified in their shape and electron density, break away from the material and will be either included into vacuoles or surrounded by pseudopodia-like cell protrusions. The velocity of degradation should be appropriate to bone modelling—is the resorption too fast it will disturb local bone regeneration. Following implantation of a fast resorbable ultraporous β-tricalcium phosphate in the distal femora of New Zealand White Rabbits Hing et al. [13] observed an inflammatory response that impaired bone apposition in the defect by degradation products. Calcium sulfate materials with very high dissolution rates also provoked a mild inflammatory reaction preventing bone formation in the defect.

Summarizing this considerations nanocrystalline hydroxyapatite should have the potential to show a better resorbability for obvious reasons. Some authors described experimental and clinical results with this material alone or in combination with other ceramic implants or morselled cancellous bone grafts [5, 14, 15, 33, 40].

2 Materials and methods

The aim of the study was to investigate the resorption behavior of a nanocrystalline hydroxyapatite and the influence of supplementation with autologous or allogenous cancellous bone on the integration of the ceramic implants and the formation of new bone. We used an animal model primarily described by Siebels et al. [31] with implantation of the specimens into the metaphysis of distal rabbit femora perpendicular to the long axis of the bone. The commercially available bone substitute Ostim® contains 25% of pure, synthetic hydroxyapatite in an aqueous solution. It is described as nanocrystalline hydroxyapatite with crystal length of about 18 nm. In comparison with other ceramic implant materials the needle-shaped crystals create a large specific surface (100 m^2/g) because of their small dimensions. Ostim® consists of a HA-crystal dispersion in water forming a high-viscosity paste neither stable in form nor load resistent but easily injectable and hydrophilic. Physiological bone apatite is generally described as plate-like, polygonal crystal with smooth surfaces and dimensions of about 25×3.5 nm in contrast [28, 38, 39].

For the animal study we used 60 female New Zealand White Rabbits. Under general anaesthesia a critical size defect of 4,0 mm diameter and 10 mm in depth was drilled into the metaphysis of distal femoral condyles perpendicular to the long axis of the bone. The hydroxyapatite paste itself has no form stability and mechanical loading therefore is impossible. In our animal model the host bone surrounding the implants undergoes physiological strain during unrestricted movement of the animals. For evaluation of bone formation and implant change with time animals had been sacrificed after 2, 4, 6, 8 and 12 weeks. For each survival time a group of 12 animals underwent operation. We applied three different types of implant: either Ostim® alone or Ostim® mixed with 100 μg autogenic cancellous bone (extracted from the drill holes) and allogenic cancellous bone, respectively. The cylindrical defects were filled with a total volume of 0,125 ml of the implant paste. Each animal received two randomly distributed implants of different types into the distal femoral condyles. Each material was implanted in eight femora thus gaining a sufficient number of specimens for statistical analysis of histomorphometric data. All animals received fluorescent dyes immediately, two and four weeks after surgery for labeling the growing bone. After sacrificing the animals the thigh bone was dissected from the surrounding soft tissue and processed for histological staining. The bones underwent a procedure with fixation in ethanol with increasing concentrations (40%–100%), two times defatting with Roticlear® and finally embedding in polymethylmethacrylate (PMMA) resin containing a softening agent and stepwise enlarging concentrations of an accelerator.

After polymerisation the bone samples were sliced perpendicular to the long axis by diamond cutting. Subsequently the specimens had been grinded and polished up to a final thickness of about 50 µm and stained with Masson′s trichrome, Ladewig, and van Gieson staining for examination in conventional light microscopy. Using a Zeiss Axioplan 2 microscope we evaluated the bone formation in a region including the drill whole and a rim of adjacent host bone. Because of lacking form stability this region might vary in extension. The fate of the implant itself was examined in light microscopy and Environmental Scanning Electron Microscopy (ESEM) delivering more detailed informations of the implant and its interface with surrounding bone tissue. In contrast to conventional transmission electron microscopy the ESEM technique allows the visualization of specimens without the necessity of any contrasting or staining procedures under only slight vacuum thus preserving the original structures and minimizing preparation artifacts at the interface of materials with different stiffness and water content. Animal testing was approved by the regional council of the federal state (approval nb. 43.2-420502/2-2-294).

3 Results

All surgical incisions healed uneventfully without infection or wound dehiscence. Generally two different patterns of integrating the nanocrystalline hydroxyapatite into the host bone could be found in our study: In the major part of animals the implants were barely resorbed at all survival times. They remained in the implantation site as a compact mass which became quickly included into connective tissue and had only sparse contact to the surrounding bone (Fig. 1). We found only slight disintegration at the periphery of these implants. Some animals had no visible decay of the HA within the study time. While the bulk implant material has only few contact points to the surrounding bone some smaller particles detached from the primary implant showed a very intense contact to bone. With increasing implantation time the hydroxyapatite became denser and changed its stainability especially in the implant periphery (Fig. 2). Only in a minor part of the animals we could observe a more extended implant decay reaching inner parts of the specimens.

Two weeks after surgical procedure an intensive formation of small fibrous bone trabeculae could be observed in nearly all animals. The surface of the new formed bone was covered by osteoid and mineralization started in the centre of the trabeculae. A direct contact of bone with the implant surface was hardly observed. The major part of implant surface was covered by soft tissue and the Ostim® paste showed a condensation of the mineral structure in its

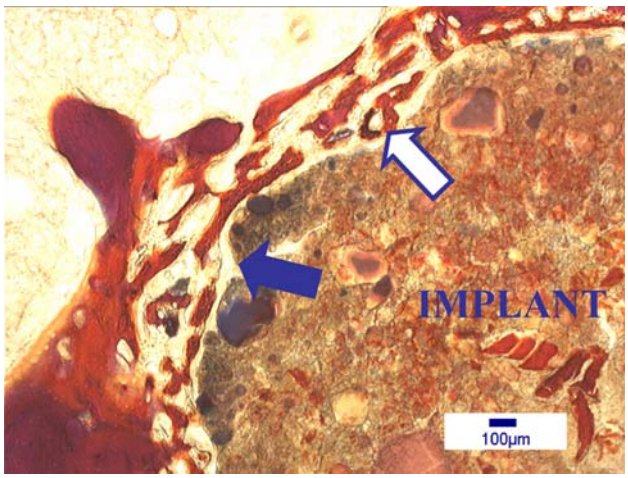

Fig. 1 Ostim® 2 weeks after implantation. The implant surface is covered by soft tissue (*filled arrow*) and woven bone (*hollow arrow*). Ladewig staining

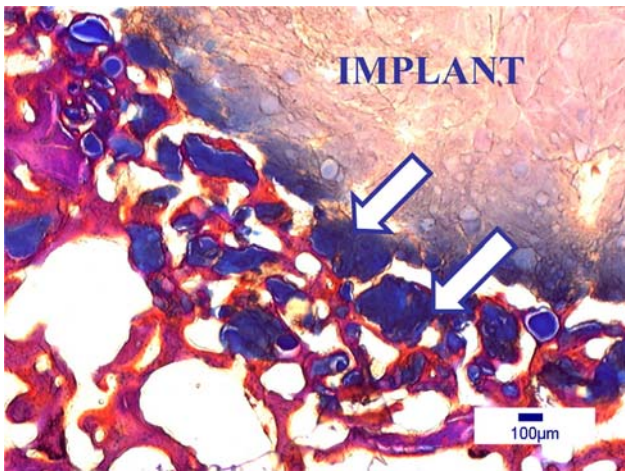

Fig. 2 Ostim® 4 weeks after implantation. *Arrows* indicate HA particles included in bone trabeculae. Ladewig staining

interior space. While the hydroxyapatite in the centre remained barely stainable we found an increased stainability at the periphery and in the regions with immediate contact to bone. The HA mineral became inhomogeneous and formed globular structures with obviously higher density (Fig. 1).

Four weeks after implantation bone trabeculae in the interface had been grown in length and diameter and contain well mineralized woven bone in the centre. Their surface was covered with osteoid and the new formed trabeculae had broad bonding sites to the surrounding cancellous bone stock. The disintegration of the implant showed only slight progress. In the majority of animals the HA remained nearly unchanged in the centre and was enclosed in a layer of fibrous connective tissue containing only few cells. An extended inflammation or

multinucleated giant cells could not be found. In a minor part of the animals implants showed a decay at the rim: numerous small, but no more nanocrystalline HA particles disintegrated from the periphery of the bulk material and had been included into the newly formed bone trabeculae. The included HA particles were stained intensely and had a compact and dense appearance. In some cases the Ostim® implant was washed out from the site of implantation and a diminishing concentration of HA was found in the proximal femur diaphysis. The scattered material appeared like the tail of a comet. In some of these animals we made an unexpected observation: in the light microscopy we saw deep zones of bone resorption, either from the endosteal surface or in the interior space of the cortical bone (Fig. 3). The induction of this pathologic bone resorption started as early as 4 weeks and could be observed up to the end of the study after 12 weeks. To proof the connection between the scattered HA particles and the pathologic bone resorption we studied these regions using the technique of Environmental Scanning Electron Microscopy. Already in the light microscopy we had remarked that the polymer resin used for embedding contains tiny bubbles in the marrow cavity near the resorption zones. These bubbles indicated a disturbance of the polymerization process. Indeed the electron microscopy revealed tiny HA particles inside the resorption lacunae far away from the site of implantation (Fig. 4).

By means of EDX-microanalysis we could indubitably proof that the mineral content of the HA particles is similar to implanted Ostim® (Fig. 5).

Six and eight weeks after implantation the resorption zones had substantially enlarged and in some animals the cortical bone was destroyed over more than half of the diameter (Fig. 6). The resorption areas were surrounded and stabilized by a strong periosteal callus. Twelve weeks after implantation we did not remark further enlargement of the resorption zones but they were still visible in nearly the half of all animals. Interestingly we did not find any clinically evident fracture so that we conclude a strong stimulation of callus formation parallel to the bone resorption process.

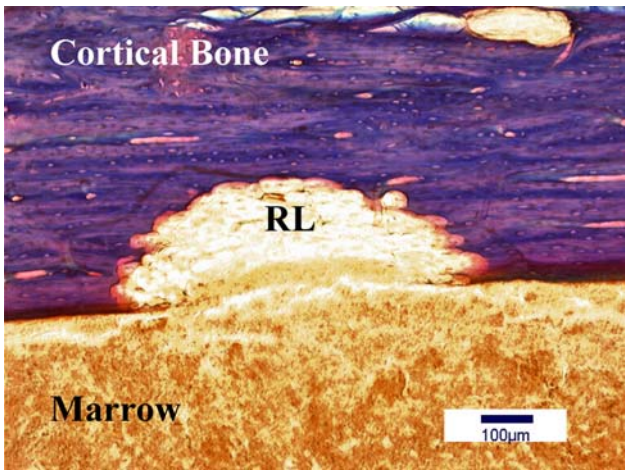

Fig. 3 Ostim® 8 weeks after implantation. Resorption lacuna (RL) at the endosteal surface. Ladewig staining

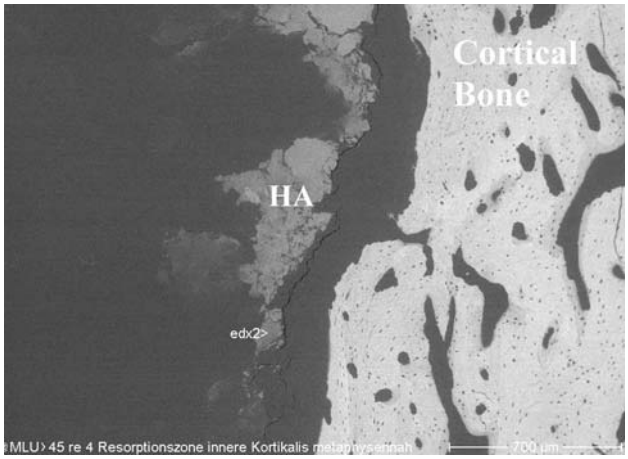

Fig. 4 Ostim® 8 weeks after implantation. Resorption lacuna at the endosteal surface. ESEM shows the HA particles forming a "tail of comet" and the adjacent resorption zone (HA)

The picture of the implant has not changed very much in the study group 6 weeks after implantation: in most animals the implant remained compact and nearly unchanged in its outer shape. The interface was dominated by fibrous connective tissue with only rare contacts to bone. Only in a minor part of the animals we could observe disintegration at the periphery with appearance of smaller particles surrounding the bulk implant like satellites. The disintegrated particles were found totally included in newly formed trabecular bone, appeared denser in comparison with the bulk implant and showed an intense stainability. The bone tissue in the interface still shows the irregular structure of woven bone.

Eight weeks following operation we could not observe any convincing progress: most animals had nearly no signs of implant resorption. As two weeks earlier the major circumference of the Ostim® was covered by connective tissue and direct contacts with bone we could hardly found. In some cases small particles had been detached from the surface and showed an intense stainability. In contrast to the bulk material these small particles found to be totally included in bone trabeculae which were in contact with the primary bone stock. The newly formed bone trabeculae in the drill hole became thicker and appeared more compact. Interestingly they are still consisted of immature woven bone.

Only in a small number of animals the Ostim® paste showed decay from the periphery up to deeper regions or the implant centre (Fig. 7).

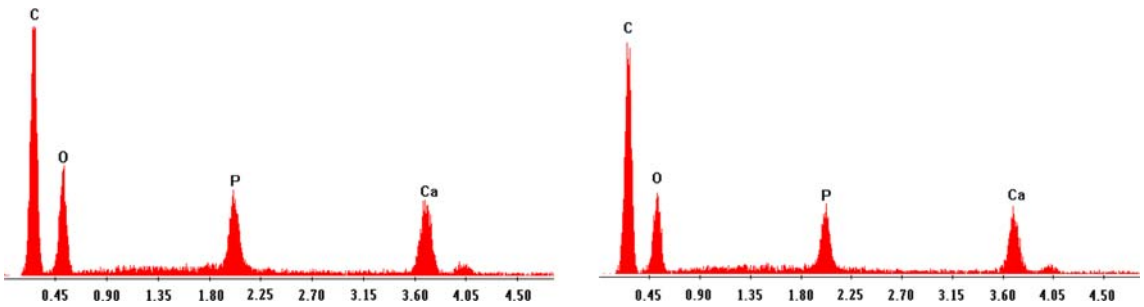

Fig. 5 Ostim® 8 weeks after implantation. Identical EDX-Analysis of the compact Ostim® implant at the defect (*left*) and Ostim® particles scattered over the marrow cavity (*right* diagram, measured at the marker "edx2" in Fig. 4)

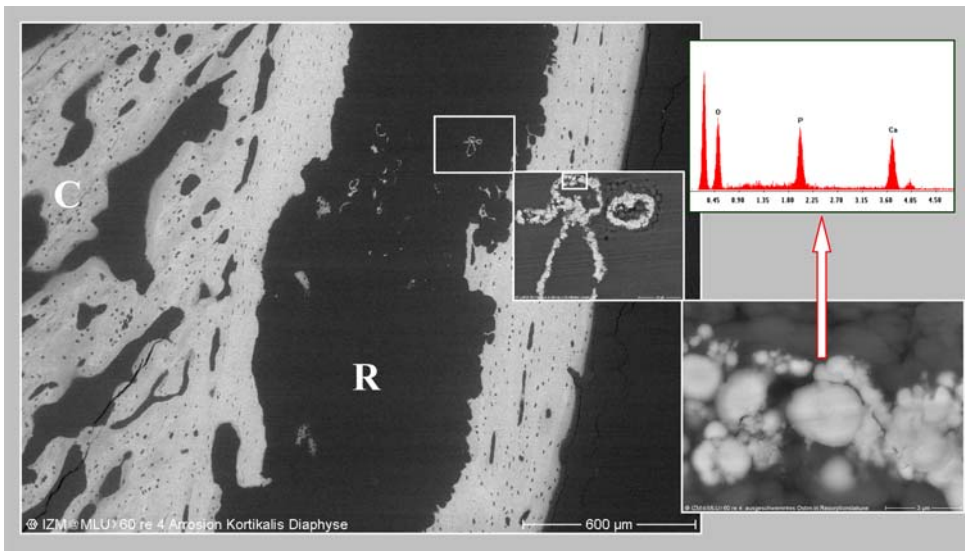

Fig. 6 ESEM analysis of Ostim® 6 weeks after implantation. Broad intracortical resorption zone (R) covered by periosteal callus (C). Inside the resorption channel we could detect agglomerates of HA-particles (smaller pictures). Small pictures show scattered Ostim® in the resorption defect with enlarging magnification. EDX analysis revealed the same mineral content of the scattered particles and the Ostim® bulk implant (small diagram)

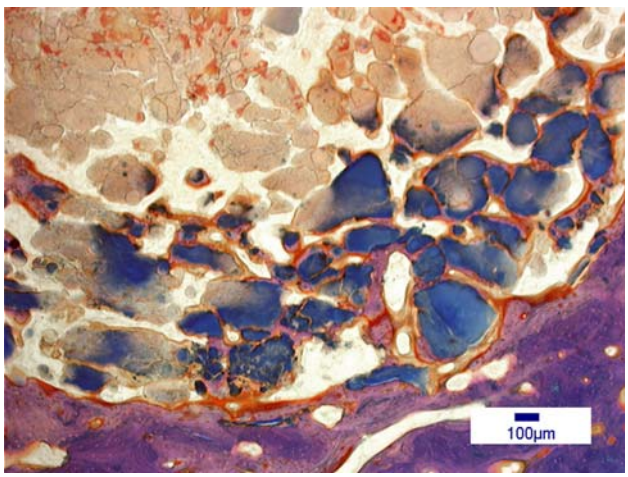

Fig. 7 Ostim® 8 weeks after implantation. The implant decay reached deeper zones of the implant, incorporation of disintegrated HA particles into bone can be found only at the periphery. Ladewig staining

The van Gieson staining showed no extended inflammatory reaction in the connective tissue surrounding the implant. It contains collagen fibres but only a very low amount of monocytic cells. Extended foreign body reaction with giant cells could not be observed.

At the last survival time 12 weeks after implantation the majority of animals still showed an only marginal detachment of small implant particles but no remarkable resorption or disintegration in the centre. The detached HA material appeared dense, compact and showed an intense stainability. HA was included in bone trabeculae of varying thickness. Now the trabeculae consisted of matured lamellar bone at least in part. The detached particles showed an interior structure with dense agglomerates similar to the bulk implant. In this vast majority of animals we found the implant nearly intact in the centre. A detachment of small particles was found only at the periphery–a picture similar to previous survival times. The bulk implant showed a smooth boundary and was covered by a thick connective tissue membrane containing lots of collagen fibres and only few monocytic cells. Extended inflammatory reaction or foreign body reactions could not be observed. The fluorescence labelling of the growing bone revealed that bone formation lasted only in the marrow cavity apart from the contact area but ceased in the interface with the Ostim® implant (Fig. 8).

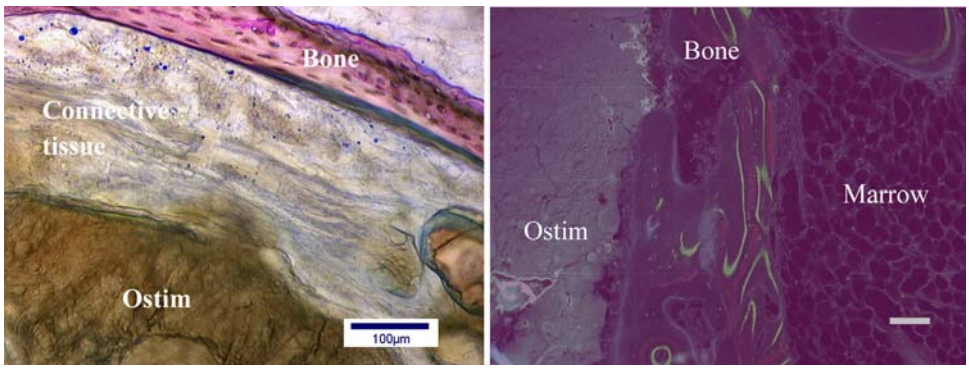

Fig. 8 *Left* The major part of Ostim® bulk implant is covered by fibre-rich connective tissue without direct bone contact, 12 weeks after implantation. Van Gieson staining. *Right* HA implant with contact to bone tissue. No bone modelling is evident at the interface while an intense remodelling takes place at the counterface. Six weeks after implantation, fluorescent labelling (calcein green: *green label*, alizarine complexon: *red label*). The bar is equivalent to 100 µm. (Color figure online)

A more extended disintegration of the implant we found only in a small number of the animals. The pattern was similar to former findings with the formation of numerous small particles which were included in thin bone trabeculae. The cancellous bone had only few contact points to the implant surface leaving gaps between the trabeculae filled with connective tissue. Mostly the decay did not reach the centre: the central part of implants remained compact and showed no signs of disintegration. Figure 9 delivers an overview of typical histological disintegration patterns at the end of our study 12 weeks after implantation:

For semiquantitative classification we divided the implant decay into five groups depending on the degree of disintegration:

grade 0: Unchanged implant without observable decay
grade 1: Only marginal decay at the surface with formation of satellite like smaller particles

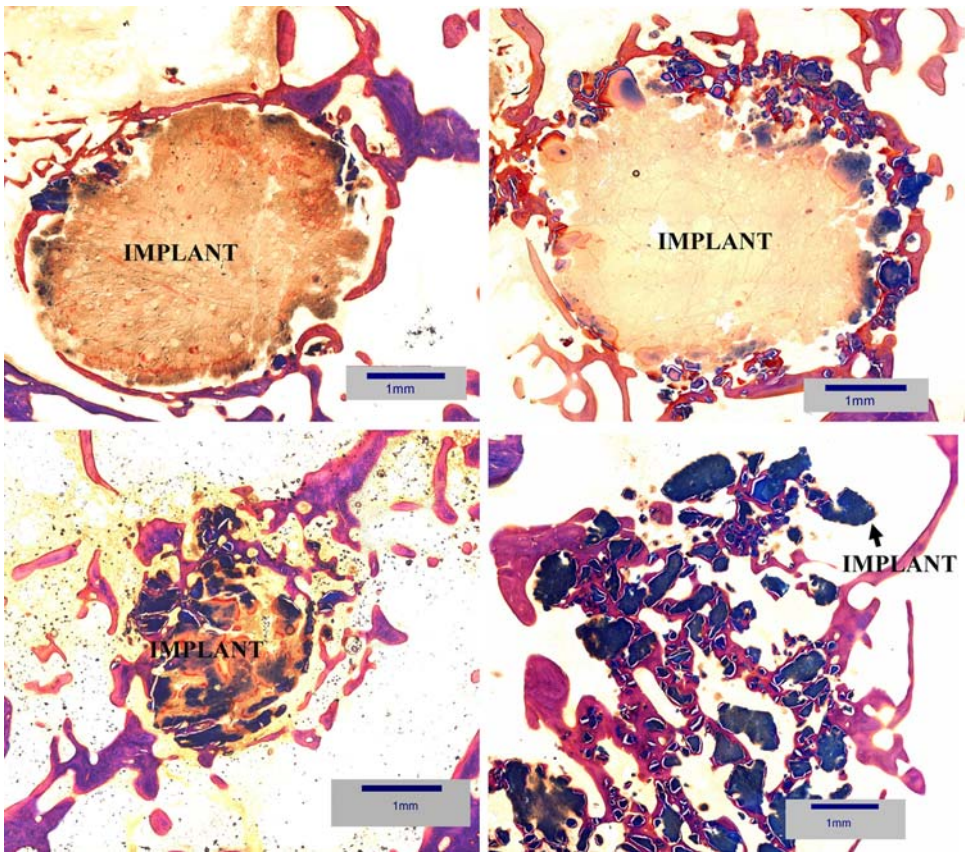

Fig. 9 Typical fate of Ostim® 12 weeks after surgery: *upper left* no observable decay of the implant and sparse bone contact; *upper right* only marginal decay with satellite-like particles, *lower left* decay to the centre of the implant, *lower right* decay to the implant centre and remarkable resorption. Ladewig staining

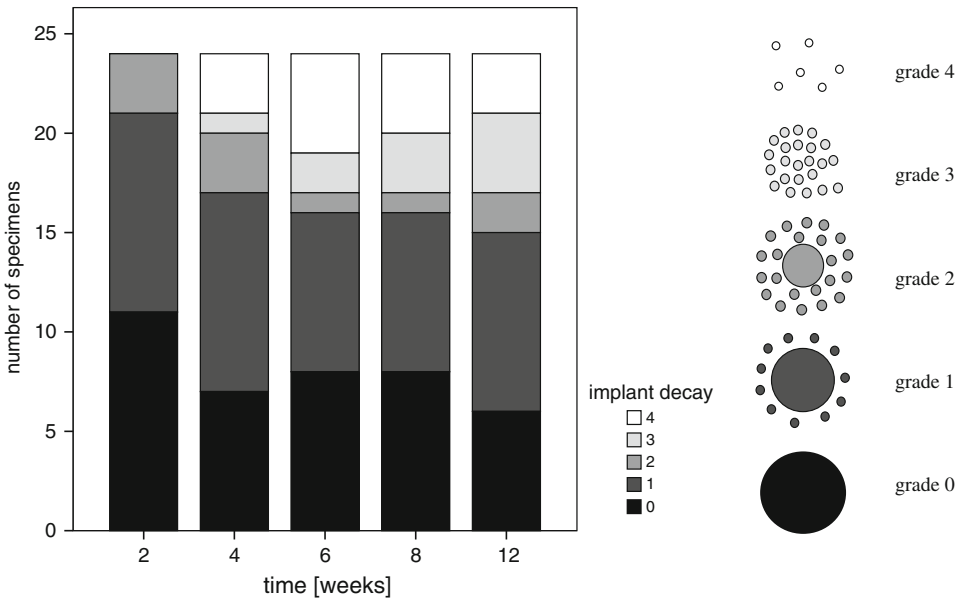

Fig. 10 Semiquantitative evaluation of implant decay during the study. *Left* Number of implants and the level of their decay at different survival times. *Right* Schematic illustration of degradation scaled with grade 0 to grade 4

grade 2: Decay down to 50% of the implant diameter
grade 3: Decay down to the centre of the implant
grade 4: Decay down to the centre and remarkable resorption

The summary of the above mentioned decay grades in Fig. 10 clearly illustrates, that we could generally observe no or only peripheral implant decay in the vast majority of the animals at any survival time. The diagram reveals that the implant decay nearly ceased beyond the fourth week after surgical procedure. We did not observe any complete resorption of the Ostim® material in our study.

To evaluate the possible influence of autogenous or allogenous bone grafts on the bone formation in the defect zone around the implant we performed a quantitative histomorphometric analysis using the image processing software KS 300 (Zeiss, Germany). By means of a computer-driven scanning table we gained a complete image of the whole implant at magnifications high enough for detailed separation of different tissue types. The newly formed bone was measured in an automated procedure and according to common morphometric parameters the relation of bone volume and total volume was computed (BV/TV). For statistical analysis we performed Mann–Whitney's *u* test using the statistical software SPSS 11.0.

Comparing the different implant combinations with or without bone we could not find any continuous influence on the osteogenesis in the drill hole by autogenous or allogenous grafts. Although differences between single animal groups occurred at distinct survival times we could not find a superiority of supplementation with bone graft. Figure 11 summarizes the histomorphometric analysis of bone formation in the drill hole after implantation of Ostim® alone or in combination with autogenous or allogenous bone.

ESEM investigations revealed a higher density of detached HA-particles included in peripheral trabeculae compared with the bulk implant material. These particles had an even higher density than the adjacent mineralized bone matrix. Smaller particles are fully included in bone trabeculae and show a direct, intimate contact to bone without interposition of soft tissue both in light and electron microscopy (Fig. 12). Major parts of these trabeculae had been remodelled into lamellar structure after 12 weeks but in the near surrounding of the hydroxyapatite still remained islands of woven bone. Only a minor part of the bulk implant circumference has immediate contact to bone in the electron microscopy and even these trabeculae with pseudopodial shaped processes are often separated by a small layer of fibrous tissue.

4 Discussion

HA is one of the mostly used bone substitute materials for reconstructive surgery of the skeleton. The chemical composition resembles to bone mineral thus providing an excellent biocompatibility and osteoconductive properties of the material. A lot of animal studies and the clinical use for many years have proven its direct incorporation into the bone [7] and even a physicochemical bounding [17]. HA implants will be integrated into bone very quickly and form an interface of very good mechanical stability with the surrounding bone. Unfortunately HA ceramics remain without further degradation once included in bone. Former investigations have shown that compact HA implants as well as granular ones [23] may enhance bone formation.

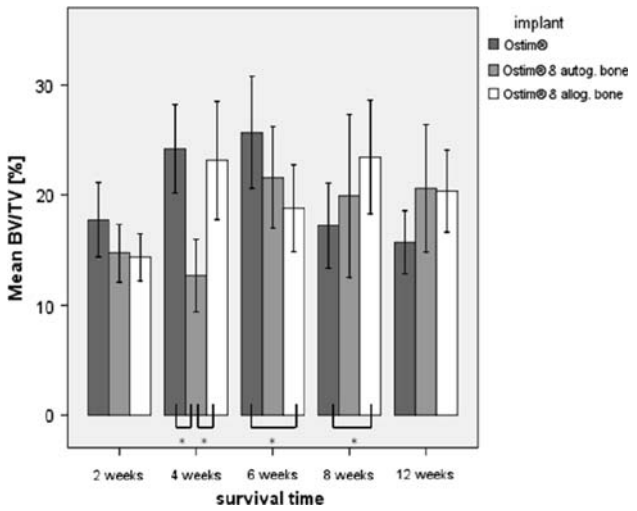

Fig. 11 Survey of the histomorphometric results of bone formation in the defect at all survival periods. The bars show the bone volume/total volume ratio after implantation of Ostim® alone or in combination with autogenous or allogenous bone. For each implant material we measured eight specimens at each survival time. *Asterisks* mark significant differences between several treatment groups but a systematic influence of bone grafting could not be proven

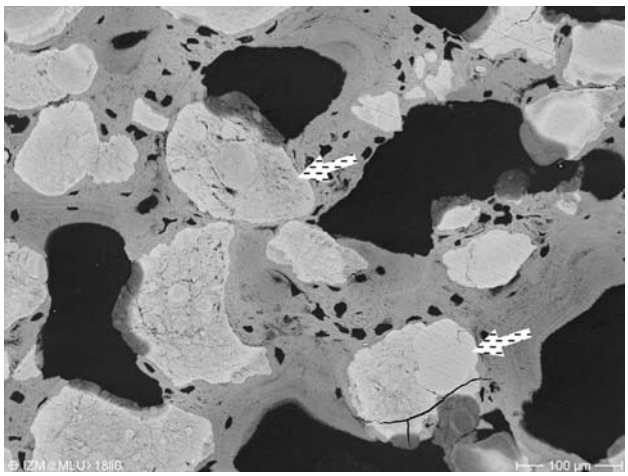

Fig. 12 Ostim® 6 weeks after implantation. Small particles of HA fully incorporated in trabecular bone (*arrows*). They show a higher density in ESEM (*lighter grey*)

The HA surface is often covered by bone without interposition of connective tissue but the material itself undergoes no further resorption if the bone healing once is finished. In contrast to HA materials an ideal bone substitute should be resorbable and disappear without any remnants after the formation of new bone. The permanent presence of stiff and brittle material may weaken the skeleton against mechanical load.

Synthetic materials mimicking the characteristic calcium/phosphate ratio of human bone hydroxyapatite are supposed to enhance bone formation in a defect [6, 20, 28]. The nanoparticular hydroxyapatite suspension Ostim® with an adequate calcium/phosphate ratio of 1.675, a small crystal size and therefore a large specific surface in the primary suspension was expected to be osteoconductive and resorbable. First experimental results of Grigorian et al. [11] with the filling of jaw defects in dogs seemed to meet these expectations with reports on good bone ingrowth.

Ostim® has been reported to enhance bone formation and to be completely resorbable after implantation in pig skulls by Thorwarth et al. [40]. The authors implanted the HA paste into critical size defects in the skull of domestic pigs either alone or in combination with 25% autogenic bone. They evaluated the implantation site by microradiography, light microscopy and histomorphometry from 3 days to 6 months postoperatively. The authors reported on a disintegration of the HA paste as early as 3 days after implantation and a nearly complete regeneration of the defects with newly formed bone within 8 weeks accompanied by a complete resorption of the implanted material. A second experimental study with implantation of Ostim® support the findings of Thorwarth et al. [40] at least in part: Busenlechner et al. [2] implanted Ostim® and other bone substitutes under titan hemispheres in monocortical skull defects of minipigs and observed the formation of new bone in the defect. Twelve weeks after surgery the coefficient bone volume/total volume reached a median of 23.3% and highly varying results of ±18.3%. This value was considerably below conventional HA powders (Bio-Oss reached a BV/TV of 38.4 ±13.3%) but significantly higher than in hemispheres without any bone substitute. In contrast to Thorwarth et al. [40] the authors did not observe a complete resorption and describe in the space between the new bone trabeculae remnants of Ostim® associated with giant cells.

Our study with implantation of Ostim® in New Zealand white rabbits confirms the latter. We could not found any case with complete resorption of the implant material up to 12 weeks after surgery. The hydroxyapatite was implanted either alone or in combination with autogenous or allogenous bone into the metaphyseal region of the distal femora. The vast majority of implants underwent only peripheral disintegration of the implanted HA paste with formation of numerous small rounded particles surrounding the bulk implant in a satellite-like manner. These particles showed an intense stainability and became included in trabecular bone within 4 weeks after implantation but once included they underwent no further resorption. The major part of the Ostim® paste remained as a compact implant mass in the defect without any remarkable resorption and mostly without disintegration in the central parts. The bulk implants were covered with connective tissue containing a lot of collagen fibres but only few cells. Fluorescence labelling could prove that bone formation in the interface

ceased 8 weeks after implantation while it continues apart from the implant in the host bone at the rim of the defect.

According to our results Spies et al. [36] found a comparable biocompatibility and osteoconductivity resulting in a good osteointegration following implantation of Ostim® into the proximal tibia of the Goettinger Minipig. Unless using the same animal species as Thorwarth et al. [40, 41] they reported on different results concerning the resorption behaviour: 6 weeks after implantation the resorption ceased and no further degradation of implants could be observed. Huber et al. [15] implanted Ostim® into a defect in the proximal radius of New Zealand white rabbits and bridged the incision with a three-hole mini plate. The authors report on well structured cortical and trabecular bone tissue among the implant material in the qualitative histological evaluation. In contrast to Thorwarth et al. [40, 41] they describe fragmented round shaped Ostim® remnants of different size in the marrow spaces of the newly formed bone 60 days after surgery. The authors could not find a complete resorption of the material and the histological pictures in the paper show connective tissue in the surrounding of the implant. Also Rothamel et al. [29] observed a fate of Ostim® similar to our results. They used the paste to fill extraction sockets of dogs immediately after tooth extraction. Three month after surgery they describe no or minor resorption of the material and a gap between the implant and the alveolar wall. In some cases Ostim® was disintegrated and in line with our results smaller particles had been included in bone and showed a higher stainability. Carmagnola et al. [4] describe a similar outlasting of non degraded Ostim® particles in the trabecular bone filling the defect zone around titanium implants in tibiae of New Zealand White Rabbits. The histological evaluation showed a decay of the HA with formation of numerous small and polygonally shaped particles that are integrated into trabecular bone at least in part. Chris Arts et al. [5] implanted a mixture of morselised cancellous bone (MCB) and 33% Ostim® into zylindrical defects of the distal femora of New Zealand White Rabbits in a procedure very similar to our study. They could find no statistically significant difference to the implantation of MCB alone. In their histological findings the authors describe great amounts of remnants and some large areas of non-osseous integrated Ostim®. These results seem to be very similar to our own observations concerning the fate of the hydroxyapatite after implantation.

With respect to the influence of adding bone grafts to Ostim® our experiments support the results of Thorwarth et al. [40, 41]: histomorphometric analysis could not show any benefit of autogenous or allogenous grafts on the bone formation in the defect. Despite of some differences in the test groups at single survival times we could not prove a continuous enlargement of bone formation lasting over the whole implantation time. At the end of our study there was no significant difference in bone formation between the animal groups with or without bone grafts. According to the work of Thorwarth et al. [40, 41] we found a good biocompatibility of the HA with the absence of extended inflammatory reactions or osteonecrosis. Foreign body giant cells were not observed, osteoclast-like giant cells could be seen only in direct contact with bone but not with the HA.

Our results support former findings of Müller-Mai et al. [22] after implantation of a nanocrystalline HA into the distal femora of rabbits. Their HA particles with a dimension of about 50×10 nm pressed into cylinders for implantation. The authors described a histological picture very similar to our observations: a minor part of the implant surface had direct bone contact and a major part was covered by soft tissue. Degradation took place only at the periphery and a substantial resorption of the material was not reported. Interestingly the authors observed that HA resorption takes places only in areas contacting the soft tissue but not in contact areas with bone. Smaller particles of the implant were found loosely scattered in the surrounding tissue. An obvious change of stainability was found in surface regions especially when contacting soft tissue. Similar to our findings they described globular particles <1 μm and structures with larger diameters near the surface of the nanocrystalline HA which they interpreted as aggregates of several or many particles. Investigating madreporic hydroxyapatite with larger grain size (1.5–2.8 and 2.8–5.6 mm) in the same animal model Müller-Mai et al. [23] found an extend of bone formation much higher than in the nanocrystalline ceramic. The bone trabeculae could grow into the pores between powder grains forming ramificated trabecular networks. The first clinical applications of high dispersed Hydroxyapatite had been made in maxillofacial surgery [1, 25]. Zuev et al. [46] filled jaw defects in 395 patients either with demineralised bone matrix or with Ostim®. The rate of complications was higher in the bone matrix group (3.6%) than in the Ostim® group (1.5%). In case of periodontal abscesses they found a benefit of the HA suspension.

More recent clinical findings of Ostim® application in the maxillofacial surgery correspond with our experimental results: Smeets et al. [33] report on the use of Ostim® in the upper jaw bone of a 60 years old patient to enhance the fixation for two dental implants. Histological findings of one implant failed 3 years after surgery revealed a slight bone marrow fibrosis and a still lasting presence of extended areas with immature woven bone. The material had not been resorbed and was only partially enclosed in bone. Major parts of the HA paste were covered by fibrous connective tissue. Gerlach et al. [10] filled jaw defects caused by jaw cysts and tumors with Ostim® and

postulated bone healing from conventional X-ray findings only and did not confirm their evaluation by histological investigation or tomography procedures. Huber et al. [14] used Ostim® to fill traumatic voids in combination with common osteosynthesis. During the removal of the fracture fixation devices they took cylindrical bone biopsies from the site of implantation 3–15 months following the surgery. They concluded from their histological findings an intimate contact between bone tissue and Ostim® unless they present histological pictures with HA particles enclosed in fibrous tissue. In accordance with other studies the authors describe focal fibrosis of the medullary space with amorphic HA in all samples

Ostim® is designed for filling unloaded bone defects by injection. As an aqueous paste it is neither stable in form nor load resistant. Therefore all above mentioned animal experiments used unloaded models only. The differences of loaded and unloaded implants must be taken into account for interpretation of animal test results. The influence of implant loading on the process of osseointegration is controversially discussed in the literature and depends on numerous factors as implant design, structure and porosity of the surface, range of micromotion and others. First investigations with loaded porous implants by Cameron et al. [3] and Ducheyne et al. [8] in the 1970s revealed that movement in the interface due to mechanical stress prevents bone ingrowth and leads to formation of fibrous tissue. Later Soballe et al. [34, 35] substantiated in sophisticated experiments that micromotions of 500–150 μm lead to formation of fibrous tissue around the implant. But Cameron et al. [3] assumed that not all movements would be deleterious and introduced the concept of a threshold micromovement. Nearly 20 years later Pilliar et al. [26, 27] concretized that micromotions of up to 50 μm do not disturb bone formation and the threshold level was estimated near 100 μm [37]

The studies of Wehrbein and Diedrich [42] and Ohmae et al. [24] in dogs revealed a more extensive bone remodeling in loaded implants compared with unloaded implants. The authors suggested that the high rate of remodeling during the osseointegration of loaded implants may result in a bone in the interface never reaching the final level of mineralization. The incompletely mineralized tissue has possibly a higher capacity for crack accumulation thus preventing microdamage [16, 19, 37]. But a higher bone turnover may influence the resorption of biomaterials unless there are no experimental results on this topic so far. Contemporary results of Serra et al. [30] show significantly better load resistance and higher stiffness of host bone in the surrounding of unloaded implants compared to loaded implants in a rabbit model. Slaets et al. [32] observed a significantly higher bone volume in unloaded compared with loaded rabbit tibiae after 6 weeks using a bicortically anchored screw model. Only the bone forming surface was found to be higher in the loaded group in the endosteal region at 4 weeks and in the periosteal region at 6 weeks. These findings support the viewpoints of Wehrbein and Diedrich [42] of a prolonged or lasting process of remodelling but they do not indicate any benefit of loading for osseointegration.

5 Conclusions

HA has proven its capability to serve as a proper biomaterial with excellent biocompatibility and osteoconductive properties [17]. The similarity of its chemical composition to the natural bone apatite is believed to be the reason for its very good biocompatibility and a direct, chemical bonding of bone tissue forming a biological and mechanical stable interface. But unless a certain influence of the manufacturing process HA is barely resorbable and remains without further changes once included in bone. The progress of scientific research and clinical experiences of the last 15 years suggest that the use of powders with small grain size could overcome the disadvantages of solid implants and lead to resorbable biomaterials for temporary substitution of skeletal defects. Since the advantage in material technology allows the manufacture of nanocrystalline HA the small dimensions of such crystals were expected to realize this aim. But in accordance with the results of many other authors we could find only a partial resorption of the nanocrystalline HA in a rabbit model. The fate of the implant was characterized by a peripheral and only partial decay of the material with a remaining central core that was included in connective tissue and has only few contact points to bone. More or less extended amounts of small HA agglomerates separated from the bulk implant and could be found in the near surrounding showing a satellite-like pattern. These small particles were mostly well included in bone and had a higher stainability. A minor part of the material was washed out and migrated into the femoral diaphysis forming a “tail of a comet” containing minute HA-particles. In some cases these particles formed very small aggregates and caused severe destructions of the cortical bone from the 4th week on. Unless no clinical thighbone fracture happened we found extended osteolysis with simultaneous endosteal and periosteal callus formation. Electron microscopy could prove the presence of HA nanocrystals in the resorption cavities. Further investigations should be made to clear up this non physiologic bone resorption. That such resorption was not described in former studies may be caused by their large distance from the site of primary implantation.

Implanting a mixture of Ostim® with allogenous or autogenous bone did not change the principal findings and

histomorphometric evaluation could not show an enlargement of bone formation in the defect. The results of other experimental and clinical studies mentioned above support our results. Besides the study of Thorwarth et al. [40, 41] all other authors reported on non resorbed remnants of nanocrystalline HA.

Summarizing our findings and the observations reported in the literature the nanocrystalline HA material Ostim® has a good biocompatibility and does not provoke any inflammatory reaction. But it is poorly resorbable and in the majority of cases Ostim® implants were included into connective or bone tissue remaining there without further resorption. They form agglomerates with an extremely low porosity becoming more and more densified with increasing implantation time. Once included in bone the material does not undergo further resorption. The lacking porosity may be a reason for poor resorption of the nanocrystalline hydroxyapatite material. Further studies should clear up this densification.

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