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# Formation of nano hydroxyapatite – a straightforward way to bioactivate bone implant surfaces

*Dedicated to Professor Dr. Hartmut Worch on the occasion of his 65<sup>th</sup> birthday*

Bioactivity has been a field of biomaterials research for more than 25 years. However, clinical application of bioactive implants is progressing rather slowly and is still limited to a few types of bone implants. The increased understanding of the molecular mechanisms underlying bioactivity has opened up new attractive opportunities to render conventional implant materials such as metals, ceramics and even polymers bioactive. In particular, biomimetic nano hydroxyapatite coatings and in-situ mineralising surfaces induced by incorporation of mineralisation seeds are applicable to a wide variety of implant types and materials. They have the potential to set new standards for the improved performance of orthopaedic implants whilst complying with the economic constraints on healthcare.

**Keywords:** Bioactivity; Nano hydroxyapatite; Bone implant; Mineralisation seeds

The term bioactivity describes the ability of a material to induce a specific biological activity. In the case of most bone implant materials a highly desirable aspect of bioactivity is the active integration of an implant by the surrounding bone tissue without the encapsulation by a soft tissue membrane. Bioactivity, therefore, is desirable in all implant materials designated for long-term function in a bony site. While bioactivity is a prerequisite for direct bone bonding and is generally regarded a promising tool to improve long-term implant function, it is not entirely sufficient – implantation conditions, implant design, and vitality factors at the implantation site need to be appropriate too, in order to exploit the full potential of a bioactive implant.

The term bioactivity was coined more than 25 years ago by Osborn [1, 2] in order to describe the difference in bone tissue reaction towards calcium phosphate based bioceramics or bioglass compared to the broadly used polymeric, metal and high-performance ceramic (alumina) implants, which are also regarded as biocompatible, but do not bond to bone directly (and therefore are not bioactive). In the mean time, much scientific effort has been expended to understand the molecular basis behind bioactivity [3–5] and on that basis to find methods to confer bioactivity to

the classical – non bioactive – implant materials that represent the overwhelming majority of orthopaedic bone implants and will certainly continue to do so in the foreseeable future – the situation for dental implants is different in many aspects. The reasons for the rather slow progression of bioactive implants towards clinical application in orthopaedic surgery are quite numerous. The most important factor is that simple biocompatible, polymeric bone cement and titanium- and cobalt-based alloys do quite well clinically, thus making it hard to prove in well-controlled clinical studies that bioactive implants would really bring a significant benefit to patients. At the same time, at least the early, bioactivated – hydroxyapatite (HA)-coated – metal implants (Fig. 1a and b) could not keep the promises of marketing people when HA-layers delaminated from the metal surfaces [6, 7]. In addition, bioactivity is more expensive. Even a simple HA-coating on a hip stem increases the number of manufacturing steps and adds to the costs. In times of cost containment in health systems all over the world and in the face of the shortage of proven clinical evidence, orthopaedic companies hesitate to invest heavily in the development, manufacturing, and clinical introduction and promotion of bioactive implants. Bioactivity of bone implants, therefore, has to be either very simple and cheap or their clinical effectiveness must be provable in straightforward trials so that they can find their way to daily clinical practice and to the market place. A combination of both would of course be ideal.

On the other hand, orthopaedic clinicians are faced with changing requirements. The number of risk patients is increasing, as is the expectation of the individual patients. To give just a few obvious examples: the population is getting older accompanied by a growing incidence of osteoporosis and revision arthroplasties with less bone left to fix implants appropriately. Patients wanting to stay more active pose higher demands on the performance of implants and especially the implant–bone interface. Arthritis patients request earlier joint replacement, since the dramatic health risks of long-term pain treatment by NSAID (Non-steroidal anti-inflammatory drugs) have been well documented [8] – consequently those patients will face more joint revisions if the durability of implants does not improve. New treatment concepts are entering the hospitals such as vertebroplasty, minimally invasive joint replacement and tissue

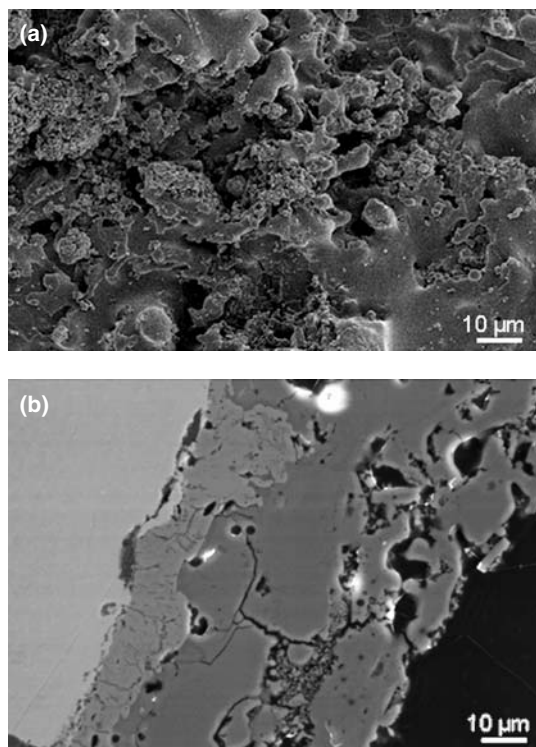


Fig. 1. (a) Electron micrograph of commercial HA-plasma-spray coating on orthopaedic implant. (b) Cross-section of a plasma spray hydroxyapatite coating (right, dark grey) on a Ti6Al4V-specimen (left, light grey). A layer of TiO<sub>x</sub> (10 μm thick; centre, grey) was used to improve the adhesion of calcium phosphate. This image was kindly provided by Prof. Götze, TU Bergakademie Freiberg.

engineering concepts. In this environment, bioactivity will adopt a key role in the improvement of the existing implant materials beyond the present state of the art in order to be able to cope with the growing demand for increased performance and increased implant lifetime.

### How does bioactivity work?

Undoubtedly, well-manufactured calcium phosphate bioceramics, calcium phosphate bone cements and bioglasses are bioactive, as are the different calcium phosphate coatings on metal implants [9–13]. Nevertheless, bioactivity is not limited to calcium phosphates or bioglasses. Gaillard [14] reported on the bioactivity or osteoconductivity of a bone-bonding polymer and Kawai et al. [15–17] showed that a chemically modified polyamide showed bioactivity. What do these materials have in common with calcium phosphates? In both cases, the polymeric materials mineralise once they are incubated in simulated body fluid (SBF). Is this step of mineralisation indicative of bioactivity? Yes. Indeed, the ability of a material to mineralise in SBF is so indicative of its biological activity that it is used as the definition for bioactivity [18]. Mineralisation itself, however, is not the determining factor for bioactivity. While all spontaneously (in SBF) mineralising materials seem to show bioactivity in vivo as well, the work of Prof. Kessler's group (München) [19, 20] with RGD(Arg-Gly-Asp)-coated PMMA-based materials has shown that a polymeric surface that definitely does not significantly mineralise in SBF also shows high bone bonding capacity, while the non modified material shows the typical non bioactive behaviour in histo-

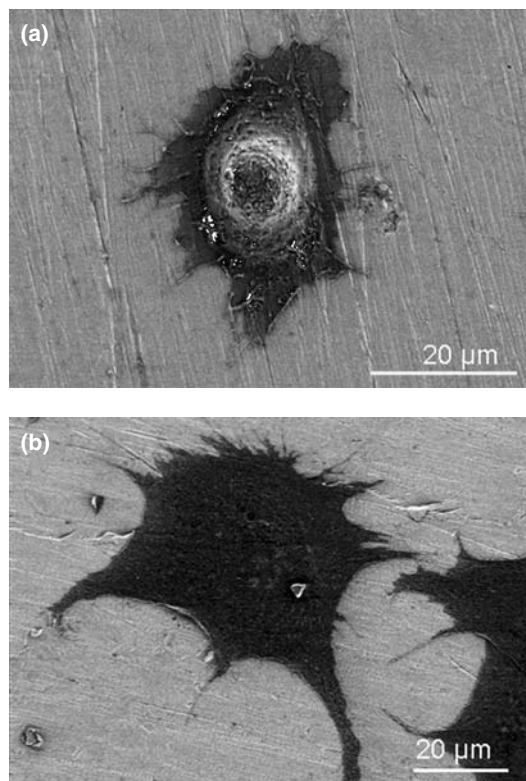


Fig. 2. SEM-micrographs of MC3T3-cells 1 hour after seeding on (a) a titanium surface and (b) a titanium surface modified with RGD.

logical evaluation. The results of Kessler demonstrate that the physiological adhesion or attachment of bone cells to a materials surface determines its bioactivity. The same holds true for titanium surfaces (Fig. 2a and b). With immobilised RGD peptides primary human osteoblasts adhere very effectively and spread on the metal surface [21]. Jansen et al. [22] investigated the influence of RGD-coated porous titanium fibre mesh implants on bone formation in the cranium of rabbits and found a significant increase in bone formation for the RGD-loaded titanium implants compared to the uncoated implants after 4 and 8 weeks.

A direct signalling via adhesion molecules even seems to be more effective than a nano-HA coating (Fig. 3) [21]. It is interesting to notice that a combination of nano-HA and immobilised RGD peptide does not have an extra effect. This observation supports the conclusion that bioactivity ad-

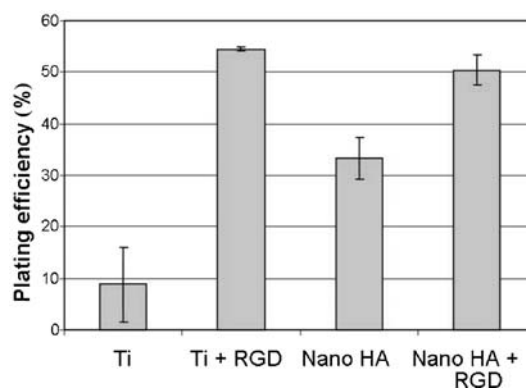


Fig. 3. Plating efficiency of MC3T3-cells after 1 h seeding on titanium, on titanium modified with RGD, on electrochemically deposited nano-HA and on electrochemically deposited nano-HA modified with RGD.

dresses one and the same specific site on the cell surface regardless whether it is based on HA or an adhesion peptide [21]. Once the osteoblasts (or pre-osteoblasts) are attached, they are able to deposit new bone matrix on the implant surface and generate the appropriate environment for further bone growth.

**Is this all one and the same basic mechanism?**

On an RGD-coated implant, bone cells adhere directly to the immobilised RGD-peptides on a material’s surface via their  $\alpha_v$ ,  $\beta_{III}$ -integrins. On a mineralising bioactive surface bone cells will probably bind by the same mechanism. In a first step – after implantation – nano-sized calcium phosphate (nano-HA) precipitates on the surface and adsorbs proteins from the surrounding body fluid, especially those involved in bone metabolism such as osteocalcin and/or osteopontin. This view is in no contradiction with the fact that all implanted materials are exposed to high concentrations of dissolved proteins, which bind almost immediately and unspecifically to the surfaces. For bioactive surfaces especially this unspecific protein binding seems to be reversible and in a dynamic equilibrium and thus these proteins are replaced by biominerals, which again are recognised by the specific binding sites of some bone proteins.

Some of these bone proteins also contain RGD-sequences, which then act as adhesion molecules for bone cells [3, 23, 24]. Nano-HA therefore can act as the binding site for bone proteins, which then are detected by the bone cell integrins and confer adhesion of bone cells to the nano-HA surface. The higher the protein binding nano-HA surface area, the more signal molecules can be absorbed and the stronger the signal for cell adhesion will be. For a bioactive implant coating, it is therefore important that the specific surface area of a synthetically produced HA reaches a value in the order of magnitude of natural bone minerals so that it presents a similar signal strength to bone cells as natural bone does.

**How to achieve nano-HA surfaces on bone implant materials**

**1. High temperature HA coating**

High temperature calcium phosphate deposition on metal implants (especially HA plasma spray) is an old and still the most popular way to render metals like titanium and its alloys, cobalt chromium alloys, or stainless steel bioactive [9, 25–27]. For many years, there has been controversy in the scientific community on the details of appropriate HA coating. The question of whether an HA coating should be thick (50–100  $\mu\text{m}$ ) or thin (<50  $\mu\text{m}$ ) or whether it should be more or less pure-phase HA or partially amorphous and – connected with this question – whether it should be more or less resistant to dissolution after implantation, has been a particular topic of debate and concern. For reasons less related to the degree of bioactivity there is some consensus on this topic today. As an HA coating is quite a poor material for load transmission, a rather thin coating (less than the roughness of the underlying metal structure) is preferred [11, 28, 29]. The solubility of the HA coating is of bigger importance for its bioactivity. The plasma sprayed surface is a rather dense structure on the microscopic scale with a

**Principal of HA coatings**

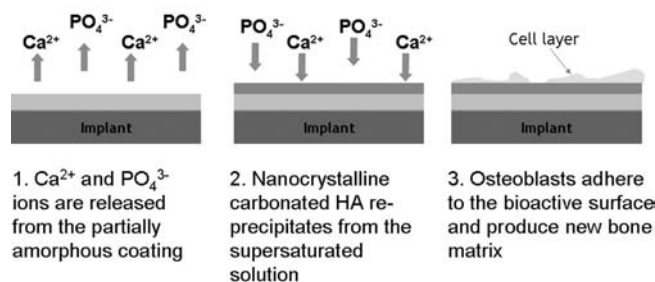


Fig. 4. Sketch showing the principle of dissolution–precipitation behaviour of hydroxyapatite coatings.

small specific surface area and has no similarity with the structure of bone minerals (Fig. 1a). After implantation some of the deposited calcium phosphate (especially the amorphous phases) dissolves in body fluid and – provided the conditions at the implantation site are favourable – re-precipitates as nano sized HA [30] (Fig. 4). In this sense, the HA plasma spray coating is not the bioactive surface in itself, but provides the ionic species of calcium and phosphates and the mineralisation seeds on the undissolved HA remnants on the metal surface for an in-situ mineralisation process resulting in nano-HA formation. Only this surface can then adsorb sufficient amounts of bone proteins to provide a strong signal for bone cell adhesion [31, 32].

From this consideration, it is therefore logical that a good plasma spray HA coating needs to have a soluble fraction to provide calcium and phosphate, but also some non or less soluble parts that can act as mineralisation seeds in order to direct the nano-HA precipitation towards the implant surface. This process of dissolution and precipitation is, however, undefined and largely beyond the control of the clinician. The effectiveness of the remaining HA to act as mineralisation seeds for nano-HA precipitation is especially questionable.

**2. Nano-HA coating from solution**

A more promising way towards increased bioactivity is the direct coating of implant surfaces with nano-HA.

In the case of metal surfaces, some methods have been described and have made the first steps towards clinical application [11, 33–38]. They have in common that they take place at ambient temperature (or 37 °C) and in aqueous solution. The main differences are in the preceding steps of implant preparation in order to generate mineralisation seeds on the metal surface, composition of the coating solution, and the application of electrochemical assistance in order to accelerate the coating procedure. The result in all cases is a more or less nano-crystalline and a more or less substituted HA coating on the metal surface (Fig. 5a and b). Such nano-HA coatings clearly have the advantage of a defined coating composition at the time of implantation, with a nano-structure already quite close to bone minerals. Given their high specific surface area, they should be able to adsorb sufficient amounts of bone proteins and trigger osteoblast adhesion. From this aspect the coating with the smaller crystal size and by direct correlation the higher surface should be the more effective or bioactive, although a

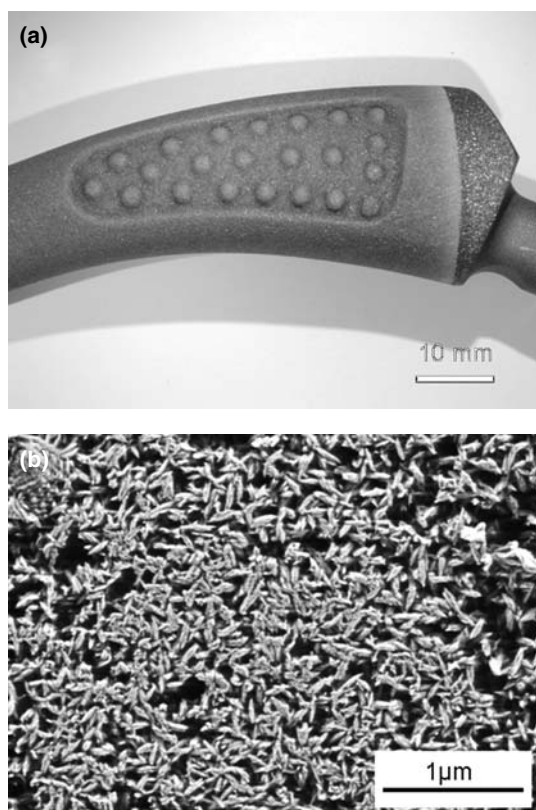


Fig. 5. (a) Micrograph of a titanium implant coated with electrochemically deposited hydroxyapatite at 37°C (Cenos BoneMaster). (b) SEM-picture of a titanium implant surface coated with electrochemically deposited hydroxyapatite at 37°C.

direct comparison by histological or histomorphometric evaluation or even clinical studies is not available. Histological and clinical data, however, seem to support the superiority of nano-HA coatings in general over HA plasma spray coatings and uncoated implants [39].

Nano-HA coatings are typically very thin ( $\leq 5 \mu\text{m}$ ) compared to HA plasma spray coatings. A potential disadvantage may be the low resistance against dissolution after implantation before bone cells have a chance to adhere to the surface. On the other hand HA is the most stable calcium phosphate under implantation conditions and the dissolution tests and considerations derived from partially amorphous HA plasma spray coatings do not apply in the same way for nano-HA coatings. Nanosized hydroxyapatite should thus be stable under implantation conditions and like bone itself these materials should only be resorbed by bone cells, or if in case of an inflammation at the implantation site, a decline in pH leads to subsequent (bone) mineral dissolution.

Technically and economically nano-HA coating compares favourably with HA plasma spray in that simple incubation tanks and, if applicable, electrical power supplies require less investment and the involved chemicals are cheap and abundantly available. Nano-HA coating is therefore also within reach for smaller companies that cannot raise the significantly higher investments for plasma spray equipment. A huge advantage is the fact that these solution coatings are applicable to complex implant structures, while plasma spray is restricted to line of sight coating.

Major disadvantages are the long coating cycles of hours and days – especially for those techniques that rely on un-

supported biomineral precipitation – combined with the fact that implants need to be incubated in aqueous solution at ambient temperatures, which implies a latent risk of microbial contamination. Great care and precautions have to be taken to handle this risk.

For metal implants, nano-HA coating will certainly become the method of choice for bioactivation. This view also includes coatings of different chemical composition such as, for example, brushite, which is converted to nano-HA after implantation. The speed at which one or more of the available methods will replace HA plasma spray coating and spread to the field of uncoated implants is as yet unpredictable. A big leap in this direction will follow more conclusive clinical data and more technical progress in coating procedures from equipment manufacturers, so that the step from the lab to the pilot plant scale can be taken more easily.

### 3. Bioactivation by mineralisation seeds

As mentioned before, bioactivation via nano-HA formation is in principle also applicable to polymeric materials. As in the case of metals, nano-HA formation on a polymer surface just requires the formation of mineralisation seeds and, at least locally, a supersaturation of calcium and phosphate ions with respect to HA. The size and shape of the HA nano-crystals can be influenced by the addition of different inorganic ions (e.g.  $\text{Mg}^{2+}$ ) [38], small organic molecules (e.g. citrate) [40] or biopolymers (e.g. chondroitin sulphate) and proteins [41–43]. Effective mineralisation seeds are especially anionic moieties such as carboxyl, sulphate, phosphate, and silicate groups on the polymer surface [16, 44, 45]. While such effects and results have been described in the scientific literature, no clinical application for solid, pre-shaped bioactivated synthetic polymers as bone implants is yet to be seen on the horizon. For the most frequently used polymeric bone implant material – acrylic bone cement – the above-described methods for bioactivation do not apply, since the final surfaces of this implant material will only form *in vivo* after implantation, when the cement sets.

Attempts towards bioactivation of acrylic bone cements are focused so far on the addition of bioactive filler materials like bioactive glass and hydroxyapatite [46–50]. Although effective/bioactive at very high filler content, the material properties change drastically and the resulting products are significantly different from conventional acrylic bone cements and are typically not used for implant fixation.

More recently, Miyazaki et al. (2003) [51] described an acrylic bone cement modified by the addition of a siloxan containing methacrylic monomer and  $\text{CaCl}_2$ . Samples of this bone cement mineralised in SBF and the material can thus be considered bioactive. However, in this case, the necessary amount of additives was too high to regard the resulting material as a good candidate for the development of bioactive bone cement, since the mechanical properties were significantly impaired at the effective concentration of additives. The data show, however, that there are interesting alternative ways for the bioactivation of acrylic bone cements. The continued interest of several research groups to render acrylic bone cement bioactive reflects the high clinical demand for bioactivation, especially for this pro-

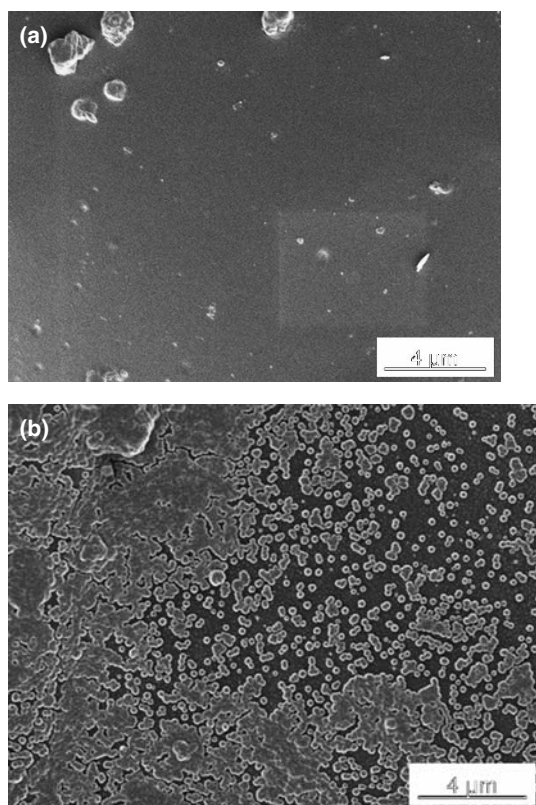


Fig. 6. SEM-micrographs of PMMA-surfaces after immersion in SBF for 24 h; (a) PMMA and (b) PMMA modified with mineralisation seeds.

duct class. Because bone cement is the material of choice in many indications where bone quality is particularly poor, it would profit considerably – and even more so than metal implant materials – from an effective bioactivation principle. Material development efforts of the author’s group are therefore focused on modifications of acrylic bone cements, which are effective in generating mineralisation seeds on the cement surface, while having no detrimental effect on the mechanical and handling properties of the cement. First experimental approaches have been focused on the addition of anionic polymers and monomers to polymeric bone cements and those materials were effectively mineralised in SBF (Fig. 6a and b). The concentration of additives could be kept at concentrations that had no apparent detrimental effect on cement handling and mechanics. Further experiments are in progress to analyse the complex interactions of cement composition and properties both *in vitro* and *in vivo*.

Bioactivation with nano-HA coating – regardless of the material employed – always has some critical medical, technical and practical aspects, some of which have been addressed above. Just to name a few (partially they also apply to HA plasma spray):

- All types of HA coatings (but especially the very thin coatings) suffer from dissolution when the implant is placed (or has to be placed) into an environment where an inflammatory process is still ongoing. Inflammatory processes, however, accompany to a more or less pronounced extent, any big intervention, as the implantation of a joint implant definitely is. The reduced pH resulting from inflammation (as low as 5.0–5.5) may then lead to an increased dissolution of the HA coating or at least prevent new HA precipitation.

- The technical issues of development, manufacturing, and regulatory approval of new bioactive coatings are by no means prohibitive, but still put a severe financial burden on bioactive coatings, especially for implants that are under price pressure already, in particular hip and knee joints.
- Bioactive coatings are susceptible to damage before and during implantation. Even touching a nano-HA coating will leave fingerprints behind that cannot be removed, since such a coated implant cannot be cleansed anymore. Nano-HA-coated implants are therefore much more sensitive to handling than conventional ones.

### Is there a solution to these issues?

There should be one, as the experiences with acrylic bone cements [51 and unpublished data from the authors’ group] suggest. It is hypothesised that nano-HA coating itself is (at least in many cases) not necessary in order to render a bone implant surface bioactive. The generation of an appropriate amount and quality of mineralisation seeds on the material’s surface ought to be sufficient. Like simulated body fluid, real body fluid is over-saturated with regard to HA, the respective ions, however, are stabilised in solution in order to prevent uncontrolled mineral precipitation in the body. Mineralisation seeds on an implant material with a higher affinity to calcium and phosphate ions than the stabilising molecules in solution will also trigger implant surface mineralisation also in the body environment. Bioactivation of implant surfaces could, therefore, restrict itself to the generation of mineralisation seeds; the formation of nano-HA would then occur *in situ* by the processes involved in bone mineralisation.

This concept would ease the development and manufacturing of bioactive implant surfaces significantly, as there will be no lengthy *in-vitro* mineralisation process involving the downstream issues (risk of microbial contamination, clean room handling, high QC-efforts). Costs would be reduced accordingly. Even the clinical performance could profit from a higher reliability, since the mineralisation seeds should be stable moieties on the implant surface and are not destroyed in an inflammatory environment as HA layers might be. In the latter case, mineralisation would

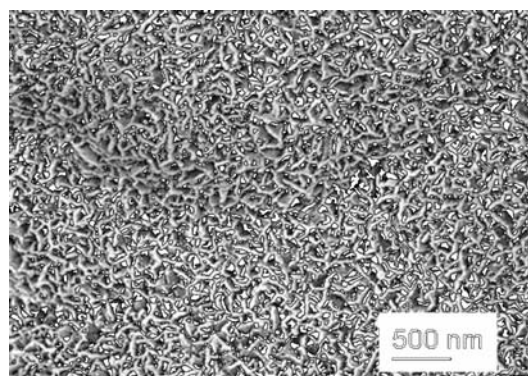


Fig. 7. SEM-micrograph of nano-HA on a NaOH-treated Ti6Al4V surface. Nano-HA was formed after immersion in SBF (Tas-SBF: Tris-buffered synthetic body fluid of 27 mM  $\text{HCO}_3^-$  concentration) for 1 week [54].

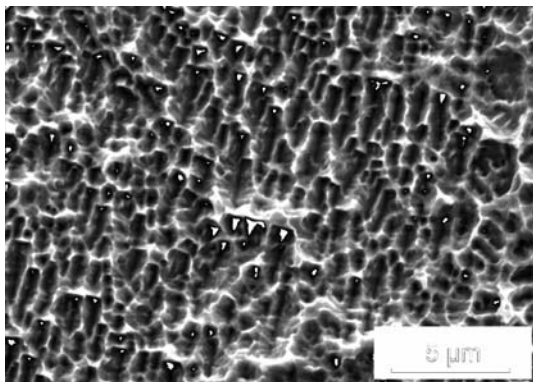


Fig. 8. SEM-micrograph of a chemically modified titanium implant surface (Osseotite<sup>®</sup>). The surface was produced by removing the native oxide layer from titanium followed by an etch-process. The resulting surface shows a substantially uniform micromorphology.

start from the mineralisation seeds as soon as the inflammatory process has come to an end and the surrounding pH-value has reached the normal level.

In the case of metal implants, Kokubo [52, 53] proposed this concept some time ago. Figure 7 shows a chemically modified titanium surface on which nano-HA has formed after immersion in Tas-SBF (27 mM HCO<sub>3</sub><sup>-</sup>) [54]. In the area of dental implants a surface treatment based on a comparable bioactivation concept has been introduced successfully into clinical practice (Osseotite<sup>®</sup>) (Fig. 8). For other metals of interest, polymers and non calcium phosphate ceramics, it will require some further development effort. Preliminary data, however, suggest the feasibility of generating inexpensive bioactivation concepts with applicability to the vast majority of bone implants.

The authors gratefully acknowledge the support of

Prof. Dr. A. Cuneyt Tas  
Department of Biomedical Engineering  
Yeditepe University, Istanbul  
for providing the SEM photograph depicted in Fig. 7 and stimulating discussions on calcium phosphate mineralisation

Prof. Dr. Jens Götze and Mrs. M. Hengst  
TU Bergakademie Freiberg  
for providing the SEM photograph depicted in Fig. 1b

Dr. A. Sewing  
R&D Biomaterials  
Biomet Deutschland GmbH Berlin  
for providing photographs and technical and clinical information on the Bonemaster<sup>®</sup> nano-HA coating

The authors are especially grateful to Prof. Dr. Hartmut Worch, TU Dresden, for many years of close cooperation in basic and applied research projects and his pioneering concepts of linking materials science with biology, which continue to be a guideline for our present work.

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(Received February 12, 2007; accepted April 27, 2007)

**Bibliography**

DOI 10.3139/146.101510  
 Int. J. Mat. Res. (formerly Z. Metallkd.)  
 98 (2007) 7; page 630–636  
 © Carl Hanser Verlag GmbH & Co. KG  
 ISSN 1862-5282

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