

Osseointegration of loaded dental implant with KrF laser hydroxylapatite films on Ti6Al4V alloy by minipigs

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Abstract. This study was performed with the objective of evaluating osseointegration of titanium alloy Ti6Al4V dental implants coated with hydroxylapatite (HA) deposited by a KrF laser. For this a KrF excimer laser and stainless-steel deposition chamber were used. The thickness of the HA films was approximately $1 \mu\text{m}$. In this investigation experimental animals minipigs were used; the implants were placed vertically into the lower jaw. After 14 weeks of unloaded osseointegration, metal-ceramic crowns were inserted and, at the same time, fluorescent solution was injected into the experimental animals. Six months after insertion of crowns the animals were sacrificed. The vertical position of the implants was checked by a radiograph. Microscopic sections were cut and ground, and the sections were examined under polarized and fluorescent light using a microscope with a charge coupled device camera. The six month long osseointegration in the lower jaw has confirmed the presence of newly formed bone around all the implants. In the experimental group, which had a laser-deposited coating, the layer of fibrous connective tissue was seen only randomly. In the control group (titanium implant without a cover) the fibrous connective tissue between the implant and the newly formed bone was observed more frequently, but this difference was not significant. © 2001 Society of Photo-Optical Instrumentation Engineers. [DOI: 10.1117/1.1357191]

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1 Introduction

Osseointegration of an implant is a basic requirement for its successful functioning in a host organism. One of the main conditions of good osseointegration is the material of the implant or that of its surface. The commercially used materials for implants are metals. Unfortunately, metals and metallic alloys, mechanically highly resistant, necessarily bring about problems of corrosion.¹ From the literature it is known that the toxicity of vanadium and its combination with aluminum (both contained in the Ti6Al4V alloy) may be connected with various neurological disorders.^{2,3} Ceramic materials are chemically inert and fragile, but some of them can be dissolved.⁴ These problems were overcome by using a metal substrate coated with a bioceramic material, in particular, hydroxylapatite (HA), since calcium hydroxylapatite, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, is one of the main inorganic chemical constituents of bones.

The samples in this study were coated by a pulsed laser deposition (PLD) method, which allows one to modify properties of the coating by changing the deposition conditions.^{5,6} For evaluation of the degree of osseointegration of the implant, polarized and fluorescent light microscopy with computer image processing was applied. That allowed us to determine the percentage of direct bone contact with the real dental shape implant under loaded conditions. The study was per-

formed with the objective of evaluating osseointegration of titanium alloy Ti6Al4V dental implants coated with hydroxylapatite deposited by a KrF laser.

2 Materials and Methods

2.1 Preparation of Implants: Deposition Conditions

For deposition of hydroxylapatite films on titanium substrates a special stainless-steel chamber was used, and all films were formed under the same deposition conditions.⁷ Before deposition, the commercially available cylindrical dental implants, 12 mm long and 3.3 mm in diameter (Figure 1), made from Ti6Al4V alloy (with a sand-blasted surface) were cleaned in acetone, toluene and in ethanol in an ultrasonic bath. A KrF excimer laser (Lambda Physik LPX 200, Goettingen, Germany, wavelength $\lambda=248 \text{ nm}$ and repetition rate 20 Hz) was used. Both, a HA target and an implant were placed into the stainless-steel deposition chamber. The laser beam was focused onto the sintered hydroxylapatite target at an angle of 45° and the target was rotated to keep the same ablation conditions. In the beginning the chamber was evacuated up to 10^{-4} mbar by a turbomolecular pump, and then was filled with an Ar–water mixture (Ar flow of 12 sccm, water vapor flow of 10 sccm). The substrate was preheated to 490°C using

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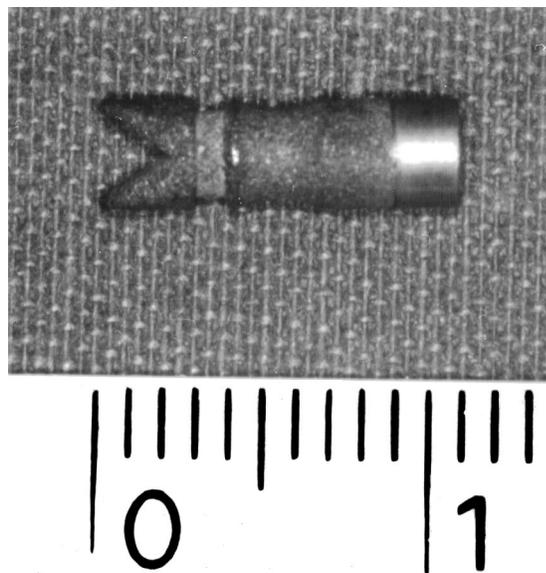


Fig. 1 Photo of the implant used (commercially available, used as a substrate under the coating).

a CO₂ laser; the deposition time was 15 min. The thickness of the hydroxylapatite layers created varied from 1.00 to 1.20 μm.

2.2 Biological Evaluation

The experimental set for osseointegration consisted of 20 specimens, cylindrical dental implants from Ti6Al4V alloy with hydroxylapatite coatings, which were formed by the pulsed laser deposition technique, and of four noncoated titanium alloy implants with the same shape as a control group.

In this investigation four minipigs, 2 years old, weighing on average 40 kg, were used, and 10 weeks before implantation their lower premolars were extracted.

After the healing process the implants were inserted using the following procedure: after inducing general anesthesia, Azaperon and Metronidat (Léčiva, Praha, Czech Republic), a local anesthesia, Mesocain, dose 4 mL per half jaw (Léčiva, Praha, Czech Republic) was applied. The minipigs were secured on the preparation table in a prone position with their mouths open. The soft tissues were incised on the crest of the premolar area of the lower jaw and the bone was denuded. The implant bed preparation was started using a round drill, followed by a pilot drill and finished with a full size drill (drilling machine Elcomed 100, W&H Dentalwerk, Burmoos, Austria). During preparation sterile saline solution was used to protect the bone from overheating. Similarly, the socket was rinsed with sterile saline solution to clean out bone detritus after which it was allowed to fill up with blood. The implant was plugged into the bone and the soft tissues were sutured in layers with plain catgut. The implants in each minipig were arranged in the following way: three coated implants were inserted into the left side of the lower jaw and two into the right side. The control implant was plugged as a middle sample on each right side to provide sufficient bone support, comparable mastication force distribution, and to be shielded by neighboring implants. Each experimental animal

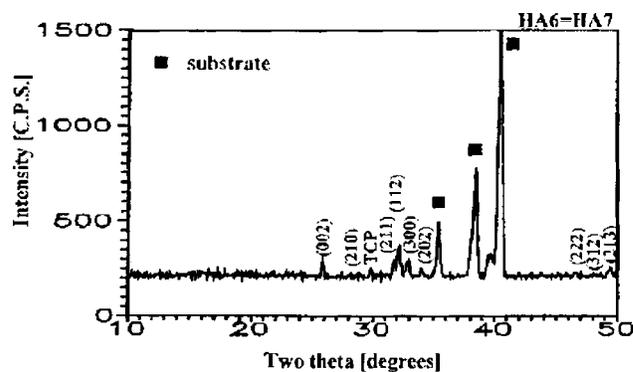


Fig. 2 XRD of an implant surface coat used in experiments.

had his own control. The implants were allowed to heal without loading for 16 weeks.

After the healing period, the implants were uncovered using general anesthesia and metal-ceramic crowns were inserted. The crowns were made from a chromium-cobalt alloy (Wiron, Bego, Germany) and a ceramic material, Vita Omega (VITA, Germany), following the manufacturer's directions. Ketac-cem Aplicap (ESPE, Germany) was used for cementing. At this stage the fluorescent solution Calcein DCAF was injected intramuscularly in a dose of 20 mg/kg to determine the state of the bone after the unloaded healing period.

After 6 months of loaded osseointegration the experimental animals were sacrificed and the blocks of bone with implants were soaked in Schaffer's solution (36% formaldehyde neutralized over CaCO₃+80% ethanol in a ratio 1:2-3) for fixing. This type of fixing solution does not leach a fluorescent label from tissues.⁸ The vertical position of the implants was checked radiographically (Trophy, Paris, France). The specimens were embedded into methylmethacrylate resin (Merck, Darmstadt, Germany), transversal microscopic sections were cut using a diamond saw blade, and then ground (both Buehler, Lake Bluff, IL) with water as a coolant to a thickness of approximately 100 μm. Five to seven sections were prepared from each implant, and the sections were examined under polarized and fluorescent light (Nikon Eclipse 600, Tokyo, Japan) with a charge coupled device (CCD) camera (Mitsubishi, Tokyo, Japan).

Computer software system (Sigma Scan and Sigma Scan Pro, Jandel, Erkrath, Germany) was used to analyze the osseointegration. Each section was viewed and the percent of contact length was calculated for each section and for the whole implant. The data obtained were compared with those of the control implant from the same animal. The significance of differences between the experimental and control groups was calculated by Student's *t* test at probability $P=0.05$.

3 Results

3.1 Mechanical and Physical

For the whole set of experiments similar x-ray diffraction (XRD) spectra of samples were observed (Figure 2), obviously due to the reproducibility of the deposition conditions.⁷ The spectra had peaks of HA, tetracalcium phosphate (TeCP)-Ca₄O(PO₄)₂, tricalcium phosphate (TCP)-Ca₃O(PO₄)₂ and peaks of CaO and TiO₂. The actual prefer-

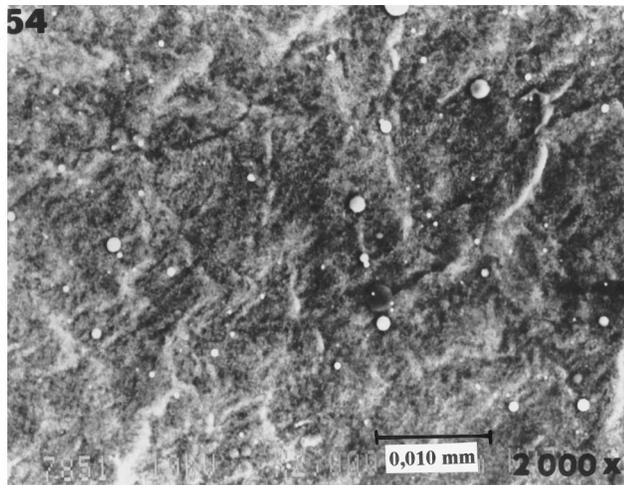


Fig. 3 SEM of implant surface used in experiments (magnification 400 \times , bar=0.025 mm).

ence of hydroxylapatite film orientations was controlled by the deposition conditions. The surface of the layers was very smooth (Figure 3), however, sometimes a few spherical smooth droplets (average diameter 0.002 mm) were always present. The formation of films was regular and was not influenced by the implant shape.

3.2 Histological Evaluation under Polarized and Fluorescent Light

Evaluations after 16 weeks of unloaded osseointegration and 6 months of loaded osseointegration in the lower jaw have confirmed the presence of newly formed bone around all the implants. Osteoclasts, macrophages or inflammatory reaction cells including phagocytes as well as regressive changes were not observed in any of the ground sections.

In the experimental group, with a laser-deposited coating, the layer of fibrous connective tissue occurred in about 22.5% of the implant body surface without making a continuous layer. Figure 4 shows visible firm contact between the bone and the implant surface with interposition of fibrous tissue (shown by the arrow). The same view under fluorescent light (Figure 5) shows a uniform distribution of the fluorescent label in the whole bone, probably as a result of a remodeling process of early formed bone. The released label can be distributed in the whole bone volume. These findings support the assumption that bone healing was already finished and at the time of sacrifice no new bone was formed. These results were supported by the observations made at higher magnifications (Figures 6 and 7); the yellow spots mark active bone cells.

In the control group (titanium implant without a cover), the fibrous connective tissue between the implant and the newly formed bone occupied 34.8% (Figure 8), i.e., more than in the experimental group which was 22.5%, especially in the middle portion of the implant. However, these differences were not significant (Student's t test with probability $P = 0.05$). The fluorescent label (Figure 9) was localized on the margin of the bone socket facing the implant and the adjacent periosteum (shown by arrows). This may suggest that at the time of sacrifice the bone had been still active.

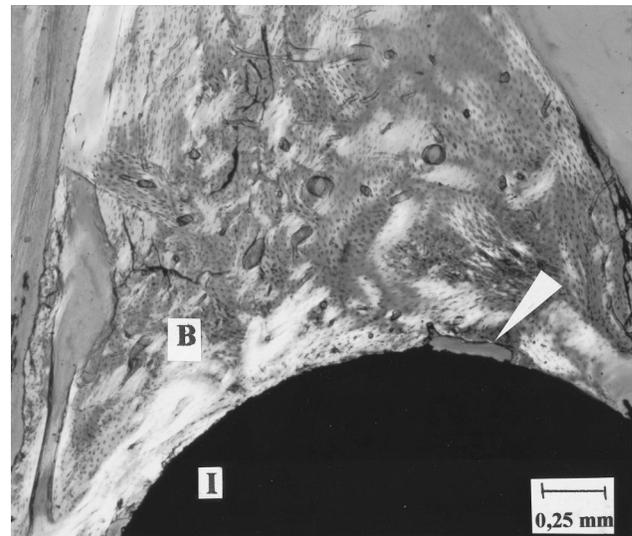


Fig. 4 Firm contact of bone to the HA coated implant surface with a small interposition (shown by the arrow) of fibrous tissue. B—bone, I—implant (polarized light, magnification 40 \times , bar=0.25 mm).

3.3 Quantitative Analysis of Percentage of Osseointegration

To simplify calculation of the percent of osseointegration, each section was divided into individual sectors. The length of osseointegrated and fibrointegrated surfaces was measured in each of these sectors, summarized for the whole implant and then calculated for the groups of experimental and control implants. Table 1 presents the contact circumferential lengths of osseointegrated and fibrointegrated surfaces of the samples and the control implants for each sector of the section.

The calculated area of the bone/implant interface varied from 65.2% (SE 13,5) for titanium implants to 77.5% (SE 10,2) for hydroxylapatite films. There was no significant difference (Student's t test with probability $P=0.05$) between

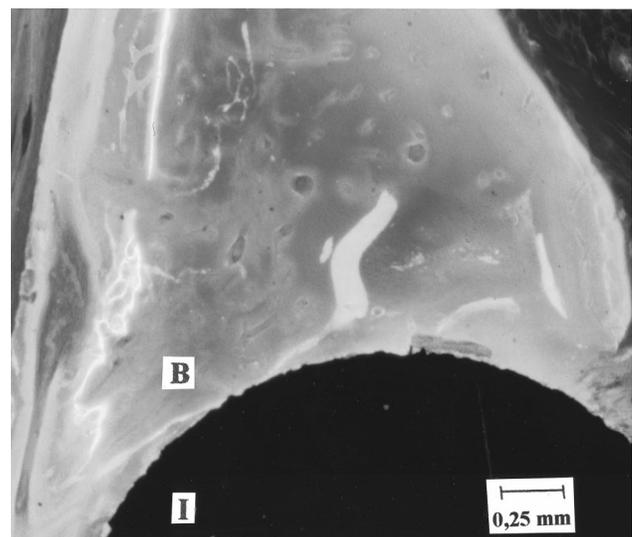


Fig. 5 Equitable distribution of a fluorescent label in bone around the HA coated implant. B—bone, I—implant (fluorescent light, magnification 40 \times , bar=0.25 mm).

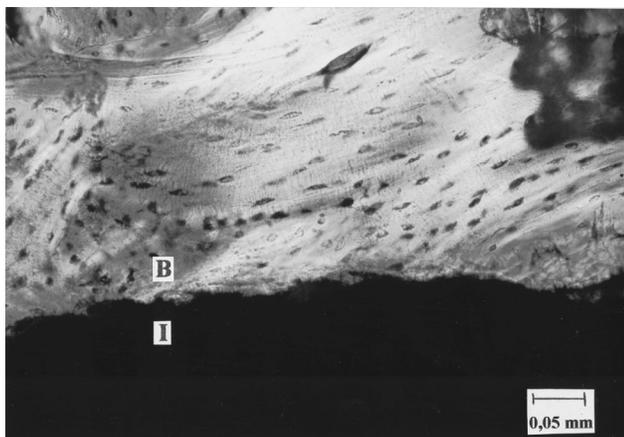


Fig. 6 Similar to Fig. 4. B—bone, I—implant (polarized light, magnification 200×, bar=0.05 mm).

the type of surface and therefore we believe that the osseointegration of all HA films and of the control titanium implants was similar.

4 Discussion

The prerequisite for successful osseointegration is sufficient width of the bone into which an implant is inserted, as well as a very precise implantation procedure.⁹ Inadequate implant methodology can be the cause of treatment failure. The reported implant failures do not signify that implants are a poor treatment option. In reality, current implant treatments are remarkably successful.

To identify the actual reason for failure several authors have tried to examine the failed dental implants. One of the most important factors causing failure is the implant itself (biomaterial failure), the second one is adherence of the remaining tissue to the implant. Lemons¹⁰ suggests that materials and biomechanical properties directly influence the tissue interface response.

For the implants with a bioceramic cover there is no strict need for tight contact with the surrounding bone, because the materials are osseoconductive and are able to attract the bone

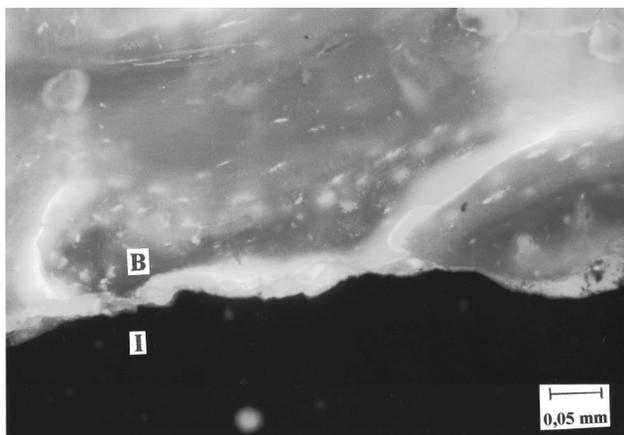


Fig. 7 Yellow spots marking active bone cells. B—bone, I—implant (fluorescent light, magnification 200×, bar=0.05 mm).

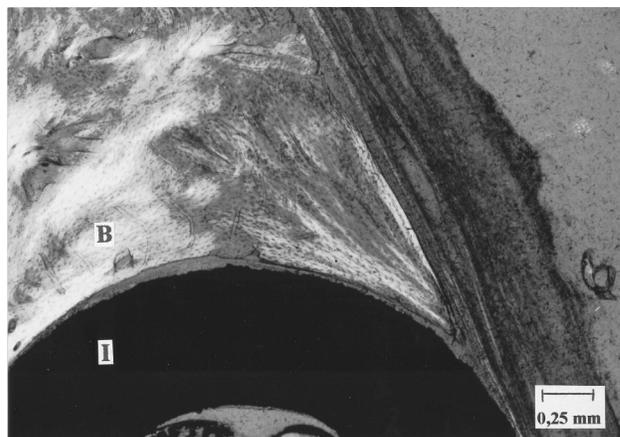


Fig. 8 Fibrous connective tissue between the control implant and the bone is more extensive. B—bone, I—implant (polarized light, magnification 40×, bar=0.25 mm).

to cross the space between the implant cover and bone bed.¹¹ Our experiment confirmed the literature data: the control implants had fibrous tissue, especially in the middle portion, which is thinner (diameter 2.8 mm) than the apex and the neck (diameter 3.3 mm) and therefore there was a primary gap between the implant and the bone socket. Such a geometry acts as an antipush-out device.

Fritz et al.¹² suggested that the bone is not fully mature and sufficiently stable up to six months of healing. Our results have shown a wholly healed bone around the coated implants at the end of the experiment. The fluorescent label was uniformly distributed in the bone around the experimental implants. In control samples the bone had still been active (after 4 months of healing and 6 months of loaded integration) at the site of fibrous interposition (the middle portion mentioned above), probably due to osseoconductive properties of the HA coating, which help to heal the lesion.

There is a hypothesis as to the necessity to protect the metal part against corrosion¹ and therefore permanently isolate the bone by covering the implant with a bioceramic layer. Some authors state that ions could be released from the tita-

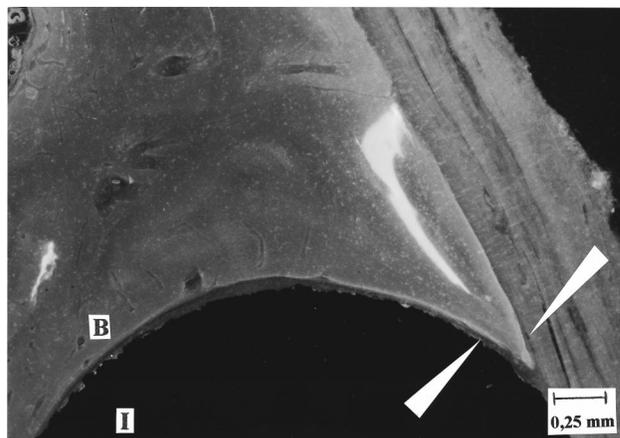


Fig. 9 The yellow label is localized in the bone margin between the periosteum and the implant (shown by arrows). B—bone, I—implant (fluorescent light, magnification 40×, bar=0.25 mm).

Table 1 Implant osseointegration.

Implant	Fibrous connective tissue (mm)		Bone (mm)		Periimplant sector area (mm)		Percent of bone integration	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Titanium	1.61	0.59	3.23	0.78	4.84	0.25	65.2%	13.5
Coated	1.29	0.62	4.15	0.62	5.44	0.13	77.5%	10.2

nium alloy thus causing some problems.^{1-3,13} On the other hand, titanium and its alloys are frequently used in implantology for a long time without problems. In the literature there were reports about better biological and worse mechanical attributes of commercially pure titanium in comparison with the titanium alloy, but both pure titanium and titanium alloy implants are available. In our previous *in vitro* experiments many different ceramic samples had shown better properties than titanium control samples.¹⁴ The titanium alloys or pure titanium are biologically compatible, but why not use new types of ceramic coverings with strong bonding to the substrate and with low degradability. At present, bioceramics, especially hydroxylapatite, are commonly used as a coating material. Its protective function is based on the high adhesion of the hydroxylapatite layer to the titanium substrate.¹⁵ Physical properties of bioceramic materials allow optimal connection of hydroxylapatite to the titanium implants.²

The method of KrF laser covering hydroxylapatite films enables one to maintain the same deposition conditions for each implant, however, some minor fluctuations in the deposition parameters may occur. HA film analyses confirm that these small fluctuations have no influence, or, if they do, then they are only very small, according to the XRD spectra. Only small fluctuations in the XRD spectra have been observed. All the films created were adherent.⁵⁻⁷ The morphology of all the samples seen by scanning electron microscopy (SEM) is very smooth, with typical bubbles.

5 Conclusion

Osseointegration of the laser deposited films has been proven in all cases and the active bone formation was visible around both HA and titanium surfaces. The degree of loaded osseointegration of HA films was higher than 75%. Under polarized light in 23% of the area the fibrous tissue interposition was visible; in noncoated implants it was especially visible in the middle portion.

Results of our study show that osseointegration of the coated layer is better (77.2%, SE 10.2) than integration of titanium alloy implants (65.2%, SE 13.5), especially in the areas with a primary gap between the implant and the bone bed, however, this difference is not significant. It should be emphasized that KrF laser coating could be a prospective method for the covering of metal implants, since the success with coated implants was similar to that with noncoated ones, and had the additional advantage of an inert and osseoconductive ceramic coating.

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