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Effect of calcium phosphate (Ca–P) coatings on trabecular bone response: A histological study

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The trabecular bone response to noncoated and fluorapatite (FA), hydroxyapatite (HA), and hydroxyapatite heat-treated (HAHT) plasma-sprayed coated implants was investigated in a goat animal model. Forty-eight cylindrical implants were inserted into the trabecular bone of the lateral and medial femoral condyles of twelve goats according to a split plot design. After an implantation period of twelve weeks, the bone-implant interface was evaluated histologically. Quantitative histomorphometrical measurements demonstrated a significant difference in bone contact between implants inserted in the lateral and medial condyles. In addition, a significant difference in bone apposition was observed between the coated and the uncoated implants. Finally, all Ca–P coatings showed reduction in coating thickness. Measurements revealed that FA and HAHT showed less reduction in coating thickness than HA coating. Despite the coating reduction, the bone remained in close contact with the implant surface. © 1995 John Wiley & Sons, Inc.

INTRODUCTION

During the last decades enormous progress has been made in oral implant dentistry. Currently, the use of oral implants may be considered an acceptable treatment concept to support dental prostheses.1–5 Although there are differences in success percentages among them, various studies have demonstrated that implant success is related directly to the quality and quantity of the available jaw bone.6 The clinical significance of the status of the host bed is best demonstrated by the observed difference in survival rate of implants inserted into the mandible and those inserted into the maxilla. For example, a recent study performed in the Netherlands demonstrated that the three-year implant survival rate for implant-retained overdentures in the lower jaw was 96.7% while the survival percentage in the upper jaw was 70.7%.7 These percentages are independent of type of cylindrical implant used. The presence of low bone quality is supposed to be the major reason for the poorer performance of maxillary implants.8,9 Lekholm and Zarb10 visualized this problem by classifying the jaw bone quality. According to their classification, the maxilla consists mainly of loose trabecular bone surrounded by a thin cortical shell, so called type III and type IV bone. In contrast, the mandible consists primarily of a core of dense trabecular bone surrounded by a thick layer of compact bone.

Despite the clinically recognized importance of the host bed for the final success of oral implants, most experimental studies have been performed in high quality cortical bone. On the basis of the observed bone adaptation in these studies, currently titanium and titanium–aluminium–vanadium alloy are considered to be the materials of choice for oral implants.11 On the other hand, it is known that the use of implants coated with a layer of calcium phosphate (Ca–P) ceramic can increase the bone apposition.12–17 Therefore it may be inferred that the application of Ca–P coatings can be beneficial for maxillary dental implants. In the present paper, we report the first results of a series of experiments conducted to test this hypothesis.

MATERIALS AND METHODS

Implant materials and coating characteristics

Forty-eight cylindrical titanium–aluminium–vanadium (TiAl6V4) implants with a length of 10 mm
were grit-blasted with $\text{Al}_2\text{O}_3$ ($Ra = 4-5 \mu m$). They were cleaned ultrasonically in propanol and dried at 100°C. Subsequently the implants were left uncoated, or a Ca-P coating approximately 50-60 μm thick was applied using a plasma-spray process. Three different coatings were produced:

1. Hydroxyapatite (HA)
2. Hydroxyapatite coating subjected to heat-treatment (650°C for 10 min) (HAHT)
3. Fluorapatite coating (FA)

Plasma spraying was performed at a current of 400 A, a voltage of 70 V, and with a working distance of 13 cm. The arc and carrier gas were nitrogen.

For the deposition of both HA-coatings, commercially available spray-dried powder (CAM IMPLANTS B.V., The Netherlands) with a mean particle-size distribution of 38 μm was used. For the FA-coating, fluorapatite powder $[\text{Ca}_5(\text{PO}_4)_3\text{F}]$ was prepared by mixing $4\text{Ca(OH)}_2$ and $3\text{H}_3\text{PO}_4$ with CaF$_2$. The powder had a mean particle-size distribution of 22 μm.

The coatings were characterized by X-ray diffraction (XRD) (Fig. 1) and infrared spectroscopy (IR) (Fig. 2). The flat defined baseline and the narrow peaks with high intensities in the diffractogram of FA are indicative of more crystalline material (95%) with little or no amorphous content as compared with the HAHT-coated (crystallinity of 65%, ratio of crystallinity to crystallinity + amorphous phases) and the HA-coated (crystallinity of 60%) implants. Analysis of the IR spectra for the plasma-sprayed HA coating reveals a broad phosphate stretching region, which is indicative of the formation of some amorphous calcium phosphate phases in the coating. The dehydroxylation that the HA coating has undergone partially disappears in the FTIR of the HAHT coating because of the heat treatment after plasma spraying.

The diameter of the implants that were submitted for plasma-spray coating was 3.9 mm, and the diameter of the implants that remained uncoated (TI) was 4.0 mm; therefore the final diameters of all implants were similar.

Before surgery, all implants were cleaned ultrasonically in 100% ethanol to remove any loose particles and then were dried at 50°C. The implants then were sterilized in an autoclave.

**Experimental design and surgical procedure**

Twelve healthy adult female Dutch goats, with an average age of 30 months and an average weight of 50-80 kg, were selected for the experimental animal model. The animals were kept in quarantine for at least 4 weeks and tested for CAE/CL arthritis.

The implants were placed into the trabecular bone of the femoral condyle. Surgery was performed under general anesthesia induced by intravenous pentobarbital (25 mg/kg-animal and atropine (0.5 mg/animal). After orotracheal intubation, anesthesia was maintained by ethrane (2–3%) through a constant volume ventilator.
To reduce the perioperative infection risk, prophylactic antibiotic Albipen® was administered for 3 days starting 1 h postoperatively.

Six of the twelve goats received *in vivo* fluorochromes that adsorb to bone mineral during the time they are present in the blood circulation. These markers were administered at timed intervals (Table I) to assess the mineralizing surfaces of the bone.

**Histological procedures**

After twelve weeks, the animals were sacrificed using an overdose of Nembutal®. After killing the animals, the femoral condyles together with the implants were excised.

Following fixation in 10% buffered formalin solution, the specimens were dehydrated by alcohol series, and, finally, embedded in methylmetacrylate. Nondecalcified thin (10 μm) sections were made using a modified diamond blade sawing microtome technique. The sections were made perpendicular to the long axis of the implant. These sections were stained with methylene blue and basic fuchsine and examined by light microscopy.

In addition to thin sections, 30 μm thick sections were prepared of the samples of the animals that received fluorochromes. These sections were rinsed with alcohol (96% and 100%), dried, and enclosed with Aquamount®. Finally, they were evaluated by fluorescense microscopy.

**Histological evaluation**

For the insertion of the implants, the animal was immobilized on its back and the hind limbs were shaved, washed and disinfected with povidone-iodine. A longitudinal incision was made on the medial and lateral surface of the left and right femur. After exposure of the femoral condyle, pilot holes were drilled in the trabecular bone. These holes gradually were widened with drills to the final diameter of the implants. The bone preparation was performed with a very gentle surgical technique and continuous internal cooling. Following the press-fit insertion of the implants, the soft tissues were closed in separate layers using resorbable vicryl 2-0 sutures. A total of 48 implants were placed: 12 Ti, 12 coated HA, 12 coated HAHT, and 12 coated FA. Each animal received 4 implants, one in each lateral and medial side of the left and right femoral condyle. The implants were placed according to a balanced split plot design to compensate for differences in bone quality and load characteristics among implantation sites.

**Table I**

<table>
<thead>
<tr>
<th>Weeks Before Sacrifice</th>
<th>Fluorochrome</th>
<th>Color</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>Tetracycline</td>
<td>Yellow</td>
<td>22.5 mg/kg (sc)</td>
</tr>
<tr>
<td>5</td>
<td>Calceine</td>
<td>Green</td>
<td>20.0 mg/kg (sc)</td>
</tr>
<tr>
<td>1</td>
<td>Tetracycline</td>
<td>Yellow</td>
<td>22.5 mg/kg (sc)</td>
</tr>
</tbody>
</table>
The percentage of bone contact

For this purpose, the microscopic images were projected on a monitor using a video camera coupled to the light microscope (magnification 12.5×). Subsequently, the amount of bone was measured for the total implant perimeter. Finally, the percentage of bone contact was defined as the length of the interfascial area where there was direct bone-implant apposition. The histological sections for the quantitative bone evaluation were chosen randomly and were representative of the trabecular bone response. Three sections were used from each implant for bone contact analysis. Results presented are based on the average value of these three measurements.

The thickness of the Ca-P coating

For this measurement again the light microscope (magnification 8×), coupled with a video camera and computer, was used. Based on discriminating differences in gray level, an image could be depicted in which the coating was isolated from the underlying metal and surrounding tissues. The coating thickness was measured on three randomly selected sections of each implant type. Subsequently, five images in each section, covering about 80% of the implant perimeter, were stored as digitized images in the computer. In these images the discrimination of the coatings by gray levels was fairly easy. Subsequently, 16 horizontal scan lines with a distance of 32 pixels were superimposed on each image, and the spacing between the coating boundaries was calculated (Fig. 3). This procedure resulted in a distance count of 80 for each implant. Finally, the data for the various coatings were merged and displayed in box and whiskers plots.

RESULTS

One goat had to be sacrificed nine days after surgery due to a broken rib. The rest of the experimental animals had no complications and appeared to be in good health during the test period. At sacrifice, no clinical signs of inflammation or adverse tissue reactions could be seen around the implants. X-rays taken parallel to the long axis of the implant showed that the implants were located in trabecular bone. Only on the outside were they surrounded by cortical bone (see Fig. 4).

Descriptive histological evaluation

Light microscopical evaluation

The light microscopic evaluation of the implants demonstrated a satisfying bone maturation around the four different implants.

The noncoated Ti implants occasionally showed an intervening fibrous tissue layer at the bone-implant interface (Fig. 5). At places where no fibrous encapsulation was present, close bone apposition to the implant surface was observed (Fig. 6).

Around the FA coated implants, an intimate bone-implant contact was formed. Sometimes, remodelling lacunae with osteoblasts were clearly visible (Fig. 7). In none of these sections did the FA coating show signs of reduction.

The bone reaction to the HA and HAHT (Fig. 8) coated implants was similar to the FA coated implants, frequently showing an intimate bone-implant contact. Also, remodelling lacunae with osteoblasts in contact with the HA and HAHT coating were observed. However, in contrast with the FA coated implants, a moderate reduction in thickness of the HAHT coating (Fig. 9) and a severe reduction of the

Figure 3. Schematic drawing of one of the five representative random images with discriminated coating on the implant surface, divided into 16 horizontal scan lines.

Figure 4. Radiograph of an implant located in the trabecular bone of the femoral condyle of the goat.
Figure 5. Histological appearance of a non-coated implant showing a fibrous tissue layer at the bone-implant interface. A: original magnification 10×, bar = 294 μm; B: original magnification 25×, bar = 118 μm.

Figure 7. Light micrograph showing a fluorapatite coated implant. An intimate bone contact at the interface can be observed. Note also the unchanged thickness of the FA coating. Original magnification 32×, bar = 32 μm.

HA coating were observed. This loss of HA and HAHT coating was not uniform and did not interfere with the intimate bone contact. Even on places where the coating was completely absent a close contact of the bone with the underlying titanium alloy implant surface still existed without signs of fibrous tissue formation or inflammatory reaction (Fig. 10).

Fluorescence microscopical evaluation

The accumulation of tetracycline and calceine labeled bone demonstrated that an active remodeling activity had taken place in the vicinity of the implants. Newly formed bone was deposited on the coated as well as on the noncoated implant surfaces. In addition, no significant differences in bone remodeling activity around the various Ca-P coated and noncoated implants were found (Fig. 11).

Figure 6. Light micrograph of an uncoated titanium implant showing an area of close bone-implant contact. No intervening fibrous tissue can be observed. Original magnification 40×, bar = 73 μm.

Figure 8. Histological appearance of a HAHT coated implant demonstrating bone deposition on the implant surface and cellular activity in the remodeling lacuna on the implant surface. Original magnification 40×, bar = 73 μm.
Histomorphometrical evaluation

Percentage of bone contact

The histomorphometrical analysis demonstrated a variation of up to 20% in the amount of bone contact among the three used sections of each implant. Tables II and III show all bone apposition data for the various implants and implantation sites. Statistical testing, using a one-way analysis of variance (ANOVA) and a multiple comparison procedure (Newman-Keuls) revealed a significant difference in bone contact between implants inserted in medial and those inserted in lateral condyles (P < 0.05). Further statistical testing indicated also that the difference in bone apposition between Ca-P coated and noncoated implants was significant (P < 0.001). No significant difference in percentage of bone contact existed among the FA, HA and HAHT coated implants.

Thickness of the Ca-P coating

In Figure 12 the results from the measurements of coating thickness are shown in box and whiskers plots, using a Tukey five number summary (0, 25, 50, 75, 100).

### TABLE II

Mean Bone Apposition (%) ± Standard Deviation of the Four Different Implant Types

<table>
<thead>
<tr>
<th>Implant Type</th>
<th>Mean % of Bone Contact ± Standard Deviation</th>
</tr>
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<tbody>
<tr>
<td>FA</td>
<td>78.4 ± 9.1 (n = 11)*</td>
</tr>
<tr>
<td>HAHT</td>
<td>79.1 ± 8.15 (n = 11)</td>
</tr>
<tr>
<td>HA</td>
<td>78.2 ± 9.4 (n = 11)</td>
</tr>
<tr>
<td>Ti</td>
<td>56.8 ± 16.9 (n = 11)</td>
</tr>
</tbody>
</table>

*The number of implants that were studied for each group.
TABLE III
Means and Standard Deviations for the Various Implants for the Two Implantation Sites

<table>
<thead>
<tr>
<th></th>
<th>FA</th>
<th>HAHT</th>
<th>HA</th>
<th>Ti</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medial</td>
<td>82.36 ± 7.2</td>
<td>80.58 ± 10.7</td>
<td>84.34 ± 5.5</td>
<td>60.82 ± 12.7</td>
</tr>
<tr>
<td></td>
<td>(n = 18)*</td>
<td>(n = 15)</td>
<td>(n = 15)</td>
<td>(n = 18)</td>
</tr>
<tr>
<td>Lateral</td>
<td>72.8 ± 10</td>
<td>76.98 ± 8.5</td>
<td>74.03 ± 11.0</td>
<td>49.34 ± 21.4</td>
</tr>
<tr>
<td></td>
<td>(n = 18)</td>
<td>(n = 18)</td>
<td>(n = 18)</td>
<td>(n = 18)</td>
</tr>
</tbody>
</table>

*The number of the measured histological sections.

is not surprising. Our study was unique, however, in that we used a very careful surgical technique that resulted in minimal bone damage during drilling and in optimal fit of the implants. Therefore the only factor in our study that could affect the final bone response to the various implant materials was the status of the host bone at the two implantation sites. The significance of this parameter clearly was shown by the observed difference in bone apposition to implants in lateral condyles as compared with implants in medial condyles. An explanation for this finding might be that the bone turn-over and the original bone mineral density of the lateral and medial condyles differ. This observation confirms once again the need of proper statistical implantation schedules in bone-biocompatibility tests.

Other problems associated with the testing of materials for bone replacements are the histological preparation and evaluation techniques. The modified sawing technique used in this study appears to be an excellent method for the preparation of light microscopical sections of hard implant materials, including the surrounding tissues. An enormous number of high quality sections of each implant can be produced, and also the staining technique used provides good contrast for differentiating between the various tissues and implant materials.

Evaluation of three representative sections taken at different levels from the same implant revealed a significant variance in trabecular bone response. This observation refutes the statement of Soballe,22 who assumed that one section from each implant is sufficient for histomorphometrical analysis. Our study indicates that completely different conclusions about the suitability of an implant material may be reached dependent upon the level evaluated.

With regard to the fluorescence microscopy, in our study no additional information was obtained about the bone apposition and remodeling processes around the implants. This observation proves that fluorescent labels are not very useful when implants are placed in press-fit conditions.

Further, we made transversal histological sections of our cylindrical implants. This made it possible to evaluate the complete circumferential implant–tissue contact area. Unfortunately, due to the different preparation steps, the top or bottom of the implants in relation to the main load direction is unknown.

DISCUSSION AND CONCLUSION

Animal models used to study cortical bone behavior in relation to implant materials are not very effective for the investigation of the behavior of maxillary oral implants. Consequently, in this study a trabecular experimental design was used to evaluate the possible beneficial bone effect of three calcium phosphate coatings in comparison with noncoated Ti-alloy implants.

Measurements of the percentage of bone contact demonstrated significantly more bone apposition to the Ca-P coated than to the non-coated implants. Other studies have demonstrated similar observations with Ca-P coated implants,19-21 so this finding

![Figure 12. Box-whisker plots showing the results from the computer-based analysis of the coating-thickness reduction for the three different coatings.](image)
Nevertheless, it cannot be excluded that the observed 58% bone contact to the bioinert noncoated titanium alloy implants is caused mainly by an unidirectional loading condition in the interface. Therefore, as also confirmed by an earlier report of Zeinek et al., a definitive statement about the behavior of trabecular bone along the surface of titanium alloy implants can be made only with the help of experiments in absolutely load free conditions. From the standpoint of bone response, this last phenomenon demonstrates the importance of the further development of coating techniques for the deposition of bioactive Ca-P ceramics on bioinert implant materials.

An interesting finding in the present study resulted from the measurement of the residual coating thickness. Although several reports already have been published about the stability of plasma-sprayed Ca-P coatings, in those studies mainly subjective parameters were used to quantify the coating behavior. Only occasionally were histomorphometric techniques applied. A drawback inherent in the quantification methods used in other studies is the presentation of the results as an overall mean of the coating thickness. It was recognized in our study that the reduction in coating thickness was not uniform, and by using the proposed analysis, this lack of uniformity was clarified. Similar to other studies, the results confirmed the relative stability of FA coatings; in contrast to other studies, however, the measurements showed a great variance in thickness reduction within each material group. For example, for the HA-plasma-sprayed implants, on some locations the coating had completely disappeared while in other areas the coating was still intact. The final clinical consequences for this variable coating behavior are not clear since we observed that the bone–implant contact was not influenced by this coating loss. Recently Kangasniemi et al. reported that plasma-sprayed Ca-P coatings should not be used in long-lasting load-bearing application. They measured the bone bonding strength of FA and HA coated titanium implants using a tensile test. Fractures always occurred in the coating–titanium interface. Consequently, irrespective of the desire to develop long-lasting coatings, it can be hypothesized that a thinner coating or a coating that shows a predictable homogenous desintegration is more favorable. This assumption is supported by the fact that Ca-P coatings show an effect only on the initial bone response. In summary, we conclude that Ca-P coatings have no negative influence on bone–implant contact, not even when the coating disappears, and that coated implants enhance the quantity of direct bone formation on the implant surface in comparison with the uncoated implants. Based on the findings of this study, we suggest that a stable screw-design implant combined with a Ca-P coating may decrease the implant failures in the maxilla. In future studies we hope to confirm this hypothesis.

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