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Initial interfacial healing events around calcium phosphate (Ca-P) coated oral implants


The bone response to different calcium phosphate (Ca-P) coated and non-coated titanium implants was evaluated in a goat animal model. Two types of Ca-P coatings have been investigated: an experimental plasma-spray bi-layered Ca-P coating (FA-HA) and an amorphous RF magnetron sputter coating (Ca-P-a). Fifty-four conical screw shaped implants were inserted in the lateral and medial femoral condyles of 18 Saanen goats. After implantation periods of 3, 12 and 24 days, the bone–implant interface was evaluated histologically and histomorphometrically. Light microscopical evaluation revealed that bone formation on the Ca-P coated implants proceeded faster. At 24 days higher percentages of bone contact were measured for both Ca-P coated implants than for non-coated implants. However, this difference was only significant for the FA-HA coated implants. On basis of these findings, we concluded that Ca-P coatings show improved bone response due to an initial difference in bone cell response.

It has generally been accepted that calcium phosphate (Ca-P) coated implants induce a faster bone adaptation and improved bone healing (Dhert et al. 1993; Cook et al. 1988; Gottlander et al. 1992; Caulier et al. 1995, 1996; Hulshoff et al. 1996a). Currently, the plasma-spray technique is the most widely used technique for the deposition of Ca-P ceramic coatings (Wolke et al. 1992; Dalton & Cook 1995). However, recently, some concerns have been raised about the safety and clinical prognosis of plasma-spray Ca-P coated oral implants (Wheeler 1996). Failures have been reported, which mainly have to do with the degradation and fatigue behavior of these coatings (Fryssinet et al. 1995; Bloebaum & Dupont 1993; Bloebaum et al. 1994). This can hamper the further use of Ca-P implants. Consequently, experiments are started to improve the biodegradation of plasma-sprayed Ca-P ceramic (Klein et al. 1993). For example, to increase the stability, additional heat treatment procedures and various Ca-P powder compositions are used. The rationale behind these modifications is, that degradation is related to the structural properties (amorphous vs crystalline) of the coating. On basis of the different studies, de Groot et al. (1994) concluded that high coating crystallinity does not improve compatibility, but rather reduces the bioactivity compared with amorphous coating. Therefore, he suggested that perhaps the most optimal coating should consist of: (a) an amorphous outer layer for the bone healing, and (b) a crystalline inner layer to reduce the degradation.

Besides plasma-spraying, also other techniques can be used to produce Ca-P ceramic coatings. For this purpose, since 1991, in our laboratory efforts have been made to the development of the so-called RF magnetron sputter method. The advantages of this process are that the deposited films are very thin (100 nm–4 μm), well-defined in structure and composition, and strongly bonded to the underlying metal substrate. Previous cell culture and animal experiments (Hulshoff et al. 1995, 1996b) already showed the biological feasibility of this type of coating.

Finally, it has to be noticed that, despite the frequently described favorable bone reaction, the reason for the beneficial effect of Ca-P ceramic is still not completely understood. It is only suggested, that events in the cellular and tissue response during the
initial healing phase are responsible (LeGeros & Craig, 1993).

Therefore, the aim of this investigation was to compare the early interfacial response to two alternative Ca-P coated implants with a non-coated control.

**Materials and methods**

**Implant materials and coating characteristics**

Fifty-four tapered, conical, screw shaped dental implants (Biocomp® Industries) were used. All implants measured 10.0 mm in length. Thirty-six implants had a diameter of 4.0 mm and 18 implants had a diameter of 3.9 mm. The implants with a 3.9 mm diameter were grit-blasted to a roughness of $R_a = 4-5 \mu m$, as determined by roughness measurements with a profilometer. They were cleaned ultrasonically in propanol, and dried at 100°C. Subsequently, these implants were provided with an approximately 60 $\mu m$ Ca-P coating using a plasma-spray process. An experimental bilayered Ca-P coating was deposited. The inner 30 $\mu m$ consisted of crystalline fluorapatite (FA) and the outer 30 $\mu m$ of amorphous hydroxylapatite (HA). The final surface roughness of these FA-HA coatings was $R_a = 9.59 \mu m$. Further, of the implants with a diameter of 4.0 mm, 18 were left uncoated (cpTi) and 18 were provided with an amorphous Ca-P (Ca-P-a) coating using a radiofrequency (rf) magnetron sputter process (van Dijk et al. 1995a, 1995b; Wolke et al. 1994). These implants were not grit-blasted, but were argon-etched before sputter coating. The produced coatings had a thickness of 2–4 $\mu m$. Final roughness for cpTi and for Ca-P-a implants was respectively: $R_a = 0.44 \mu m$ and $R_a = 0.40 \mu m$. The final diameter of all implants was 4.0 mm ($\pm 0.1 \text{ mm}$). The chemical composition of the coatings was confirmed by X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR) and Rutherford backscattering spectrometry (RBS) (Hulshoff et al. 1996a).

Before implantation, all implants were sterilized by autoclave.

**Experimental design and surgical procedure**

Eighteen healthy, mature (2–4 years of age), female Saanen goats, weighing about 60 kg were used. Prior to the experiment, blood samples of the goats were taken to ensure that the animals were Caprine Arthritis Encephalitis free. All animals were housed in a stable.

The implants were inserted into the trabecular bone of the femoral condyle. The operation was performed under general anaesthesia. The anaesthesia was induced by an intravenous injection of pentobarbital and maintained by ethrane 2–3% through a constant volume ventilator, administered through an endo-tracheal tube. The goats were connected to a heart monitor. To reduce the risk of peri-operative infection, the goats were treated according to the following doses of antibiotics:

- **before the operation:** Albipen 15%, 3 ml/50 kg s.c.
- **one day after the operation:** Albipen LA, 7.5 ml/50 kg s.c.
- **three days after the operation:** Albipen LA, 7.5 ml/50 kg s.c.

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, 1.6 mm pilot holes were drilled. These holes were gradually widened with different drills to the final diameter (4.0 mm) of the implant. The bone preparation was performed with a very gentle surgical technique, using low rotational drill speeds (max. 450 rpm) and continuous and external cooling. Following insertion of the implant the skin was closed using Vicryl 3–0 sutures.

A total of 54 implants were placed: 18 uncoated cpTi, 18 coated FA-HA, and 18 Ca-P-a implants. Each goat received 3 implants. The implants were inserted following a balanced split plot design, with one vacant position.

The protocol did foresee to kill 6 goats after 3 days, 6 after 12 days and 6 after 24 days using an overdose of Nembutal®.
Initial interfacial healing and Ca-P coated oral implants

Histological procedures

Following the death of the goats, the femoral condyles were excised and fixed in a 10% buffered formalin solution. Then the remaining tissue blocks were dehydrated in a series of ethanol (70–100%). Finally, they were embedded in methylmethacrylate. Using a modified diamond blade sawing microtome technique (van der Lubbe et al. 1988; Klein et al. 1994), non-decalcified thin (10 μm) sections were made. These were stained with methylene blue and basic fuchsine to be examined by a light microscope.

Histological evaluation

The trabecular bone response to the implants was assessed histologically. First, for all implantation periods a descriptive evaluation was performed. Second, for the 24 days implants also histomorphometrical measurements were done. Therefore a computer based image analysis system (TCL-image) was used. Microscopic images were projected on a monitor. For this purpose, a video camera was coupled to the light microscope (magnification 2.5×1.25). In this way the percentage of direct bone contact at the interface for 4 different areas of interest was determined. These areas were (Fig. 1):

1. along three coronal screw threads
2. at the smooth middle part
3. along three apical screw threads
4. along the apex.

All quantitative measurements were performed for 2 different sections per implant. Presented results are based on the average of these measurements.

Results

During the experimental period, 1 of the goats of the 24-day group died because of an acute peritonitis caused by a pancreatitis. The other animals remained in good health during the various test periods. At sacrifice, no clinical signs of inflammation or adverse tissue reaction could be seen. All implants were still in situ at sacrifice.

Light microscopical evaluation

After 3 days of implantation no difference in tissue response between the various implants could be observed (Fig. 2). The original hole, that was drilled to place the implant, could be recognized very well. In some cases the implant did not reach the bottom of the hole. Then, the available space around the implant and between the original bone trabeculae was filled with splinters of bone as left by the drilling procedure. Further, blood coagulum, primitive bone marrow cells, and undifferentiated inflammatory cells were visible. Only few multinucleated giant cells were present.

After 12 days of implantation, around all implant materials a callus of woven bone was formed, which bridged the existing space between bone trabeculae and implant surface. Extensive networks of active osteoblasts were seen. Bone formation was not restricted to the interface. Trabeculae of the surrounding bone, that were not traumatized by drilling, also showed an active formation of new bone. Still inflammatory cells were seen, though in a lesser extent than after 3 days of implantation. Despite this similarity in overall reaction, a clear difference existed in interfacial response between coated and non-coated implants (Fig. 3). For both types of Ca-P coated implants osteoid appeared to be present in a greater abundance compared with non-coated implants.

After 24 days of implantation, both types of Ca-P coatings showed a lot of bone contact (Fig. 4). Even
Fig. 3. Light micrographs of both types of coated implants after 12 days of implantation. A. FA/HA coating: newly formed bone is covering the implant. B. Ca-P-a coating: osteoid (light gray zone with open stars) is present in the screw threads. A. bar = 100 μm, B. bar = 40 μm.

Fig. 4. Light micrograph of an FA/HA coated implant after 24 days of implantation. Around the FA/HA coated implants the original structure of bone trabeculae around the interface is maintained. New bone formation is also formed in trabeculae that were not traumatized by drilling. Original magnification ×2.5, bar = 400 μm.

if the implant apically was not in contact with the original bone, a thin layer of bone was often covering the implant. On the other hand, we noticed that the appearance of the surrounding bone for FA-HA and Ca-P-a implants was different. In a region with a width of about 500 μm, the bone around the Ca-P-a implants was more dense than around FA-HA implants (Fig. 4). For the FA-HA implants, the bone in this area was more similar to the original bone. In contrast with the coated implants, the cpTi implants did not show much bone contact. Further, around all implants still some inflammatory cells could be seen. The plasma-sprayed coating also showed signs of superficial coating degradation, as characterized by the presence of fragmented particles (Fig. 5). This degradation process could not be associated with the occurrence of inflammatory cells.

Histomorphometrical evaluation

The results of the bone contact measurement after 24 days of implantation are listed in Table 1. In all areas, the average percentage of bone contact appeared to be higher for the Ca-P coated implants than for the cpTi implants. Nevertheless, statistical analysis using a one-way analysis of variance and Tukey multiple comparison procedure revealed that this difference was only significant (P<0.05) for the FA-HA coated implants. In addition, the results show that the amount of bone contact in areas 3 and 4 was lower than in areas 1 and 2.

Table 1. Mean bone apposition (% ± standard deviation after 24 days of implantation (number of implants is 5)

<table>
<thead>
<tr>
<th></th>
<th>Area 1</th>
<th>Area 2</th>
<th>Area 3</th>
<th>Area 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>cpTi</td>
<td>41.56</td>
<td>(±31.46)</td>
<td>25.44</td>
<td>(±10.82)</td>
</tr>
<tr>
<td>Ca-P-a</td>
<td>68.25</td>
<td>(±31.56)</td>
<td>44.11</td>
<td>(±40.62)</td>
</tr>
<tr>
<td>FA-HA</td>
<td>90.85</td>
<td>(±8.72)</td>
<td>84.71</td>
<td>(±4.98)</td>
</tr>
</tbody>
</table>
Discussion and conclusions

In this study the influence of different Ca-P coatings on initial bone healing of screw type oral implants was investigated. Therefore Ca-P coated implants and non-coated cpTi implants were inserted in the trabecular bone of the femur of goats for implantation periods of 3, 12 and 24 days. It was found that Ca-P coated implants favor the early bone response. Already after 12 days of implantation the bone forming process had proceeded faster for the coated implants. This could not completely be confirmed in the bone contact measurements. Due to a wide variation in bone contact percentage, statistical significance could only be demonstrated for the plasma-spray coated implants. Nevertheless, our findings do corroborate with the experiments of other investigators, although they all used longer implantation periods. For example, Gottlander et al. (1992) found more direct bone contact to plasma-sprayed hydroxyapatite-coated implants compared to non-coated controls 6 months after placement in the femur of rabbits. In another study, Clemens et al. (1996) observed increased bone apposition to various types of calcium phosphate coatings within 6 weeks of implantation using the same animal model as in our study. This improved bone response even occurred when a 2 mm gap was created around the implants. On the other hand, it has to be emphasized, that our results do not agree with a recent study of Dhert et al. (1996). They placed plasma-spray Ca-P coated and non-coated Ti implants into the cortical bone of the tibial metaphysis of rabbits. After 3, 7, 18 and 28 days of implantation, almost similar percentages of bone contact were measured for all implants. This discrepancy with our observations can be due to the used implant location. It is known, that trabecular and cortical bone have a different healing response (Burr et al. 1993). Also, the implant location (condylus vs metaphysis) can have an influence (Dhert et al. 1991).

Considering the Ca-P sputter coatings, the present results confirm most of our earlier in vitro and in vivo studies (Hulshoff et al. 1995, 1996a, 1996c). Only in one study (Hulshoff et al. 1996c), we failed to demonstrate the beneficial effect of amorphous Ca-P sputter coatings. In this study, sputter coated implants were inserted in the maxillary trabecular bone of goats. This bone is of very low density. As we suggested, this negative result was probably due to the difference in trabecular bone quality between the goat maxilla and femur. As a result, the amorphous coating dissolves too fast. Unfortunately, this hypothesis cannot be confirmed with quantitative data about the in vivo dissolution behavior of Ca-P sputter coatings in various bone locations. The films are too thin for the currently available analytical techniques.

Despite the great difference in surface roughness between both types of Ca-P coating, the sputtered implants showed a high amount of bone contact. Several studies demonstrated already, that implant surface topography affects the bone biocompatibility (Buser et al. 1991; Wennberg et al. 1995a,
Hulshoff & Jansen

1995b, 1996). In a recent study, Gottfredsen et al. (1995) even claimed that a rough surface is a pre-requisite for the successful implantation in bone of low quality or quantity. Consequently, it can be supposed that roughening of the implant surface together with a thin Ca-P sputter coating will increase the bone apposition to the same level as for rough plasma-sprayed coatings. More research is necessary to confirm this theory.

Notwithstanding the better response to the Ca-P coated implants we have to mention that this difference in surface roughness can also be responsible for the lower amount of bone contact to the cpTi implants. This suggestion is supported by the findings of Courtney et al. (1995) and Gomi et al. (1993). They demonstrated that the amount and distribution of mineralized bone matrix is influenced by the sub-stratum surface roughness.

The difference in bone contact between areas 1 and 2 and areas 3 and 4 is probably due to the surgical procedure. In the histological evaluation we observed that the implants were not always in contact with the bottom of the drilled hole. The apical part of the implant has a conical shape. Incomplete positioning resulted in a small apical gap between implant and bone. Evidently, this reduced fit of the implant can be responsible for the lower percentage of bone apposition.

Finally, some critical remarks have to be made about the degradation of the used bilayered FA-HA coating. The amorphous outer layer was designed to improve the bone reaction. The purpose of the crystalline inner layer was to stay in place in order to maintain the optimal functioning of the implant. On the basis of the bone contact measurements, it appears that the amorphous layer indeed fulfilled its role. On the other hand, we observed that the resorption was associated with a fragmentation process of the coating. Although this could not be related with a cellular response, it cannot be excluded that at the long-term the released particles will lead to irritation. Further, from the point of bone bonding, the use of a non-resorbable layer is also uncertain. Especially, because there are some indications that maintenance of Ca-P coatings can endanger the final fixation of the implants due to delamination phenomena (Kangasniemi et al. 1994).

Supported by the results, we conclude that: (1) the stronger bone response to Ca-P ceramic coatings is due to an initial difference in bone cell response, and (2) RF magnetron sputtered Ca-P coatings can improve the biological capacity of oral implants. Further studies have to be performed to optimize the sputter technique. These experiments have to focus on the influence of coating composition (structure and Ca/P ratio), the clinically required coating thickness and the influence of the implant surface topography.

Acknowledgements

These investigations were supported by the Netherlands Technology Foundation (STW). The implants were provided by Biocomp Industries, Medemblik, The Netherlands. The authors thank R. P. J. Wils for his surgical skills and assistance during the animal experiments, K. van Dijk for the preparation of the RF magnetron sputter coatings, and A. F. M. Leijdekkers-Govers for the preparation of the histological sections.

Résumé

La réponse osseuse à différents implants en titane recouverts ou non de phosphate de calcium (Ca-P) a été évaluée sur la chèvre. Deux types de recouvrement de Ca-P ont été analysés: Un recouvrement expérimental par plasma spray en double couche de 30 µm d'épaisseur de fluoroapatite et d'hydroxyapatite (FA-HA) et un recouvrement amorphe de phosphate de calcium (Ca-P-a). Les autres implants étaient en titane non-recouvert. Cinquante-quatre implants en forme de vis coniques ont été insérés dans les condyles fémoraux latéraux et médians d'âge-huit chèvres Saanen. Après une période d'implantation de trois, douze et vingt-quatre jours l'interface os-implant a été évalué histologiquement et histométriquement. L'évaluation au microscope optique a révélé que la formation osseuse autour des implants recouverts de Ca-P se faisait plus rapidement. Après vingt-quatre jours de pourcentages plus importants de contact osseux étaient mesurés pour les deux implants recouvert de Ca-P qu'au niveau des implants non-recouverts. Cependant cette différence était seulement significative pour les implants recouverts FA-HA. Sur la base de ces découvertes les recouvrements Ca-P produisent des réponse osseuses améliorées dites à une différence initiale dans le réponse cellulaire osseuse.

Zusammenfassung


Resumen

Se evaluó la repuesta de hueso a diferentes implantes de titanio cubiertos y no cubiertos de fosfato cálcico (Ca-P) en un modelo animal de cabra. Se investigaron dos tipos de cubiertas de Ca-P: una cubierta experimental de dos capas de plasma de Ca-P (FA-
HA) y una cubierta amorfa de chisporroteo de magnetrón RF. Se insertaron 54 implantes rosados de forma cónica en los cóndilos lateral y medial del fémur de 18 cabras Stannen. Después de un período de implantación de 3, 12 y 24 días se evaluó histológicamente e histomorfométricamente la interfa hupe implantante. La evaluación de microscopía óptica reveló que la formación de hueso en los implantes cubiertos de Ca-P se produjo con mayor rapidez. A los 24 días se midieron mayores porcentajes de contacto óseo para ambos implantes cubiertos de Ca-P que para los implantes sin cobertura. De todos modos esta diferencia fue solo significativa para los implantes cubiertos de FA-HA. En base a estos hallazgos concluimos que las cubiertas de Ca-P muestran una respuesta ósea mejorada debido a una diferencia inicial en la respuesta celular del hueso.

**References**


