JOURNAL OF MATERIALS SCIENCE: MATERIALS IN MEDICINE 2 (1991) 63–70

Biodegradation of four calcium phosphate ceramics; *in vivo* rates and tissue interactions

M. M. A. RAMSELAAR, F. C. M. DRIESSENS, W. KALK
Department of Oral Function and Prosthetic Dentistry, Dental School, University of Nijmegen, The Netherlands

J. R. DE WIJN
Department of Biomaterials, State University of Leiden, The Netherlands

P. J. VAN MULLEM
Department of Oral Histology, University of Nijmegen, The Netherlands

To prevent exposure of artificial tooth root implants, a resorbable root implant may be developed, that in time will resorb in a vertical direction at the same rate as the alveolar ridge does after the loss of the natural teeth. Implants of four calcium phosphates: rhenanite, β-tricalcium phosphate, hydroxylapatite and magnesium-whitlockite were measured during *in vivo* resorption and their interactions with the surrounding tissues at experimental periods of 6 weeks and 3 months were investigated. It was shown that a sequence of progressive resorption *in vitro* does not correlate with the resorption rates found in this *in vivo* experiment. *In vivo* hydroxylapatite was found to be less resorbable than magnesium-whitlockite and rhenanite less resorbable than β-tricalcium phosphate. Tissue interactions showed that resorption of the calcium phosphates was positively related to the number of osteoclast-like cells and did not completely correlate with the resorption measurements insofar that most rhenanite implants showed a more reactive peri-implant with the largest number of osteoclast-like cells, strongly affecting the implant surface. In contrast, two rhenanite implants showed intimate contact with bone after initial resorption. Because of this divergent reaction with rhenanite, further *in vivo* investigation on this material is proposed.

1. Introduction

In an attempt to preserve the volume of edentulous alveolar ridges, many studies have been performed on the effectiveness of artificial non-resorbable tooth root substitutes. For this purpose these substitutes have been implanted in empty tooth sockets immediately after the extraction of natural teeth. The results of these studies are divergent. Several studies report success in preserving the volume of the edentulous alveolar ridge as long as the root substitutes can be retained [1–5]. Other studies report early exposure and loss of the root substitutes [6–10], presumably due to continuing alveloar bone resorption [6, 7, 10]. As up to now this resorption could not be completely counteracted, it has been thought possible to avoid exposure by implanting a resorbable tooth root substitute that in time is able to resorb at the same rate and in the same direction as the edentulous alveolar bone does by its vertical resorption. The remaining part of the resorbing implant will keep serving as a space-maintainer between the socket walls, thereby preserving the width of the alveolar ridge.

Because of its generally accepted compatibility with host tissues, this resorbable tooth root substitute may be composed of calcium phosphate layers. In an apical direction each subsequent layer will have a lower resorption rate to correspond with the decreasing resorption rate of the alveolar bone.

The purpose of this study was to determine the *in vivo* resorption of four calcium phosphates, to study the interactions with the surrounding tissues, and to gain more information about calcium phosphate resorption.

2. Materials and methods

The materials under investigation were, in sequence of progressive solubility *in vitro*, magnesium-whitlockite (Mg-W), hydroxylapatite (HA), β-tricalcium phosphate (β-TCP) and rhenanite (Rh). HA and β-TCP have been subject in many studies, Mg-W and Rh are less known. Data on Mg-W and Rh are given by Terpstra *et al.* [11] and Ando and Matsuno [11, 12], respectively. The *in vitro* solubilities of the first three materials were obtained by theoretical calculation at pH 7, the *in vitro* solubility of Rh is based on experimental dissolution at pH 7 in distilled water.

From each material, bars were made by a sintering process of wet powder compaction, followed by firing at the appropriate temperatures during the required time. Manufacturing conditions and the main features of the calcium phosphates are given in Table I. The

0022–2461/91 $03.00 + .12

TABLE I Manufacturing conditions and main features of the applied calcium phosphates

	Chemical formula	Starting powders	Sintering conditions: temperature (°C) time (h)	*In vitro* solubility in H_2O at pH 7 ($g\,l^{-1}$)	Micro porosity (%)	Ca/P ratio
Mg-W	$Ca_{2.5}Mg_{0.5}(PO_4)_2$	MgO (Merck 5866) $CaCO_3$ (Merck 2066) $CaHPO_4$ (Baker 0080)	1400 16 in air	0.09	25	1, 25
HA	$Ca_{10}(PO_4)_6OH_2$	$CaCO_3$ (Merck 2066) $CaHPO_4$ (Baker 0800)	1300 16 in air	0.13	25	1, 67
β-TCP	$Ca_3(PO_4)_2$	$CaCO_3$ (Merck 2066) $CaHPO_4$ (Baker 0080)	1150 1 week in air	0.17	35	1, 5
Rh	$CaNaPO_4$	$CaHPO_4$ (Baker 0080) $NaHCO_3$ (Merck 6329)	1300 16 in air	1.00	10	1

X-ray diffraction proved the products to be single phase. Compaction pressure 490 N mm^{-2}.

resulting bars were machined into cylinders 7 mm high and 5 mm diameter.

To measure the *in vivo* resorption, cylinders of the calcium phosphates were mounted in acrylic tubes (Fig. 1) and sandpapered flush at both ends. Before implantation, profile measurements were performed at both ends with a thermomechanical analyser (Mettler TMA 40, Fig. 2a), a device that is able to distinguish differences in height up to tenths of a micrometre. These measurements were taken at five points. Two metal pins inside the edges of the acrylic tubes served as zero reference points. The differences in height with these reference points were measured on three points of the calcium phosphate cylinders, two points on the border and one point in the middle, all in line with the zero reference points (Fig. 2b). Each measurement was repeated five times on the same profile line which was supposed to be representative for the whole exposed surface.

For the site of implantation, the forehead (parietal and frontal bone) of 3 month old pigs (*Sus scrofa*) was chosen. Eight animals were used, distributed over two equal groups of 6 week and 3 month experimental periods. Each animal received four acrylic tubes, containing equal numbers of two different calcium phosphate cylinders. Altogether 32 tubes were implanted, containing eight cylinders of each calcium phosphate.

For the tissue interaction study, each animal received another two single cylinders of the kind of calcium phosphates as present in the tubes. The distribution of the cylinders over the implant area is shown in Fig. 3. Prior to implantation all implants were ethylene oxide sterilized.

Under general anaesthesia, 5 and 10 mm wide and 5 mm deep cavities were drilled in the implantation site in which the tubes and single cylinders were snugly inserted. One end of the implants was placed in direct contact with bone at the bottom of the cavities, the other end protruded 2 mm from the cavities to become covered by soft tissue after the operation. This gave the opportunity to study a possible difference in resorption rate by soft tissue at the upper side and bone tissue at the lower side. In this study another opportunity was offered to gain more information on the question of whether resorption of calcium phosphates

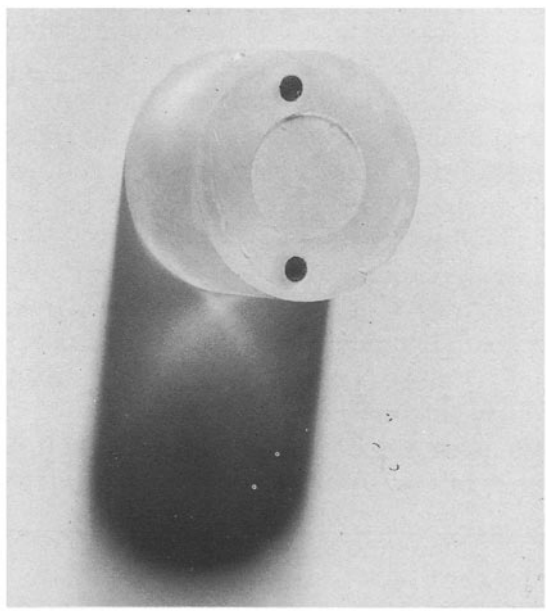

Figure 1 Acrylic tube containing a calcium phosphate cylinder. The metal pins inside the edge of the tube serve as zero reference points.

is merely a matter of physico-chemical dissolution by extracellular body fluids or that the presence and activity of tissue cells is an important factor. Therefore, in each animal two acrylic tubes containing different calcium phosphates, were covered at the upper end with a millipore filter (pore diameter 0.22 μm) to avoid cell passage towards the calcium phosphates, but leaving body fluids unhindered (Fig. 3). After insertion of the implants, the periosteum and epidermis were replaced and sutured. All animals were administered an antibiotic (Albipen), 10 cm^3 at day 0, 2, 4 and 6 after the operation. At the end of the experimental periods the animals were sacrificed and blocks of tissue containing the implants were sawn out. Twenty-eight tubes were retrieved by cleaning them carefully from surrounding tissue and filters. No firm fixation was encountered. Post-implantation profile measurements at the two surfaces of the cylinders inside the tubes were again performed as described above. These measurements were compared with the pre-implantation profile measurements and

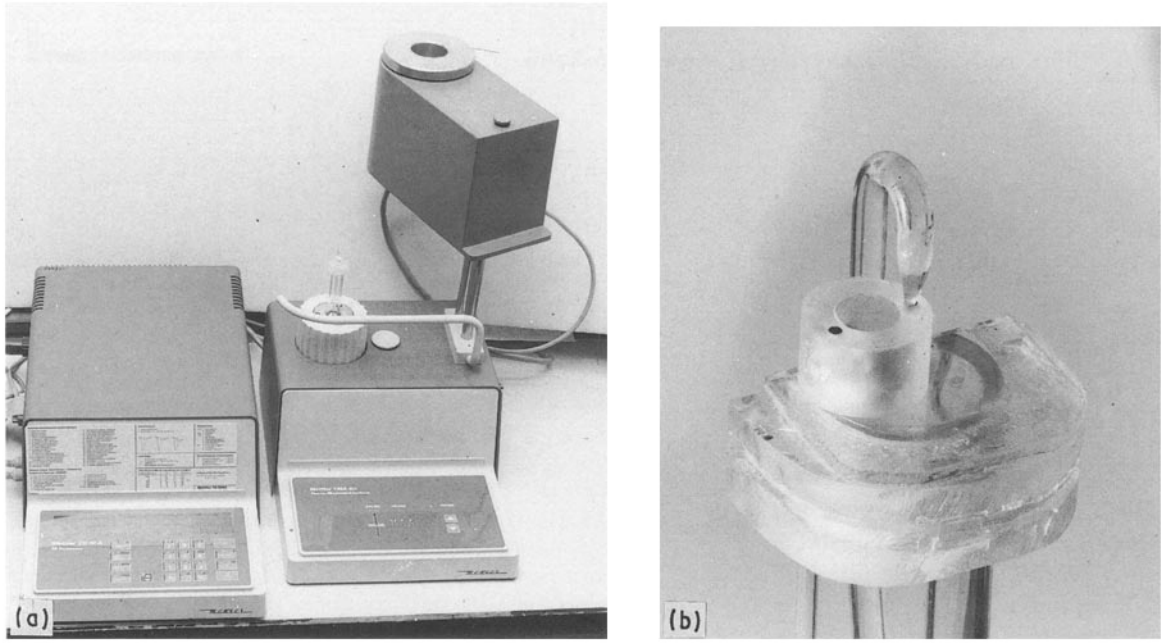

Figure 2 (a) Profile measurements are performed with a thermo-mechanical analyser (Mettler TMA 40). A close up of the quartz measuring probe is shown in (b). With the quartz measuring probe the surface of the calcium phosphate containing tube is probed on five points to measure the differences in height between the points.

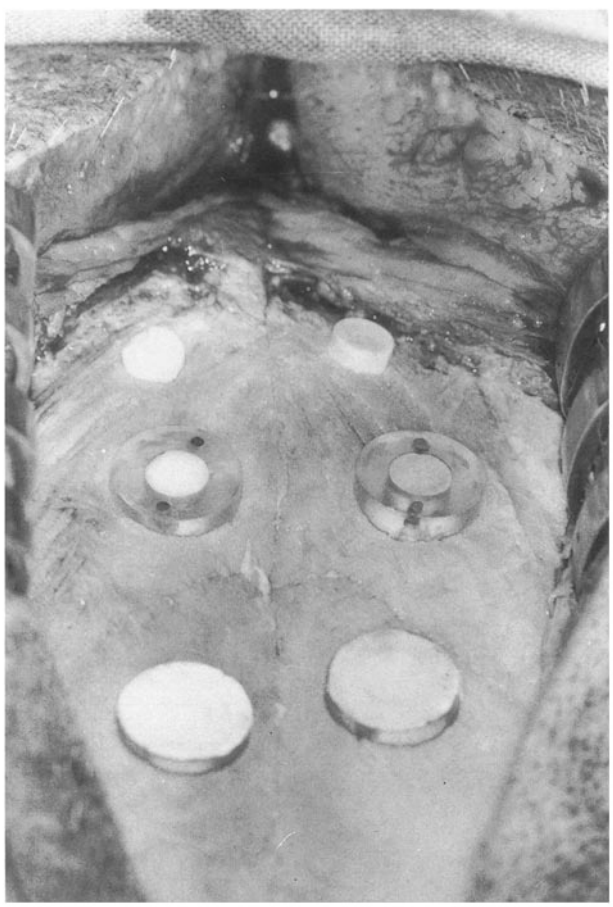

Figure 3 Distribution of the calcium phosphate cylinders over the implantation area. The two lower tubes are covered by millipore filters, those in the middle are uncovered. The upper single cylinders are destined for the tissue interaction study.

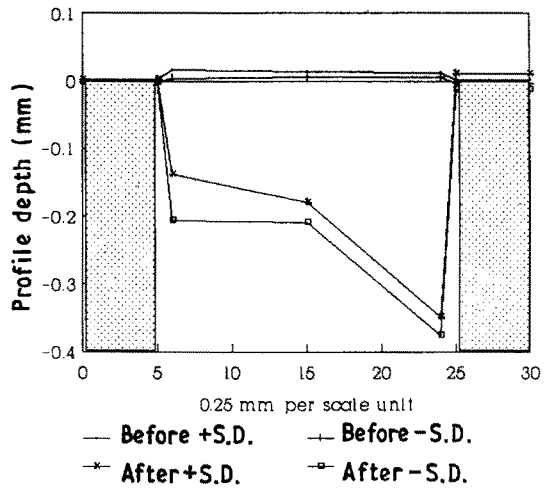

Figure 4 Resorption at the bone side of a rhenanite cylinder after 3 months, represented by the surface between the profile lines before implantation (upper lines) and after the experimental period (lower lines).

the average amount of resorption obtained by calculating the mean distance between the corresponding measuring points on the profile lines before and after the experimental periods (Fig. 4). The mean distance between these profile lines of all similar types of ceramic cylinders with identical experimental periods and the same initial tissue contact (bone side, soft tissue side, filter-covered side), were again averaged to result in the final measure of *in vivo* resorption of each type of calcium phosphate within a certain experimental period and equal initial tissue contact.

Of the 32 tubes, only 59 surfaces became available for profile measurements (Table II). In contrast to the previous 28 tubes, four tubes, two containing rhenanite (3 months) and two containing β-TCP (6 weeks), appeared to have formed a tight bond with the surrounding tissues at one or both sides and as a result could not be separated from these tissues without causing damage to the surfaces meant for profile measurements. To learn the nature of this bond, after

TABLE II Number of surfaces of the calcium phosphates per experimental period and tissue side, available for profile measurements.

	Bone side		Soft tissue side		Filter side	
	6 weeks	3 months	6 weeks	3 months	6 weeks	3 months
Rh	4	2	3	1	1	2
β-TCP	2	4	2	3	2	1
HA	4	4	3	2	1	2
Mg-W	4	4	4	2	—	2

profile measurements at non-bonded sides, these implants were added to the tissue interaction study and without decalcification were embedded in polymethylmethacrylate, sawed and Giemsa stained. Evaluation was done by light microscopy.

Owing to visual leakage of some filters, showing soft tissue growth underneath, a number of tubes containing one Rh, one β-TCP, two Mg-W and one HA cylinder(s) was added to the group of tube surfaces having initial contact with soft tissue. For the tissue interaction study, the 16 blocks containing the single cylinders were fixed immediately by immersion in 4% neutral formaldehyde solution. All Rh and β-TCP containing blocks and one Mg-W containing block were decalcified in 5% formic acid and embedded in JB4. Sections of 7 μm thick were cut and haematoxylin–toluidine blue–acid fuchsin stained. Because of difficulties in decaleifying the other Mg-W and HA containing blocks, these blocks were embedded in PMMA and sawn into 20–40 μm thick sections and Giemsa stained. Evaluation of all undecalcified sections was also done by light microscopy.

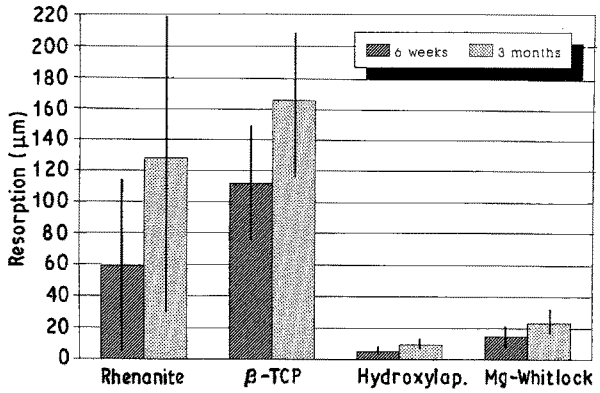

Figure 5 Resorption rates of the four calcium phosphates at the bone side after 6 weeks and 3 months. Vertical lines: ± 1 S.D.

TABLE III Statistical significant differences ($p < 0.05$) in resorption between some materials in relation to different situations by applying a Student *t*-test. Because of the relatively small number of observations the reliability of these differences can be doubted

Calcium phosphate	Exp. per.	Situation	*t*	d.f.[a]	*p*
β-TCP/Mg-W	6 weeks	bone side	5.03	4	0.01
β-TCP/HA	6 weeks	bone side	6.58	4	0.01
β-TCP/HA	6 weeks	soft tissue side	3.25	3	0.05
Rh/Mg-W	3 months	bone side	6.99	4	0.01
Rh/HA	3 months	bone side	2.85	4	0.05
β-TCP/Mg-W	3 months	bone side	5.66	6	0.002
β-TCP/HA	3 months	bone side	6.27	6	0.001
Mg-W/HA	3 months	bone side	3.28	6	0.02
Mg-W	3 months	bone side/ soft tissue side	5.13	4	0.01
Mg-W	3 months	soft tissue side/ filter side	8.02	2	0.02

[a] d.f. = degrees of freedom.

3. Results

3.1. Resorption measurement study

3.1.1. Resorption at the bone side (Fig. 5)

After 6 weeks the Rh, β-TCP, HA and Mg-W surfaces showed, rounded off to the nearest integer, average resorption values of 59, 112, 5 and 14 μm, respectively (S.D. 53, 37, 3, 7, respectively). After 3 months these values increased, in the same order, to 128, 165, 9 and 23 μm (S.D. 96, 50, 3, 8). In both periods Rh and β-TCP were much more resorbed than HA and Mg-W. A Student *t*-test showed some significant differences in resorption between the materials after the 3 month period (Table III). After the 6 week period only β-TCP showed a significant difference in relation to HA and Mg-W. In contrast to the sequence of progressive solubility *in vitro* β-TCP appeared to be more resorbed than Rh and Mg-W more than HA.

3.1.2. Resorption at the soft tissue side (Fig. 6)

After 6 weeks the resorption values for Rh, β-TCP, HA and Mg-W were 79, 122, 5 and 44 μm (S.D. 53, 56, 4, 39), respectively, whereas after 3 months these values were, in the same order, 44, 91, 10 and 53 μm (S.D. 41, 6, 3). These values seem to indicate that except for

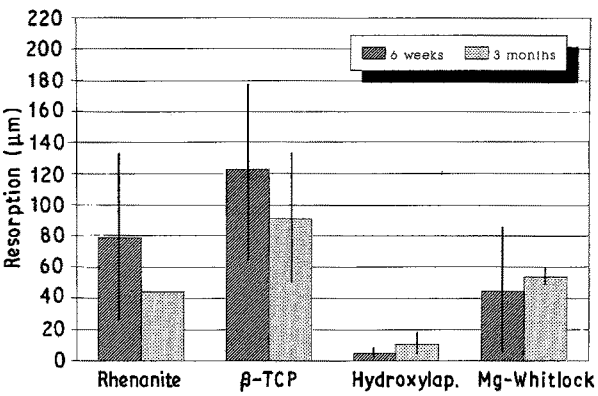

Figure 6 Resorption rates of the four calcium phosphates at the soft tissue side after 6 weeks and 3 months. Vertical lines: ± 1 S.D.

HA, resorption after 6 weeks at the soft tissue side has been higher than at the bone side. However, the observed differences were not statistically significant. After 3 months a reversed picture was observed. While the resorption of HA and Mg-W had increased in comparison to the 6 week period at this side, the resorption of Rh and β-TCP had increased. In comparison to the bone side after 3 months, only Mg-W showed a significantly higher degree of resorption.

Furthermore, the difference in resorption between HA and Mg-W after 3 months had increased with regard to the difference between the two at the bone side.

3.1.3. Resorption at the soft tissue side underneath a filter (Fig. 7)

In almost all cases resorption has been reduced. The resorption values after the 6 week period for Rh, β-TCP and HA were 16, 28 and 7 μm, respectively. Owing to the low number of observations, only the S.D. for β-TCP was obtained, being 12. No filter-covered Mg-W cylinder was available at the 6 week period. At the 3 month period the resorption values were, in the order Rh, β-TCP, HA and Mg-W, 5, 7, 6 and 12 μm (S.D. for Rh, HA and Mg-W were 0, 3 and 6, respectively. As at the soft tissue side, a reduction in resorption of Rh and β-TCP was observed when compared to the 6 week period. Except for Mg-W at the 3 month period, no significant differences were found in relation to either the bone side or the soft tissue side at both periods.

3.2. Tissue interaction study

3.2.1. Rh, 6 week period

Except for some places showing contact with bone, the implants were surrounded by a wide soft tissue layer containing a large number of osteoclast-like cells in contact with the implant surfaces. The activity of these cells was clearly directed towards the implant material, obviously causing resorption (Fig. 8). The original straight surfaces became rather notched. Inflammatory reactions were not observed. Where the upper end of the implant was left 2 mm above the bone surface with the purpose to become surrounded by soft tissue after the operation, both implants as well as all other implants in this study tended to be overgrown by bone, due to the high growth tendency of the still very young animals. This bone was separated from the implant by a soft connective tissue layer.

3.2.2. Rh, 3 month period

Compared to the 6 week period, the amount of osteoclast-like cells had increased proportional with the notching of the implant surface. The peri-implant area

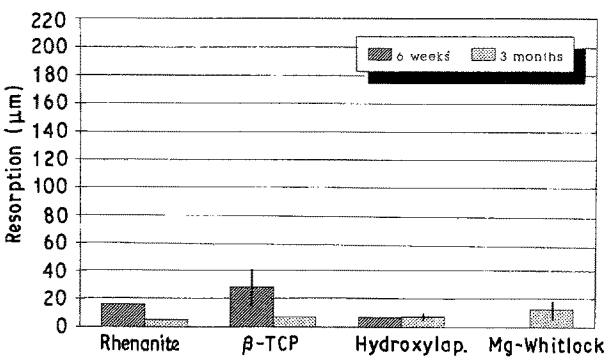

Figure 7 Resorption rates of the four calcium phosphates underneath a millipore filter after 6 weeks and 3 months. Vertical lines: ± 1 S.D.

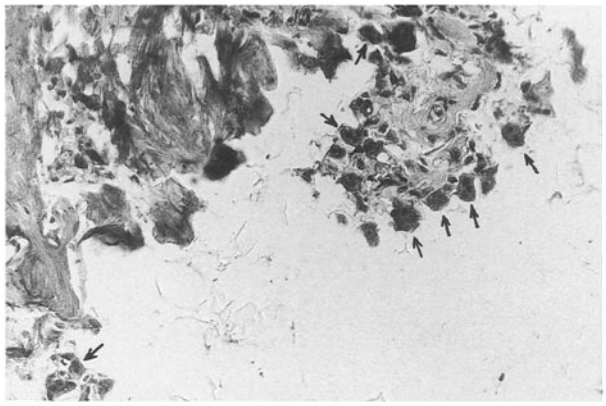

Figure 8 Rhenanite after 6 weeks. Where there is no contact with bone, the implant is surrounded by connective tissue, containing osteoclast-like cells in contact with the implant surface. (Arrows, osteoclast-like cells; decalcified section; × 42.)

now contained remnants of poorly definable tissue and implant material. However, inflammatory reactions were not found. Contact with bone was almost completely absent.

3.2.3. Two Rh containing tubes from the resorption measurement study, 3 month period

The Rh cylinders inside the tubes showed an intimate contact with bone at both ends without any soft tissue layer in between. It could be observed that initial resorption of the material took place before but obviously had stopped (Fig. 9). No osteoclast-like cells were found.

3.2.4. β-TCP, 6 week period

Both implants were surrounded by a less wide soft tissue layer than was seen with Rh. One implant had contact with bone limited to one small place. The amount of osteoclast-like cells against the implants was less when compared with Rh after 6 weeks and found at more regular distances. Both implants were clearly notched, a sign of resorption. Some diffuse inflammatory reactions were seen at protruding parts of the implants.

3.2.5. Two β-TCP containing tubes from the resorption measurement study, 6 week period

Intimate contact between bone and β-TCP had originated. Lacunae at the surface, a sign of initial resorption, were more circumscript than with Rh. These lacunae were all filled with soft tissue (Fig. 10).

3.2.6. β-TCP, 3 month period

Both implants were surrounded by a soft tissue peri-implant, decreasing in width when compared to Rh. Also the amount of osteoclast-like cells against the implant material had decreased.

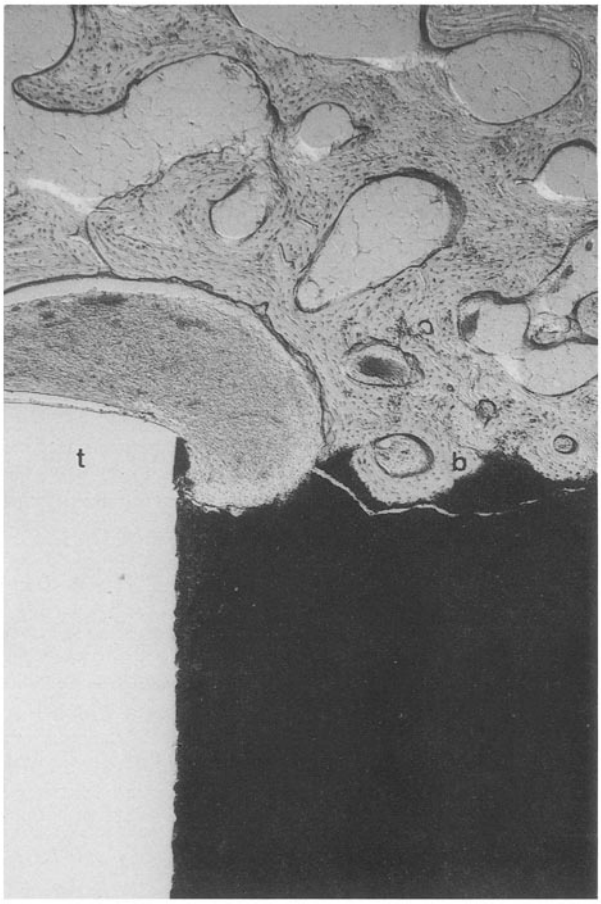

Figure 9 In contrast to Fig. 8, rhenanite (black) is shown after 3 months to have intimate contact with bone, while initial resorption has taken place. This implant cylinder was originally meant for profile measurements. No resorptive cells were found. (b, bone; t, acrylic tube; undecalcified section; × 10.)

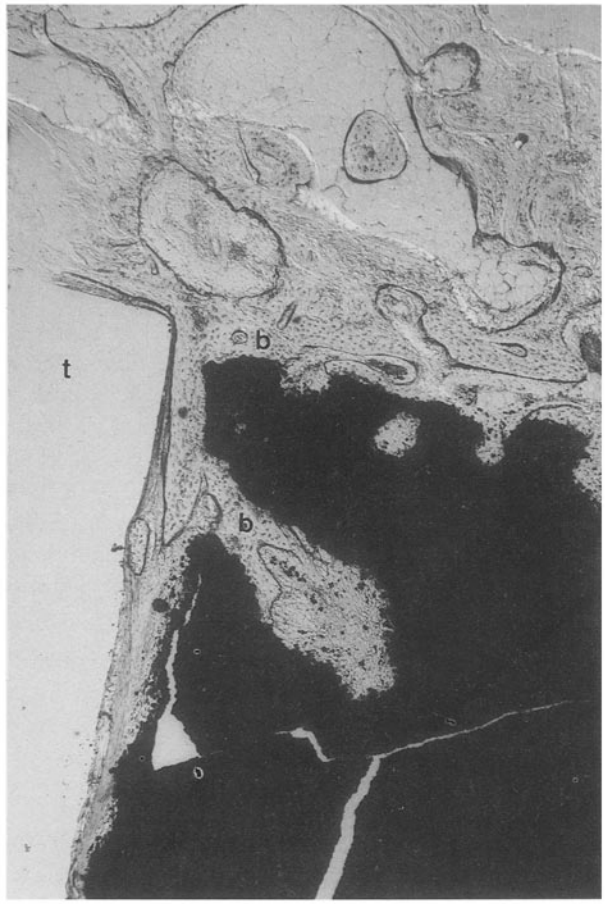

Figure 10 Two β-TCP cylinders (black) after 6 weeks and originally destined for resorption measurements, showed intimate contact with bone after initial resorption. (b, bone; t, acrylic tube; undecalcified section; × 10.)

Compared to the 6 week period, notching of the implant surface had not increased. Both implants showed a focal inflammatory reaction.

3.2.7. HA, 6 week period

Much more bone was seen to be deposited against the implant at various places while at other places a thin soft tissue peri-implant surrounded the material. The number of osteoclast-like cells was low. Many of these cells seemed to be in a less active state. The implant surface remained fairly straight. Inflammatory reactions were not found (Fig. 11).

3.2.8. HA, 3 month period

This period was comparable to the 6 week period. Contact with bone was observed at many places.

3.2.9. Mg-W, 6 week period

Except for one place, the implants were still separated from bone by a soft tissue peri-implant. Osteoclast-like cells were equal or slightly fewer in number when compared with β-TCP but definitely less when compared with Rh. Notching of the implant surface still occurred and it could be clearly seen that detached particles had been deposited in the peri-implant.

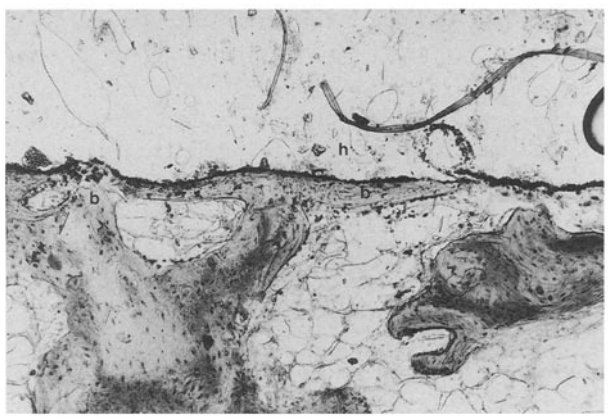

Figure 11 After 6 weeks HA had bone deposited at various places. (b, bone; h, HA; undecalcified section; × 16.)

3.2.10. Mg-W, 3 month period

The difference from the 6 week period is that several places showed bone in contact with or close to the implants (Fig. 12).

The soft tissue peri-implant was smaller than with β-TCP and Rh, poorer in resorptive cells against the implant and free of inflammatory reactions.

4. Discussion

At both bone side and soft tissue side, including filter-covered implants, a similar sequence in progressive

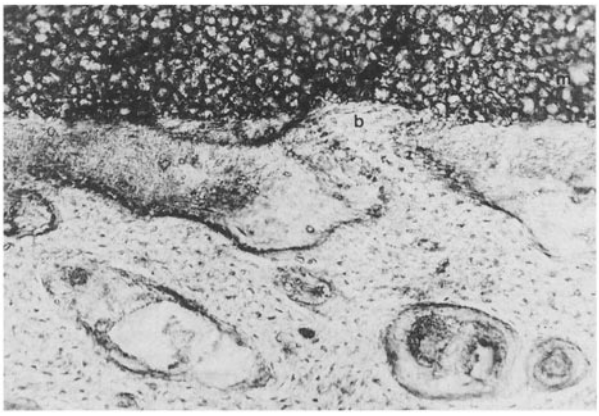

Figure 12 Mg-W after 3 months showing some contact with bone. (b, bone; m, Mg-W; undecalcified section; × 26.)

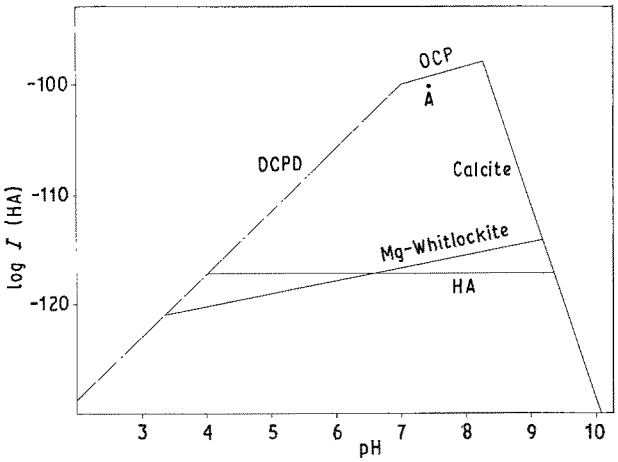

Figure 13 Relative position of the solubility lines of precipitated HA and sintered Mg-W in a plot of the negative logarithm of the ion-activity product of hydroxyapatite, log I(HA) versus pH. The location of blood plasma is about point A. DCPD, dicalcium phosphate dihydrate; OCP, octocalcalcium phosphate. This figure is composed of solubility lines already given by Driessens and Verbeeck [13].

resorption is observed, in increasing order HA, Mg-W, Rh and β-TCP. The conclusion that *in vivo* 25% microporous Mg-W is resorbed at a faster rate than 25% microporous HA is corroborated by the tissue interaction study. It is, however, not in accordance with the expectation of Driessens and Verbeeck [13] that the lower *in vitro* solubility of Mg-W correlates with its *in vivo* resorption rate. The reason for this reverse *in vivo* behaviour of Mg-W may be found in several factors, for instance the extracellular pH, which normally is about 7.4 [14]. According to Driessens and Verbeeck, only at a pH value below 6.5 would Mg-W be less soluble than precipitated HA, while above that value the reverse has been found [13] (Fig. 13). Accepting that in this *in vivo* experiment the osteoclast-like cells are real osteoclasts, acid production by the osteoclasts may be expected to have lowered the extracellular pH value [15]. As shown in the tissue interaction study, the number of osteoclast-like cells around the HA implants was less than around the Mg-W implants. The average pH value around Mg-W might have been lower than around HA, but still above 6.5, making HA resorb at a slower rate than Mg-W. The differences in solubility between Mg-W and HA, as taken over the relevant pH range, are relatively small (Fig. 13) however, and might have been overruled by other factors of influence on calcium phosphate resorption, such as sinter conditions, density or pore diameter.

Another factor which is considered to be of influence on the *in vivo* resorption of calcium phosphates is the Ca/P ratio [16–18]. In this study it is shown that 10% microporous Rh with an initial Ca/P ratio of 1, is less resorbed than 35% microporous β-TCP with an initial Ca/P ratio of 1.5. This leads to the supposition that microporosity is of greater influence on calcium phosphate resorption than Ca/P ratio. In fact, the Ca/P ratio cannot properly serve as a standard for *in vivo* resorbability of calcium phosphates. The addition, for instance, of metal oxides other than calcium oxides in the structure of the calcium phosphates, decreases the Ca/P ratio but may make the material less resorbable, as is the case with Mg-W in the present study, and has also been experienced by Klein *et al.* [19].

In the tissue interaction study the resorption of all four materials was confirmed, the originally straight surfaces of the implants being more or less affected. The estimated resorption, number of osteoclast-like cells and peri-implant width increased in the order HA, Mg-W, β-TCP and Rh. In the same order, the amount of bone deposition on the materials decreased. According to the histological picture, Rh is more resorbed than β-TCP. This finding seems to be contradicted by the profile measurements, but the difference between Rh and β-TCP was not statistically significant. On the other hand, in these profile measurements all three *in vivo* situations showed the same sequence in decreasing resorption rates, Rh being less resorbed than β-TCP, favouring the conclusion that Rh is less resorbable than β-TCP. Still, such a conclusion can only be drawn with reserve, making further investigation on *in vivo* resorption of Rh necessary.

Whether the observed resorption values will increase with time is hard to predict. As experienced by van Blitterswijk *et al.* [20], HA resorption might decrease with time because of the increasing amount of bone deposition on the implant surface, leading to a reduction in the number of resorptive cells in contact with the implant surface. This decrease in resorption might also apply for the two Rh implants originally meant for resorption measurements and showing intimate bone contact. No resorptive cells were found in these cases. Also deposition of carbonate containing apatite on calcium phosphate material as reported by Heughebaert *et al.* [21] may influence the resorption values with time by making the surfaces less soluble.

Mineral transformation of calcium phosphates *in vivo* has been reported recently in several studies [21–23]. To find an explanation for the divergent reactions of the surrounding tissues with Rh and β-TCP, sections of the two cylinders showing intimate contact with bone, were investigated by electron microprobe, X-ray diffraction and infrared spectrometry, in order to detect any quantitative and qualitative alteration in the mineral composition of the

materials, which could have led to the more favourable tissue interactions. The results of that investigation will be reported elsewhere by Driessens *et al.* [24].

The application of a millipore filter appears to reduce calcium phosphate resorption, indicating that the presence and activity of tissue cells is an important factor in calcium phosphate resorption. This is in agreement with the findings in other studies [17, 25]. Resorption of HA underneath a millipore filter does not differ much from resorption at the bone side and the soft tissue side. This may be attributed to the fact that HA by itself is so scarcely resorbable that the influence of soft tissue cells is not well measurable.

The number of observations for statistical analysis was rather small. Therefore hardly any significant difference between resorption underneath a filter, at the soft tissue side and at the bone side could be found (Table III). In general, in this study, the results of a Student *t*-test, to distinguish between differences in resorption at the three sides cannot be regarded as very reliable. A low number of observations might also explain why Rh (one observation) and β-TCP (three observations) at the soft tissue side after 3 months and after this period underneath a filter (Rh two observations, β-TCP one observation) show a lower resorption rate. This may be regarded as coincidental and not as a result of deposition of newly formed material as reported by others [21, 22]. Also, the relatively high standard deviations with Rh at the bone side after 6 weeks and 3 months may be attributed to the low number of observations.

Macrophages and foreign-body giant cells were not observed. Resorption of the material was considered to be accomplished by cells very much resembling osteoclasts. Similarly shaped cells were sometimes found against resorbing old bone in Howships lacunae. As the identity of these cells was not verified by electron microscopy, it was preferred to use the name "osteoclast-like cells" instead of osteoclasts.

For the development of a partially resorbable tooth root implant made of calcium phosphates, it may be necessary to vary the resorption rates. This may be done by modifying the factors influencing *in vivo* resorption of calcium phosphates (e.g., microporosity) and/or making composites of the calcium phosphates.

Acknowledgements

The authors thank Mr H. Schaeken, Ing., Department of Material Sciences, for manufacturing the calcium phosphates, Mr Th. Arts and his staff, Central Animal Laboratory, for their surgical assistance and devoted care for the animals. Mr S. Nottet and Mrs P. Helfrig, Department of Oral Histology, are gratefully thanked for preparing the microscopic sections.

References

1. H. W. DENISSEN and K. DE GROOT, *J. Prosthet. Dent.* **42** (1979) 551.
2. H. VELDHUIS, T. DRIESSEN, H. W. DENISSEN and K. DE GROOT, *Clin. Prev. Dent.* **6** (1984) 5.
3. J. W. QUINN, J.N. KENT, R. G. HUNTER and C. M. SCHAFFER, *JADA* **110** (1985) 189.
4. P. KANGVONKIT, V. J. MATUKAS and D. J. CASTLEBERRY, *Int. J. Oral Maxillofac. Surg.* **15** (1986) 62.
5. H. R. STANLEY, M. B. HALL, F. COLLAIZZI and A. E. CLARK, *J. Prosth. Dent.* **58** (1987).
6. D. H. BELL, *ibid.* **56** (1986) 322.
7. H. J. KWON, M. EL DEEB, TH. MORSTAD and D. WAITE, *J. Oral Maxillofac Surg.* **44** (1986) 503.
8. A. N. CRANIN, E. RONEN, R. SHPUNTOFF, G. TOBIN and J. B. DIBLING, *J. Biomed. Mater. Res.* **22** (1988) 1165.
9. I. M. BROOK, W. SATTAYASANSKUL and D. J. LAMB, *Br. Dent. J.* **164** (1988) 212.
10. M. M. A. RAMSELAAR, P. J. VAN MULLEM and J. R. DE WIJN, *J. Dent.* **14** (1986) 202.
11. R. A. TERPSTRA, F. C. M. DRIESSENS and H. G. SCHAEKEN, *Z. Anorg. Allg. Chem.* **507** (1983) 206.
12. J. ANDO and S. MATSUNO, *Bull. Chem. Soc. Jpn* **41** (1968) 324.
13. F. C. M. DRIESSENS and R. M. H. VERBEECK, 'Relation between physico-chemical solubility and biodegradability of calcium phosphates", in "Implant Materials in Biofunction, Advances in Biomaterials" Vol. 8 (Elsevier Science, Amsterdam, p. 105.
14. J. A. BERNARDS and L. N. BOUWMAN, "Fysiologie van de mens" (Bohn, Scheltema and Holkema, Utrecht, Antwerpen, 1988) p. 261.
15. R. BARON, L. NEFF, D. LOUVARD, P. J. COURTOY, *J. Cell Biol.* **101** (1985) 2210.
16. C. P. A. T. KLEIN, K. DE GROOT, A. A. DRIESSEN and H. B. M. VAN DER LUBBE, *Biomaterials* **6** (1985) 189.
17. K. KÖSTER and H. HEIDE, *Biotech. Umschau* **2** (1978) 226.
18. W. WAGNER, U. W. WAHLMANN, E. STENDER and D. HEIDEMANN, "Tissue reaction and biodegradation behavior of various calcium phosphate materials", abstract, at the European Congress on Biomaterials, Bologna (1986).
19. C. P. A. T. KLEIN, K. DE GROOT, A. A. DRIESSENS and H. B. M. VAN DER LUBBE, *Biomater.* **7** (1986) 144.
20. C. A. VAN BLITTERSWIJK, J. J. GROTE, W. KUYPERS, G. J. G. BLOK-VAN HOEK and W. TH. DAEMS, *ibid.* **6** (1985) 243.
21. M. HEUGHEBAERT, R. Z. LEGEROS, M. GINESTE, A. GUILHEM and G. BONEL, *J. Biomed. Mater. Res.* **22** (1988) 257.
22. G. DACULSI, R. Z. LEGEROS, E. NERY, K. LYNCH and B. KEREBEL, *ibid.* **23** (1989) 883.
23. R. Z. LEGEROS, I. ORLY, J. KAZIMIROFF and G. DACULSI, *J. Dent. Res.* **67** (1988) 177.
24. F. C. M. DRIESSENS, M. M. A. RAMSELAAR, A. L. H. STOLS, H. G. SCHAEKEN, P. J. VAN MULLEM and J. R. DE WIJN, *J. Biomed. Mater. Res.* (1990) submitted
25. W. DE HOLLANDER, P. PATKA, C. P. A. T. KIEIN, "Radiochemical follow up of biodegradation of calcium phosphate ceramics for bone replacement", abstract, at the 3rd World Biomaterials Congress, Kyoto, Japan, 1988.

Received 25 January
and accepted 9 August 1990