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Chemical reactions of calcium phosphate implants after implantation *in vivo*

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Sintered microporous cylinders of hydroxyapatite (OHA), tertiary calcium phosphate (β-TCP and rhenanite ($CaNaPO_4$) were implanted in the bone of the forehead of the domestic pig (*Sus scrofa*). Implants together with the surrounding bone were retrieved after 6 and 12 weeks. X-ray diffraction showed that OHA and β-TCP maintain their crystal structure upon implantation. However, rhenanite is transformed completely into an apatite within 6 weeks. This apatite later incorporates sodium and carbonate. Both β-TCP and rhenanite implants showed some resorption but were otherwise covered with new bone. Electron microprobe analysis showed that the mineral at the interface had a Ca/P ratio characteristic of new bone. At a certain distance from the interface lower Ca/P ratios were found, characteristic of precursor phases of bone mineral. This suggests that the deposition of new bone starts, at least partially, from the surface of the implant. Therefore, β-TCP as well as rhenanite may be called an osteoconductive biomaterial.

1. Introduction

In the past two decades many studies have been devoted to the biocompatibility of calcium phosphates as implant materials. The main purpose has been to develop biocompatible and possibly osteogenic materials for the replacement or augmentation of bone tissue. To date only materials from the ternary system $CaO–P_2O_5–H_2O$, such as sintered hydroxyapatite (OHA) or tertiary calcium phosphate (TCP; either the β- or the α-form), have been studied, although calcium phosphates containing carbonate, sodium, magnesium, potassium, sulphate and/or even zinc might also be safe for use as implant materials [1, 2].

The emphasis in implant studies with calcium phosphates has been on the implant quality and the histology of the surrounding bone structures. As far as the implants are concerned, a distinction has been made between hydroxyapatite ceramics as "non-resorbable" and β-TCP as well as α-TCP as "resorbable" implants [3–7]. Comparative studies [7–10] have shown that the rate of resorption of such implants was

$$\text{OHA} < \beta\text{-TCP} < \alpha\text{-TCP} \quad (1)$$

It should be noted that even densely sintered single-phase OHA is somewhat resorbable [11].

Explanations of the *in vivo* behaviour of calcium phosphate implants have been formulated primarily in terms of their *in vitro* solubility [12–15] or their *in vitro* relative rates of dissolution [16]. In fact, the relative solubilities and rates of dissolution *in vitro* in contact with aqueous solutions of a certain pH vary according to Equation 1. However, it was pointed out in [15] that extracellular fluid is normally supersaturated with OHA as well as β-TCP, so that dissolution of such implants by mere contact with body fluids can hardly be envisaged. Therefore, the cellular activity of osteoclasts which are able to decrease the pH and, hence, the degree of saturation of the body fluids in their environment, is a necessity in order to obtain the biodegradation of these implants [15] as observed in numerous animal studies. This in part explains why calcium phosphate implants do not lead to pathological calcifications in soft tissues [17] or to changes in the calcium or phosphate concentrations of blood serum [18, 19].

The question of possible reaction paths in the interaction between calcium phosphate implants and the surrounding body fluids has hardly been touched upon up to now. Driessens [15] made it clear that the reason why sintered OHA (a real oxyhydroxyapatite [20]), which has a very high solubility, resorbs at a lower rate than β-TCP can be understood only as being due to a transformation of its surface layer. Such a transformation occurs *in vitro* when sintered OHA is dispersed in dilute phosphoric acid solutions; thereby a surface layer with the probable composition

$$Ca_9(HPO_4)(PO_4)_5OH \quad (2)$$

is formed [21]. Recently, it has been shown that, both *in vivo* and in tissue culture, crystals of a carbonated

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apatitic mineral [22] grow on OHA implants, which confirms the earlier hypothesis [15].

The purpose of this study was to investigate the chemical composition and the crystal structure of retrieved implants made of several synthetic calcium phosphate ceramics. Table I gives a compilation of their chemical composition and crystal structure before implantation. Sintered OHA was included in the study as a reference material. It is known that β-TCP dissolves congruently in dilute phosphoric acid without the transformation of the surface layer [23, 24]. Renooij *et al.* [10] hypothesized that β-TCP is transformed into an apatite upon implantation. They observed a loss of 25–30% of their strontium-85 marker within 22 weeks, which seemed somewhat higher than the actual histological resorption rate. They proved that the biodegradation of TCP was cell-mediated. The only thing known about rhenanite is that it has a relatively high rate of dissolution in water, even around pH 7. This is the reason why it has been advocated as a fertilizer.

2. Materials and methods

Sintered OHA as well as β-TCP were formed by making appropriate mixtures of $CaCO_3$ (Merck number 2066) and $CaHPO_4$ (Baker 0080) and pressing in a cylindrical mould. Heating of the cylinder of OHA was carried out slowly up to 1300 °C and held at that temperature for 16 h under a stream of air. For TCP heating was carried out at 1150 °C for 1 week under a stream of air. For rhenanite $NaHCO_3$ (Merck 6329) was additionally used and heating was carried out at 1300 °C for 16 h under a stream of air. The ceramics were cooled slowly in the furnace to room temperature. X-ray diffraction of the products proved that they were single phase, whereas microprobe analysis showed them to have the correct Ca/P ratio.

Cylinders of these materials were implanted in the foreheads of domestic pigs (*Sus scrofa*) as described in [25]. Implants together with the surrounding bone were retrieved after either 6 or 12 weeks. Two rhenanite implants from one pig and two β-TCP implants from two pigs, originally meant for resorption measurements, could not be separated from the surrounding tissues without causing damage to the implant surfaces. Therefore, histological slices with a thickness of 40 μm were prepared from these four implants in coherence with the surrounding bone (see Tables II–IV, rhenanite slices nos 88016/88017 and β-TCP slices nos 88014/88015). Histological evaluation of these sections was reported in [25]. In this study X-ray diffraction with CuK_α radiation and Ni filter (Philips X-ray diffractometer) and analysis with a Camebax electron microprobe type MBI (Cameca, Paris, France) operated at 20 kV and 3 nA beam current were carried out on some of the slices. Also, some of the material was scraped off for an infrared spectrum in KBr tablets (Perkin–Elmer type 457) in order to detect whether it contained carbonate.

From three other pigs, three retrieved rhenanite cylinders (see Table II; samples nos I and IV from one pig, sample no. V from another pig) and one OHA cylinder (sample no. III from yet another pig), for which resorption measurements had previously been done [25], were additionally taken for X-ray diffraction analyses.

For comparison with the *in vivo* implantation experiments, an *in vitro* test was also carried out in which samples of rhenanite were immersed in saline solutions (0.9% NaCl) adjusted to pH 8, 7 or 6.

TABLE I Calcium phosphates used for implantation

Abbreviation	Formula	Crystal structure	Porosity (%)
OHA	$Ca_{10}(PO_4)_6(OH)_2$	Apatite [2]	25
β-TCP	$Ca_3(PO_4)_2$	Whitlockite [2]	35
Rhenanite	$CaNaPO_4$	β-Rhenanite [32]	10

TABLE II Crystal structure of implanted and retrieved calcium phosphates

Implant	Sample	Implanted structure	Period of implantation (weeks)	Retrieved structure	Remarks
$CaNaPO_4$	I	β-Rhenanite	6	Apatite	Resorption
OHA	III	Apatite	12	Apatite	Slight resorption
$CaNaPO_4$	IV	β-Rhenanite	6	Apatite	Resorption
$CaNAPO_4$	V	β-Rhenanite	12	Apatite	Resorption
β-TCP	88014	Whitlockite	6	Whitlockite	Some resorption
β-TCP	88015	Whitlockite	6	Whitlockite	Some resorption
$CaNaPO_4$	88016	β-Rhenanite	12	Apatite	Resorption, bone ongrowth
$CaNaPO_4$	88017	β-Rhenanite	12	Apatite	Resorption, bone ongrowth
OHA	87069	Apatite	12	Apatite	Slight resorption

TABLE III Quantitative composition (at %) at some spots in two slices retrieved from rhenanite implants

Slice	Spot[a]	Position	Na	Mg	P	Ca	O	Ca/P
88016	6	Bone ongrowth	0.15	0.17	2.30	2.76	94.62	1.20
	5	Bone ongrowth	0.51	0.25	4.11	4.94	90.19	1.20
	4	Interface[b]	1.59	0.07	10.22	14.34	73.78	1.40
	3	Interface[b]	2.15	0.08	11.44	16.32	70.01	1.43
	2	Interface[b]	1.71	0.08	7.51	10.03	80.68	1.34
	8	Middle of implant	3.30	0.00	12.33	17.70	66.66	1.44
	9	Middle of implant	1.83	0.00	10.81	16.39	70.97	1.52
88017	5	Bone ongrowth	0.82	0.57	4.60	5.13	88.88	1.12
	3	Bone ongrowth	0.56	0.33	6.42	9.32	83.37	1.45
	9	Interface[b]	2.20	0.00	9.37	13.21	75.22	1.41
	1	Interface[b]	0.30	0.24	5.69	7.65	86.13	1.34
	2	Interface[b]	2.11	0.09	8.53	11.71	77.57	1.37
	7	Middle of implant	1.47	0.08	5.99	9.18	83.27	1.53
	6	Middle of implant	1.24	0.00	9.08	14.04	75.65	1.55
	8	Middle of implant	1.85	0.00	9.13	13.44	75.57	1.47

[a] The measuring time was 1 min.
[b] At the side of the bone ongrowth.

TABLE IV Quantitative composition (at %) at some spots in two slices retrieved from β-TCP implants

Slice	Spot	Position	Na	Mg	P	Ca	O	Ca/P
88014	5	Bone ongrowth	0.00	0.00	1.09	1.38	97.53	1.27
	6	Bone ongrowth	0.14	0.04	0.87	1.08	97.87	1.24
	1	Interface[a]	0.20	0.00	6.94	10.50	82.36	1.51
	2	Interface[a]	0.36	0.00	6.05	9.41	84.18	1.56
	3	Interface[a]	0.35	0.00	6.76	11.39	81.50	1.68
	7	Middle of implant	0.27	0.00	7.22	10.25	82.26	1.42
	8	Middle of implant	0.48	0.00	10.25	14.62	74.65	1.43
88015	4	Bone ongrowth	0.00	0.00	1.28	1.47	97.24	1.15
	3	Bone ongrowth	0.12	0.11	2.04	2.56	95.17	1.25
	6	Interface[a]	0.00	0.08	7.34	12.21	80.29	1.64
	2	Interface[a]	0.00	0.06	7.94	11.94	80.06	1.50
	1	Interface[a]	0.33	0.13	8.82	12.90	77.82	1.46
	5	Middle of implant	0.20	0.00	7.51	11.39	80.90	1.52

[a] At the side of the implant.

3. Results and discussion

In Table II the results of X-ray diffraction studies before implantation and after retrieval are shown. It appears that OHA and β-TCP implants maintained their crystal structure during the implantation period. However, rhenanite was completely transformed into an apatite within 6 weeks of implantation (see Table II and Fig. 1). This is corroborated by the quantitative analysis with the electron microprobe as shown in Table III. Rhenanite seemed to have lost most of its sodium and also either to have gained calcium or to have lost phosphate. The final Ca/P ratio was close to 1.5 (Ca/P = 1.50 ± 0.05) and the crystal structure was undoubtedly that of apatite. The resolution of the X-ray diffraction pattern was distinctly better than that of natural bone mineral and somewhat poorer than that of dental enamel when scanned under the same operational conditions. In the middle of the rhenanite implant it also appeared to be better than at the interface with the bone ongrowth. The peak positions in the X-ray diffraction pattern of the rhenanite implant resembled closely those of the apatite in dental enamel. The infrared spectrum of the retrieved rhenanite implant showed the presence of carbonate (Fig. 2). The carbonate content was high at the periphery of the implant and decreased at the centre. As followed from the infrared spectra of successive scrapings, it rose in that centre with the time of implantation. Hence, rhenanite was transformed into a sodium- and carbonate-containing apatite upon implantation. Not only resorption of the implant occurred [25], it was even preceded by a relatively quick transformation of the implant into apatite, which was complete within 6 weeks. Later the carbonate content of that apatite increased.

Around the rhenanite implant, about 20 μm from the interface with the bone ongrowth (Table III), the bone mineral contained some sodium and magnesium and had a Ca/P ratio of 1.38 ± 0.04. This is characteristic of young bone [2]. At a much larger distance from the implant in the bone ongrowth sodium and magnesium also occurred, but the Ca/P ratio was somewhat lower: 1.25 ± 0.12. This is characteristic of the precursor mineral in newly formed bone [26–28].

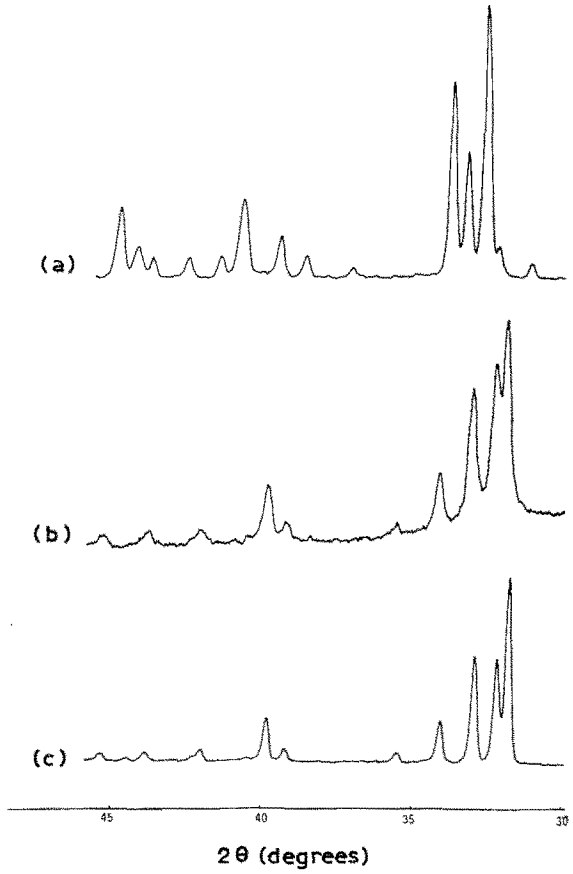

Figure 1 X-ray diffraction pattern (b) represents an X-ray diffractogram of rhenanite sample V, 3 months after implantation. Compared with (a), rhenanite before implantation, the peak positions are more in agreement with those of (c), sintered hydroxylapatite.

Hence, these data indicate that the bone ongrowth on the implant started, at least partially, from the surface of the implant. For this reason rhenanite may be called an osteoconductive biomaterial. The Ca/P ratio of the rhenanite before implantation was 1.00 ± 0.01.

As far as the β-TCP implants are concerned, they showed some resorption [25] and mostly maintained their crystal structure (Table II), but the electron microprobe analysis showed that the mineral had lost some calcium and gained some sodium upon implantation (see Table IV). This is conceivable in view

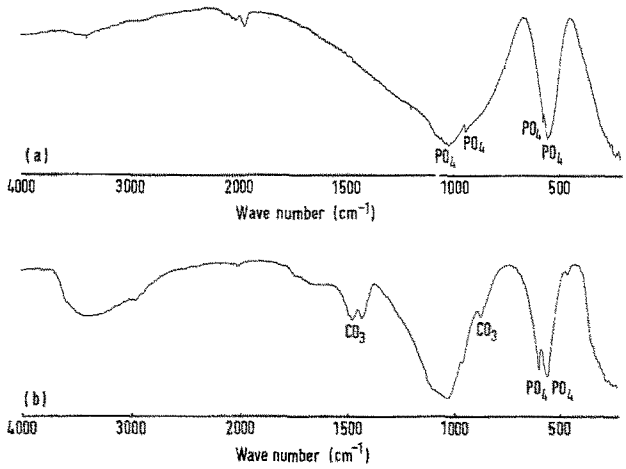

Figure 2(a) Infrared spectrum showing that rhenanite before implantation does not contain carbonate and (b) infrared spectrum of rhenanite sample V, 3 months after implantation, showing the presence of carbonate.

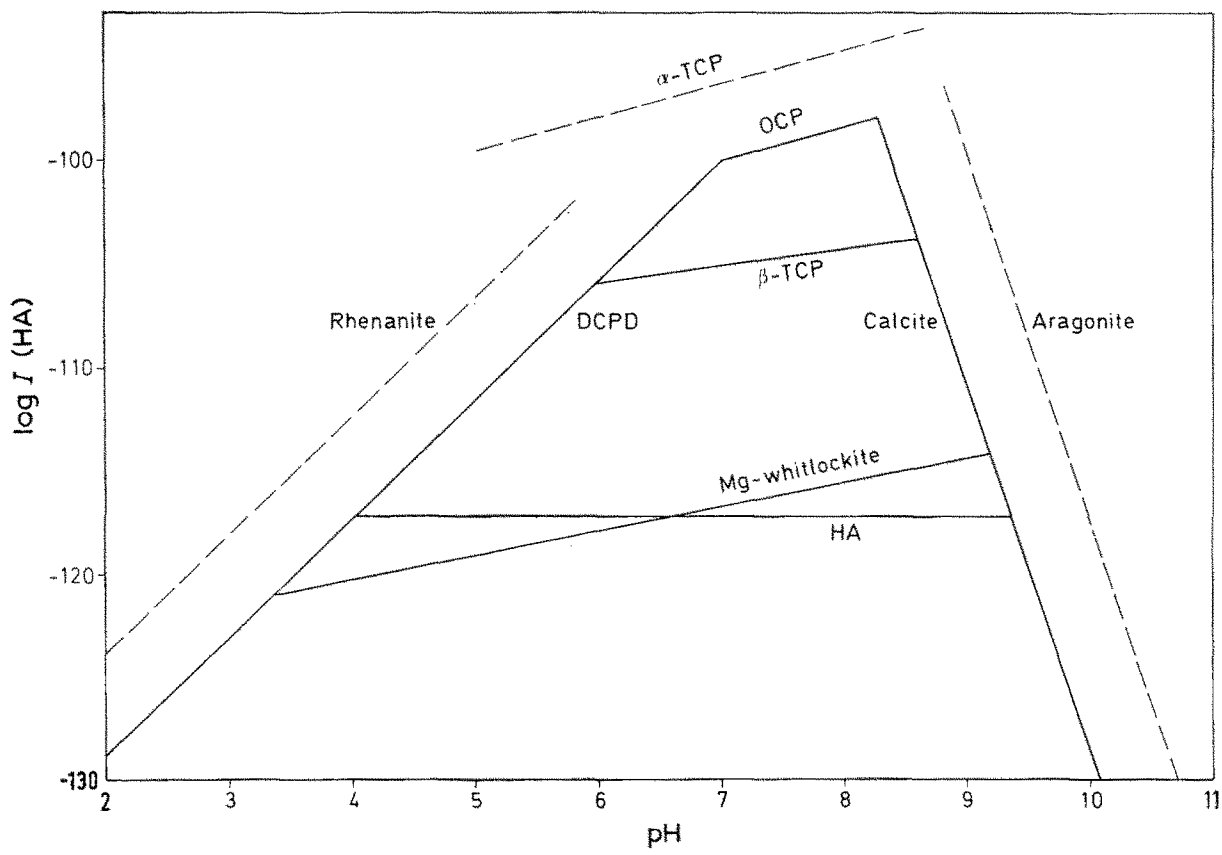

Figure 3 Solubility product diagram in which the negative logarithm of the solubility product of hydroxyapatite log I(HA) is plotted as a function of pH. The solubility lines for dicalcium phosphate dihydrate (DCPD), octocalcium phosphate (OCP), calcite, β-TCP, or tricalcium phosphate and magnesium whitlockite are given as well as the tentative solubility lines for rhenanite, α-TCP, and aragonite.

of the fact that both β-$Ca_3(PO_4)_2$ and $Ca_{10}Na(PO_4)_7$ have the whitlockite structure and form a continuous series of solid solutions [29]. This was further corroborated by the fact that the retrieved implant did not contain carbonate according to its infrared spectrum.

Around the β-TCP implants the composition of mineral at the interface with the bone ongrowth and that within the bone ongrowth at a certain distance from the implant showed the same pattern (Table IV) as around rhenanite implants (Table III). For the same reasons β-TCP might also be called an osteoconductive biomaterial.

The complete transformation of rhenanite into apatite upon implantation is of particular interest. Similarly, Guillemin *et al.* [30] reported the transformation of natural aragonites into apatite upon implantation, whereas Oohnishi *et al.* [31] reported that the β-TCP particles of a calcium phosphate cement transformed also into an apatite upon implantation. In this study we attempted to transform rhenanite *in vitro* into an apatite by submersion in an aqueous solution resembling the inorganic composition of extracellular fluid. We did not succeed in this transformation when the pH was around 8. However, when the pH was decreased to a value of around 6 by bubbling CO_2 through the aqueous solution, we succeeded in transforming rhenanite into an apatite *in vitro*. This might indicate that cellular activity and CO_2 production by the living cells activate the transformation of rhenanite into apatite *in vivo*. The abovementioned transformation of calcium phosphates upon implantation shows a certain pattern which depends on the position of their solubility isotherms in a logarithmic plot of their solubility product as a function of pH (Fig. 1). The usefulness of such plots has been illustrated extensively elsewhere [32]. The pattern of reaction *in vivo* appears to be such that those calcium phosphates having a solubility higher than that of DCPD, OCP or calcite are transformed into an apatite resembling bone minerals, whereas those with a solubility lower than that of DCPD, OCP or calcite retain their crystal structure after implantation.

4. Conclusion

X-ray diffraction, electron microprobe analysis and infrared spectroscopy were used to evaluate the changes occurring in, on and around calcium phosphate implants. Furthermore, application of knowledge about the dynamics of bone mineral in vertebrates [25–27] was used to draw conclusions about the osteoconductive potential of such implants. In this study it was found that rhenanite is transformed into an apatite within 6 weeks after implantation and that both β-TCP and rhenanite may be called osteoconductive biomaterials.

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