JOURNAL OF MATERIALS SCIENCE: MATERIALS IN MEDICINE 5 (1994) 219–224

In vitro testing of plasma-sprayed hydroxyapatite coatings

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Hydroxyapatite-coated coupons were tested in various physiological media. Immersion in Ringer's solution showed that heat-treated coatings displayed a weight gain but the as-sprayed coatings underwent a weight loss. Dissolution of the coating was measured by weighing the specimen before and after ageing. Immersion of hydroxyapatite coatings showed the appearance of small spheres that were identified by X-ray diffraction as hydroxyapatite. Changes in coating morphology were detected and the coating degradation mechanisms are discussed. This paper thus looks at the morphology, composition, crystallinity and dissolution of hydroxyapatite coatings aged in Ringer's solution.

1. Introduction

In vitro tests can be performed in a cultured cell medium or a salt-containing solution. The first type of test assesses biocompatibility by observing the behaviour of cells in the presence of the material. The types of response that indicate toxicity are cell death, reduced cell adhesion, altered cell morphology, reduced cell proliferation and reduced biosynthetic activity [1]. Salt solutions which attempt to duplicate body conditions are used to observe the behaviour of biomaterials after certain immersion periods. The second type of test is solely used to study material changes.

Biological implants require a surface that is compatible with the body environment. Thermal spraying is a well-known technique that has been chosen to produce coatings varying from 50 to 400 μm thick for biological applications. The process is clean and the high rates of deposition allow coatings to be produced fairly rapidly. Since thermal spraying is a direct line-of-sight process, isolated areas on an implant can be coated or the entire surface of a complex geometry can be coated by rotating the object. Any substrate can be used but for reasons of practicality, ceramics or metals are chosen. The process enables control over properties, such as porosity, surface morphology, roughness, composition and crystallinity which in turn influence chemical, physical and mechanical properties.

Hydroxyapatite is well known as a biocompatible material. It has the ability to bond to osseous and epithelial tissue and is accepted by muscle tissue. The reason for its acceptability lies in a composition similar to the mineral phase of bone. Bone consists of collagen and hydroxyapatite. Synthetically produced hydroxyapatite is very similar to that found in the body except that it does not contain the small additions of sodium, magnesium, fluorine and potassium. Hydroxyapatite ceramics become integrally incorporated into the body upon implantation.

Most biocompatibility studies have focused upon *in vivo* performance of the material. Hydroxyapatite has been implanted into guinea pigs, rats, cats, dogs, goats and people. Little *in vitro* research has been reported where biocompatibility is tested with cultures, and even less is documented about the effect on the properties of hydroxyapatite after immersion for different time periods.

Coatings have been produced with different morphologies and one of the common characteristics is the presence of cracks and pores. These features are generally interpreted as being detrimental to the mechanical properties of the coating but their presence is sometimes favourable. Cracks may form to relieve residual stresses which otherwise would lead to premature failure, and pores may be important by arresting propagating cracks. These two characteristics are important in high-stress applications since they determine the life of the component, but they may be accepted in low-stress applications.

2. Experimental procedure

Twenty-four hydroxyapatite coatings of about 200 μm thickness were produced by plasma-spraying 55–85 μm hydroxyapatite powder on to 316 stainless steel substrates (surface area about 281 mm^2). The plasma gas consisted of argon and helium. Powder

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was transported from a Metco 3 MP powder feeder at a feed rate of 3 g min^{-1} with argon as a carrier gas set to a flow rate of 3 l min^{-1}. A power level of 34 kW and a stand-off distance of 22.5 cm were chosen.

A 20 l Perspex tank held the Ringer's solution (Table I). After preparation of the Ringer's solution it was immediately heated to 37.5 °C and carbogen gas added to adjust the pH to 7.35, thus preventing the formation of any precipitate. The liquid composition was continually adjusted by the automatic addition of distilled water.

The hydroxyapatite coatings were either left in the as-sprayed condition or heat-treated at 800 °C for 2 h. Hydroxyapatite-coated and uncoated 316 stainless steel coupons were weighed before immersion in the Ringer's solution. The 316 stainless steel reference coupons accounted for weight changes which were not associated with the ceramic coating. The coupons were removed and placed in an ultrasonic bath to remove any loose debris after periods of 1 day, 2 days, 4 days, 1 week, 2 weeks, 4 weeks, 8 weeks and 12 weeks. The coupons were dried at 180 °C for 2 h and the following tests conducted. The weight change of the coating was determined. The roughness of the coating was measured using a Surtronic 3P roughness tester. The composition and amorphicity were determined from X-ray diffraction patterns between 2θ angles of 20 and 60°. Finally the ceramic surface was sputter-coated with gold and examined by scanning electron microscopy. An acceleration voltage of 10 kV was used to minimize any surface charging phenomenon.

3. Results

3.1. Dissolution measurements

The reference 316 stainless steel coupons showed a maximum variation of 0.01 and 0.02% in weight change for the as-received and heat-treated coupons, respectively, after immersion for up to 16 weeks. As-sprayed and heat-treated coatings behaved differently. The as-sprayed coating showed a weight loss which increased with time (Fig. 1). The initial weight loss was rapid, followed by dissolution at a lower rate. A maximum decrease of 30% of the coating weight was recorded. The heat-treated coatings showed a weight increase with ageing time (Fig. 1). After a period of 8 weeks the maximum weight gain was achieved. This corresponded to a value of 9% of the coating weight.

TABLE I Salts used to make up 20 l of Ringer's solution

Salt	Mass (g)	mol l^{-1}
NaCl	80.00	13×10^{-2}
KCl	7.40	4.96×10^{-3}
$MgCl_2 \cdot 6H_2O$	4.00	9.84×10^{-4}
$NaH_2PO_4 \cdot 2H_2O$	3.20	1.14×10^{-3}
$CaCl_2$	5.80	2.61×10^{-3}
$NaHCO_3$	40.00	2.38×10^{-3}

3.2. Roughness measurements

The r.m.s. roughness of all coatings was 10 ± 1 μm. The roughness was not influenced by ageing in physiological media.

3.3. Composition and crystallinity measurements

X-ray diffraction (XRD) determined that the as-sprayed coatings were amorphous. The XRD traces exhibited low peaks that were spread over a large range of d spacings. Heat-treated coatings, however, displayed well-defined peaks that represented crystalline material (Fig. 2). An increase in peak height was detected (Fig. 3) after ageing the as-sprayed coatings.

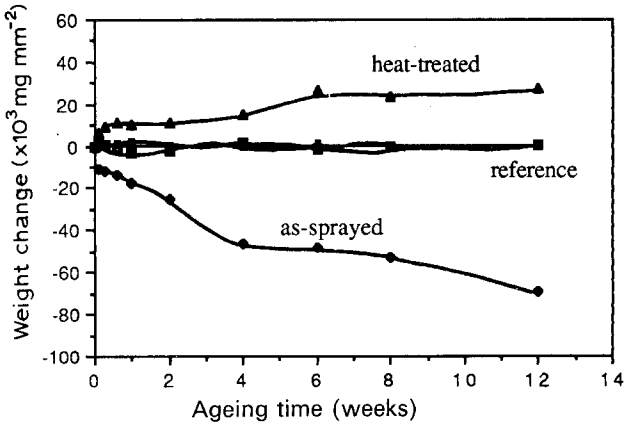

Figure 1 Weight changes for (◆) as-sprayed, and (▲) heat-treated samples aged in Ringer's solution; (■) reference samples.

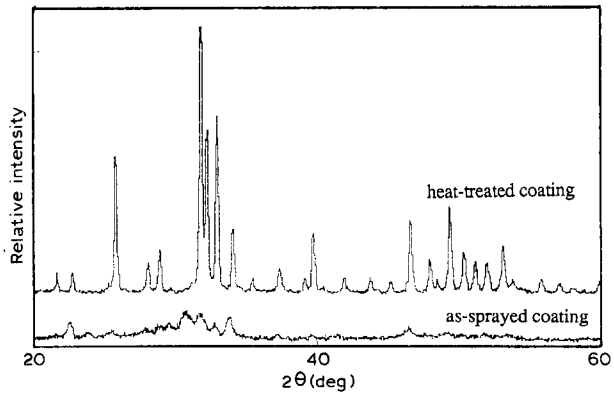

Figure 2 X-ray diffraction patterns of amorphous (as-sprayed) and crystalline (heat-treated) coatings.

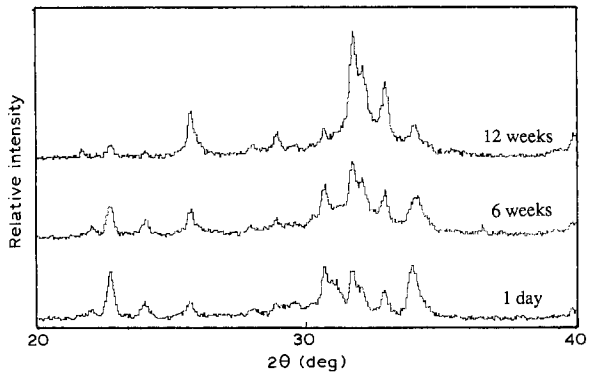

Figure 3 Increase in crystallinity shown as an increase in peak height for amorphous coatings after various ageing times.

An increase in crystallinity had occurred at 12 weeks and the pattern looked identical to a heat-treated coating. X-ray diffraction on the heat-treated coatings did not show any difference after ageing in Ringer's solution for 12 weeks.

3.4. Morphological examination

3.4.1. As-sprayed coatings

There were noticeable changes in the coating morphology after ageing in Ringer's solution. The as-sprayed coating exhibited a surface with well-molten splats, fine cracks and small 1 μm unmolten particles present on the surface (Fig. 4a). This morphology changed after ageing in Ringer's solution for 1 day. Individual splats exhibited cracking (Fig. 4b) which caused rough edges to be formed. Sometimes splat fragments could be found in the close proximity of the cracked splats. Any cracks in the coating did not show a change in size. Small pores could also be identified in the splats.

After 2 days the number of fractured splats had increased considerably. The surface appeared more rough with increased ageing time, although this change could not be measured by surface profilometry. Large regions of material were removed from many locations within the coating. Fractured splats were found in the valleys of the general topography and these features are typical of those seen in Fig. 4c. The side-elevation view of a region where many splats had deposited on one another showed that all the splats were fractured. Three to six micron pores were found in individual splats. These pores always exhibited rounded edges and were sometimes circular.

After 4 days of ageing many splat layers could be observed as the holes increased in size; this phenomenon is similar to pin-hole corrosion. The fractured sides of splats that were originally rough in appearance now exhibited smooth sides and gave a rounded appearance to the whole splat. One week of ageing (Fig. 4c) did not bring about much change except that small 2 μm spheres agglomerated on the smooth surface of splats. These small spheres contained calcium and phosphorus and were only found in localized regions on the coating.

The same surface morphology was observed for up to 4 weeks at which point the cracked and rounded splats appeared much smaller. These rounded splats were found over all the coating (Fig. 4d). There was a significant change in the appearance of the surface after 6 weeks. Small (2–5 μm) calcium- and phosphorus-containing spheres were more numerous on the surface and after another two weeks (i.e. total of 8 weeks) a dense blanket of these small spheres formed (Fig. 4e). The original splats were not visible at 12 weeks since the small spheres covered the whole surface of the coating (Fig. 4f).

3.4.2. Heat-treated coatings

Initially the heat-treated coating appeared relatively clean with spheres or small particles present (Fig. 5a). These features are quite typical of thermally sprayed coatings and represent the residue of particles which have undergone a severe splat process. The surface cracks were about 0.8 μm wide and were larger than for the as-sprayed coating (0.4 μm wide). The cracks opened up to twice this size after ageing for 1 day (Fig. 5b). One additional day did not bring about any other changes. Four days of ageing, however, showed that cracks within individual splats were causing delamination of the coating. Features of half-lamellae were observed. These were still intact with the underlying

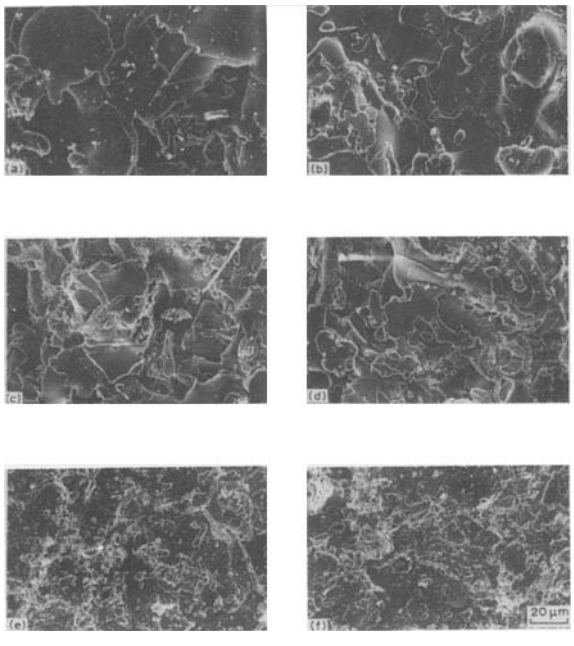

Figure 4 Surface morphology of as-sprayed hydroxyapatite coatings after being aged for (a) 0 days, (b) 1 day, (c) 1 week, (d) 4 weeks, (e) 8 weeks and (f) 12 weeks in Ringer's solution.

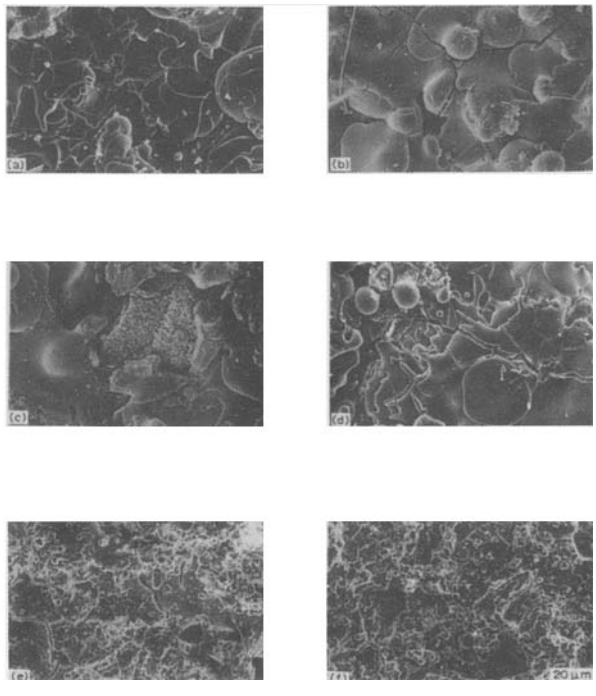

Figure 5 Surface morphology of heat-treated hydroxyapatite coatings after being aged for (a) 0 days, (b) 1 day, (c) 1 week, (d) 4 weeks, (e) 8 weeks and (f) 12 weeks in Ringer's solution.

coating (Fig. 5b). Occasionally a rough surface was observed where part of a lamella had been removed from the surface. Sections of lamella that had de-adhered from the coating were not present on the surface. After 1 week, the lamellar cracking frequency increased and sections within the coating showed cracking that divided the surface into approximately 10 μm sized fragments. This phenomenon was only observed over part of the coating surface. Some cracks were 2 μm wide and did not propagate into the underlying coating (Fig. 5c).

Small square blocks were revealed in those locations where rust-like spots were observed in the coating that had been aged for 1 week. These blocks were identified as being composed of mainly calcium, phosphorus and a trace of iron. Some smooth well-molten surfaces were still cracked with no evidence of further changes (Fig. 5d) after 4 weeks. Some locations, however, were covered with 1 μm sized spherical artifacts. These calcium-containing spheres were not uniformly distributed over the coupon surface. The calcium to phosphorus ratio of the spheres increased after 8 weeks and their size increased to 5–10 μm (Fig. 5e), so that they dominated the surface features. Microanalysis detected only traces of potassium and chlorine on the surface. Small calcium-containing spheres were detected on the flat surface and at cracks. Growth of some calcium-rich crystals was observed after 12 weeks. These 80 μm sized crystals (Fig. 5f) were detected at several sites. The remaining surface was densely populated with the 5–10 μm spheres. Few cracks could be observed after ageing for 12 weeks due to the obliteration of surface features by the dense population of spheres.

4. Discussion

Hydroxyapatite coatings behave differently depending on the post-treatment after plasma spraying. Amorphous (as-received) coatings show a weight loss which levels out after about 8 weeks to 30 wt %. Coatings that are heat-treated at 800 °C for 2 h, on the other hand, show a weight gain of 9 wt % after 8 weeks of ageing in Ringer's solution.

Hastings *et al.* [2] and Harris [3] have reported that amorphous coatings are undesirable for implantation since they are believed to be resorbed by bone tissue. In the present study, the X-ray diffraction results from the amorphous coatings show an increase in hydroxyapatite peak heights after *in vitro* ageing and this suggests an increase in crystallinity. Klein *et al.* [4] have also established that the amorphicity of plasma-sprayed hydroxyapatite coatings is replaced by a more crystalline structure after 6 weeks *in vivo* and 3 months *in vitro* ageing. The amorphous and the heat-treated coatings will be discussed in turn.

4.1. Amorphous (as-received) coatings

Klein *et al.* [4] show that there is a loss of calcium for hydroxyapatite, tetracalcium phosphate and tricalcium phosphate coatings. This loss decreases with time and after about 4 weeks there is minimal dissolution. Dissolution measurements, (Fig. 1) taken over a period of 12 weeks show that the rate of dissolution decreases after 4 weeks but then increases slightly at the 6-week mark. The amount of dissolution corresponds to 28.4% of the coating weight for the sample at 12 weeks. The loss in thickness corresponds to 47 μm if a density of 3.17 $g\,cm^{-3}$ is assumed. This compares well with the dissolution value of 50 μm provided by De Groot [5] who performed *in vivo* studies. Whether there is still any dissolution of the coating after 12 weeks is not known, but the blanketing effect of the small spheres developed on the coating seems to suggest that further dissolution is minimized. On the basis of inspecting the coating at 12 weeks, it appears that the coating would be covered with more spheres after longer immersion times until the surface is totally covered. No further dissolution would be expected after this stage. According to Osborn [6] the bone grows up to hydroxyapatite in 12 weeks and so the dissolution phenomenon shown (Fig. 1) is a reasonable estimate of coating behaviour in the body, disregarding the effect of other constituents in the physiological system (such as enzymes and proteins) on the coating.

The model proposed (Fig. 6a) shows the development of different stages in the microstructure. The initial coating has some cracks and these are indicated by dark lines. Cracks are both interlamellar and intralamellar. Upon immersion the number of cracks increases and a shift in peak positions in X-ray diffraction traces is evidence for internal (or residual) stresses being released. These small coating fragments can be carried away by the solution when cracks sufficiently separate the individual splats. The third stage also shows that weight loss may occur when surface splats become rounded and smaller in size. The final phase observed in the ageing of as-received specimens is the

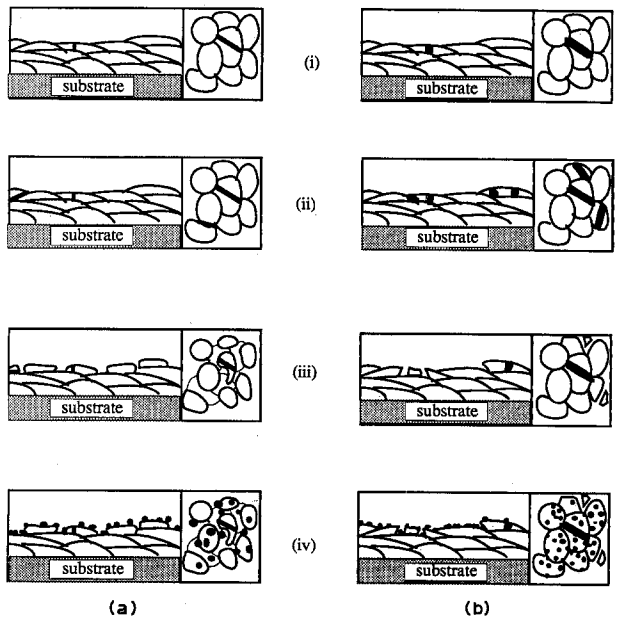

Figure 6 Proposed model showing the changes in the microstructure of (a) as-sprayed and (b) heat-treated specimens after *in vitro* ageing. Profile and planar views are presented of (a) as-received coatings and (b) heat-treated coatings.

appearance of small calcium- and phosphorus-rich spheres.

The appearance of these micro-balls coincides with an increase in the height of the hydroxyapatite peak (XRD) and a decrease in the dissolution of the coating. This implies that the micro-balls are hydroxyapatite nucleating and growing on the surface after about 6 weeks. Hyakuna *et al.* [7] have found that a calcium phosphate layer consisting of 1 μm sized balls forms on the surface of sintered hydroxyapatite and Adam *et al.* [8], who aged tricalcium phosphate in water at 80 °C, found that the outer layer transforms to hydroxyapatite. A coating that contains tricalcium phosphate could thus have hydroxyapatite forming through reaction 1 below. The mechanism by which crystalline hydroxyapatite forms on amorphous hydroxyapatite is not known.

$$4Ca_3(PO_4)_2 + 3H_2O \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 2Ca^{2+} + 4OH^- \quad (1)$$

4.2. Heat-treated (crystalline) coatings

Heat-treated coatings exhibited an increase in specimen weight with ageing time in Ringer's solution. For the specimen aged for 12 weeks there was an increase of 5.3 wt % which corresponds to a thickness increase of 1.6 μm (if the density is taken as 3.17 $g\,cm^{-3}$) and porosity is ignored. There is no change in terms of roughness and phase composition after *in vitro* ageing for 12 weeks.

In vitro ageing of heat-treated coatings differs from that of as-received coatings. Upon heat treatment the cracks increase in size and this is indicated with thicker lines, in Fig. 6b. The number of cracks increases upon immersion in Ringer's solution but not as much as with the as-received coatings. Some segments of coating become dislodged and are removed while other cracked splats remain. The geometry of a cracked splat remains jagged at the previous location of the crack. The last stage observed is the presence of hydroxyapatite spheres on the surface of the coating. There is no preferential location for these spheres and they are found on all the features of the coating, such as cracks, interlamellar boundaries and on splats.

The surface morphological changes are not conclusive with regard to understanding the weight gain after immersion. Two mechanisms are suggested for this increase in coating weight. A weight gain of 2 mg after an ageing time of 1 day suggests that some of the weight gain could be attributed to moisture absorption. All of the moisture could not be removed in the drying stage of the coating assemblies after removal from the Ringer's solution because a higher temperature is necessary [9, 10]. The hydroxyapatite spheres are believed to contribute to the increase in weight at later stages. The spheres appear 2 weeks earlier for the as-sprayed coating and have a semi-amorphous structure. This has a direct implication in the rate of bonding of bone to the coating. The bonding to bone occurs more rapidly when the surface film is formed at a faster rate [7].

The cracking of the individual splats is probably due to the release of residual stresses that form during the coating process and upon cooling [11, 12]. The X-ray diffraction patterns show that after ageing there is a decrease in the hydroxyapatite main peak *d* spacing, and this change may result from the presence of residual stresses prior to ageing. The heat treatment gives rise to a different mode of residual stress release. Heat-treated hydroxyapatite coatings show that the cracks, which are initially about 0.5 μm wide, increase to about 1.5 μm in size after ageing, whereas the as-sprayed coatings do not show any indication of crack opening.

5. Conclusion

The *in vitro* behaviour of hydroxyapatite coatings depends on the thermal history of the coating after thermal spraying. As-sprayed amorphous hydroxyapatite coatings begin to dissolve in Ringer's solution and this loss decreases with time. Hydroxyapatite coatings heat-treated at 800 °C for 2 h display a weight gain. The as-sprayed coating shows changes in the coating morphology with coating time. Individual lamellae crack and separate from the coating. Those lamellae still intact show evidence of dissolution. After a period of 8 weeks small hydroxyapatite spheres cover the surface of the as-sprayed and heat-treated coatings. These morphological changes are expected to influence the rate of bone bonding. Therefore post-treatment of hydroxyapatite coatings is seen as a technique to alter or control implant–tissue interactions.

Acknowledgements

The authors wish to thank the CSIRO/Monash University Collaborative Grant Fund that has enabled much of this work to be performed. Karlis Gross has been supported by a Monash Research Scholarship.

References

1. C. J. KIRKPATRICK and C. MITTERMAYER, *J. Mater. Sci. Mater. Medicine* **1** (1990) 9.
2. G. W. HASTINGS, D. DAILY and S. MORREY, in Proceedings of 1st International Bioceramic Symposium, Kyoto, Japan, April 26, 1988 "Bioceramics", edited by H. Oonishi, H. Aoki and K. Sawai (Ishiyaku EuroAmerica, Tokyo, 1990) p. 355.
3. D. H. HARRIS, in "Overview of Problems Surrounding the Plasma Spraying of Hydroxyapatite Coatings", pp. 419–423 of "Thermal Spray Research and Applications", Edited by T. F. Bemecki, ASM International Cleveland, Ohio, 1990.
4. C. P. A. T. KLEIN, W. J. A. DHERT, J. M. A. DE BLIECK-HOGERVORST, J. G. C. WOLKE and K. DE GROOT, in Proceedings of 7th Cimtec World Ceramic Congress, "Ceramics in Substitutive and Reconstructive Surgery", Book of abstracts titled – "Ceramics Today – Tomorrow's Ceramics", (Techna s.r.l., Faenza, Italy) p. 119.
5. K. DE GROOT, *Interceram.* **4** (1987) 38.
6. J. F. OSBORN, *Biomed. Technik* **32** (1987) 177.
7. K. HYAKUNA, T. YAMAMURO, Y. KOTOURA, M. OKA, T. NAKAMURA, T. KITSUGI, T. KOKUBO and H. KUSHITANI, *J. Biomed. Mater. Res.* **24** (1990) 471.
8. P. ADAM, A. NEBELUNG and M. VOGT, *Sprechsaal* **121** (1988) 941.

9. S, SHIMABAYASHI and M. NAKAGAKI, *Nippon Kagaku Kaishi* **3** (1978) 326.
10. H. FUREDI-MILHOFER, V. HLADY, F. C. BAKER, R. A. BEELE, N. W. WIKHOLM and J. S. KITTELBERGER, *J. Colloid Inter Sci.* **70** (1979) 1.
11. M. K. HOBBS and H. REITER, *Surface Coatings Technol.* **34** (1988) 33.
12. J. W. WATSON and S. R. LEVINE, *Thin Solid Films* **119** (1984) 185.

Received 21 January
and accepted 5 April 1991