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STRUCTURAL CHANGES OF PLASMA SPRAYED HYDROXYAPATITE COATINGS DURING IN-VITRO TESTING

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ABSTRACT: The in-vitro behavior of hydroxyapatite (**HAp**) coatings depends on the thermal history of the coating after thermal spraying. As-sprayed amorphous coatings degrade in **Ringer's** solution. Heat treatment at **800 °C** for **2** hours produces a crystalline coating which upon immersion in physiological solution displays a greater stability. Coatings are characterized for surface morphology, composition, crystallinity, roughness, and weight loss. The as-sprayed coating shows changes in the coating morphology with immersion time. Individual lamellae crack and separate from the coating. Those lamellae still intact show evidence of dissolution. After a period of **8** weeks small **HAp** 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 **HAp** coatings is seen as a technique to alter or control implant-tissue interactions.

KEYWORDS: hydroxyapatite, amorphous, crystalline, **Ringer's** solution, degradation mechanisms, microstructure, structure.

In-vitro testing is one means of determining the suitability of a material for implantation. Samples are immersed in a physiological medium which is usually a cell culture or solution mimicking the composition of blood. Changes in the coating, the solution or the cellular activities surrounding the implant can then be examined. A brief description of the various techniques will be given.

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The type of physiological media used determines the type of information that can be collected. When cell cultures are employed, the response of various cell types is analyzed which gives some information as to how the implant will behave within the body. Each cell type can be used separately or in combination, but the use of more than one cell culture makes it difficult to distinguish the changes which take place on the cells by the material or from the interaction between the two cell types. Dissolution of the material affects the morphology and nature of these cells and the response can be reported.

Another approach which is more simple and does not require the use of an incubator is the measurement of dissolution products in the physiological medium. Samples are immersed in a chemically prepared medium and the solution is analyzed to determine the ions that have gone into solution from the biomaterial. This test gives general information about the dissolution or degradation of the implant but is not sufficient to determine the mechanisms by which these ions pass into the solution. The mechanism is vitally important in determining how the crystallinity, composition, microstructure, porosity, and other properties of the bioceramic coating influence the deterioration of the coating.

The third technique which can be used to assess the suitability of an implant also entails soaking the samples in a physiological solution which is mostly referred to as a saline solution. This solution can be one with many components or just the few essential salts which can be found in blood. The samples are immersed and then removed to determine the changes in weight, surface morphology, phase composition, purity, roughness, and other characteristics which may determine the material's suitability as a biomaterial.

METHODS

Powder Preparation

Hydroxyapatite (HAp) powder was manufactured by the wet method using orthophosphoric acid and calcium hydroxide as the reactants [1]. The acid was added to the alkaline solution of calcium hydroxide at a determined rate to control the kinetics of reaction. The obtained gel was then dried in an oven and the dried cake crushed and sieved to a particle size distribution of 55-85 μm . This powder was then calcined at 800 $^{\circ}\text{C}$ to obtain a crystalline powder for plasma spraying.

Coatina Production

A Metco 3MP powder feeder was used to deliver the prepared powder with an argon feed gas to the 3MP Plasmadyne plasma spraying gun operating at 34 kW with argon and helium. Stainless steel substrates (316 grade) with a surface area of 281 mm^2 were prepared by grit blasting before coating with HAp. The torch was set to about 20 cm from the substrate and a robot was employed to apply an even coating to a thickness of $200 \pm 10 \mu\text{m}$. To avoid excessive heating of the substrate, compressed air was used for cooling.

Structure Control

The crystallinity of the coating can be decreased by using different plasma spraying parameters. It has been shown that a greater torch to substrate distance but a smaller particle size and power level lead to a lower crystallinity [2]. Crystallinity in the present experiment was controlled not by altering the spraying parameters but by heat treating an amorphous coating at 800 °C to yield a crystalline coating. This process of crystallization of the as-received coating was chosen so that the structures would be identical and thus changes in the performance of the coating could be related solely to the difference in crystallinity.

In-vitro Testing

Ringer's solution was prepared according to the method provided elsewhere [3]. Carbogen gas was added and the flow rate regulated to achieve a pH of 7.35. The purpose of carbogen (a medical grade gas consisting of 95% oxygen and 5% carbon dioxide) was not only to control the pH but also to replicate the dissolved gases found in the body. A pH meter immersed in the solution constantly monitored the pH. Finally, an immersion heater was included in the arrangement to raise the temperature to 36.6 °C and to constantly circulate the solution. The physiological solution was closely matched to the environment of the human body.

Sample Examination

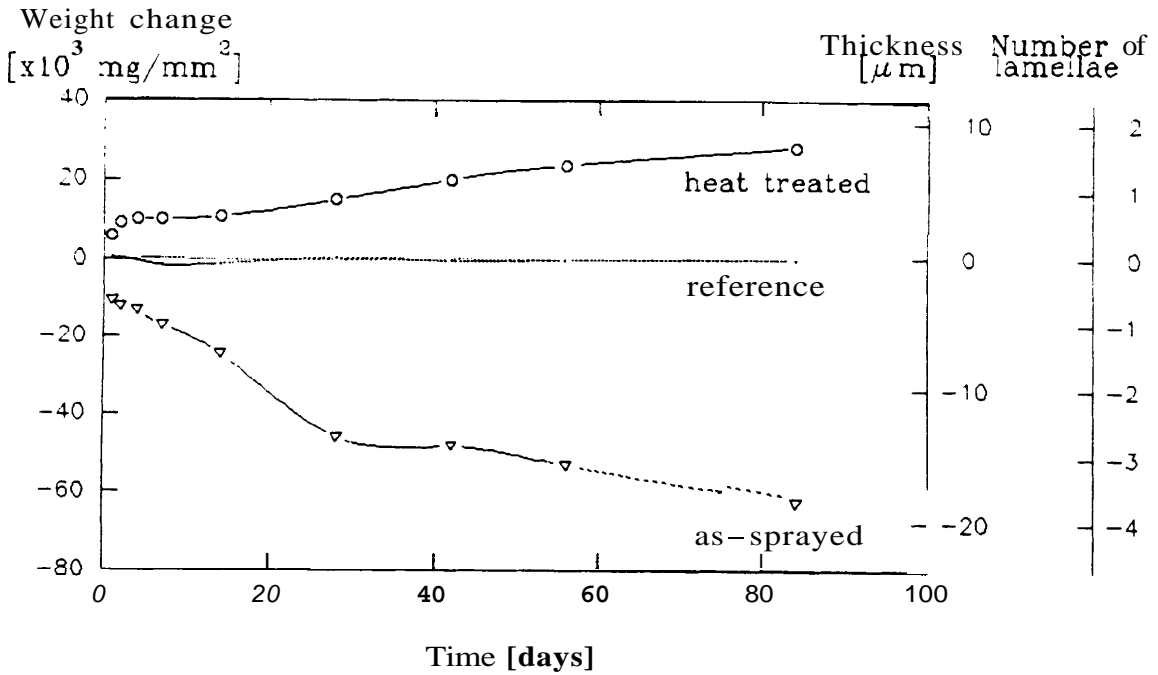
The reference coupons, as-sprayed coatings and heat treated HAP coatings were all weighed before immersion in Ringer's solution. After periods of 1 day, 2 days, 4 days, 1 week, 2 weeks, 4 weeks, 8 weeks, and 12 weeks the coatings and reference samples were removed, treated in an ultrasonic bath to remove any loose debris from the coating surface and then dried in an oven at 180 °C. The coating surface was inspected by scanning electron microscopy, composition and amorphicity were determined by X-ray diffraction, and roughness was measured with a Surtronic 3P roughness tester. Weight changes were measured to within 0.2 mg. A Cu K α source radiation was used for X-ray diffraction.

RESULTS

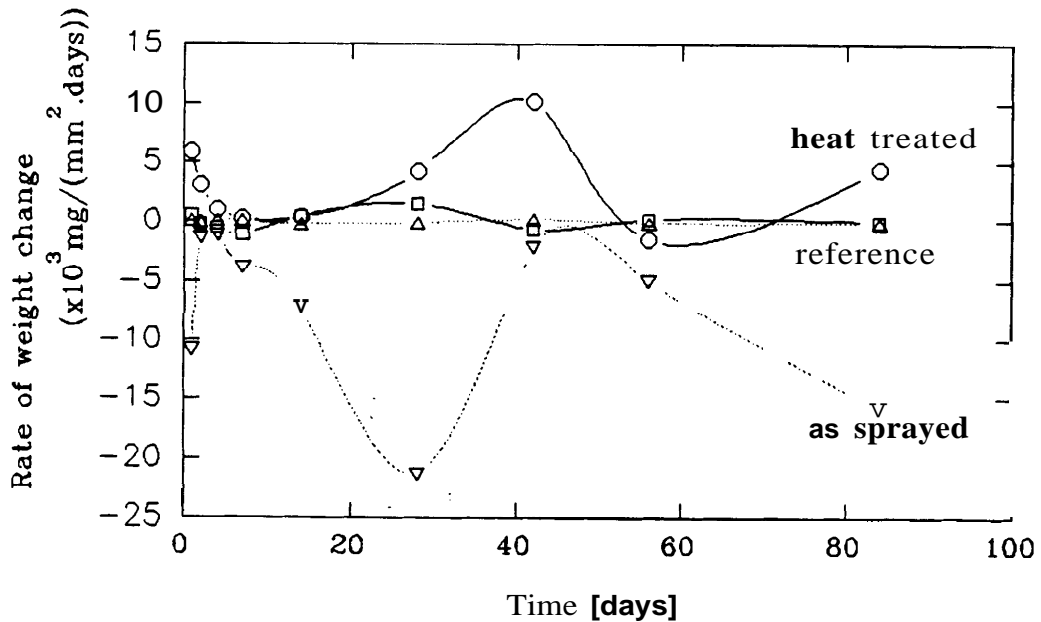
Weight Measurements

The heat treated and as-received stainless steel samples (with no coatings) showed a maximum variation of 0.02% after immersion in Ringer's solution. The heat treated stainless steel samples showed slight traces of corrosion after a period of 4 weeks. Coatings with different thermal histories also displayed a variation in behavior in the physiological solution. The as-sprayed coating showed a loss in weight with time (Fig. 1) which was most apparent in the first 4 weeks. A maximum decrease of 30% of the coating weight was recorded after 12 weeks immersion. The heat treated coatings showed a weight increase

which amounted to 9% of the coating weight. Most of the weight increase occurred in the first 8 weeks.



(a)



(b)

FIG. 1--(a)Weight change and the (b) rate of weight change for as-received and heat treated samples aged in Ringer's solution. The degradation of the coating is also assessed in terms of thickness and number of lamellae.

Roughness Measurements

The r.m.s. roughness of the grit blasted substrates varied between 3 and 4 μm . After heat treatment of the as-sprayed coating, the roughness did not change but remained at a value of 9.1 μm . No roughness changes were detected after immersion for the heat treated coating however the as-sprayed hydroxyapatite coating displayed a maximum variation of 1.9 μm (Fig. 2).

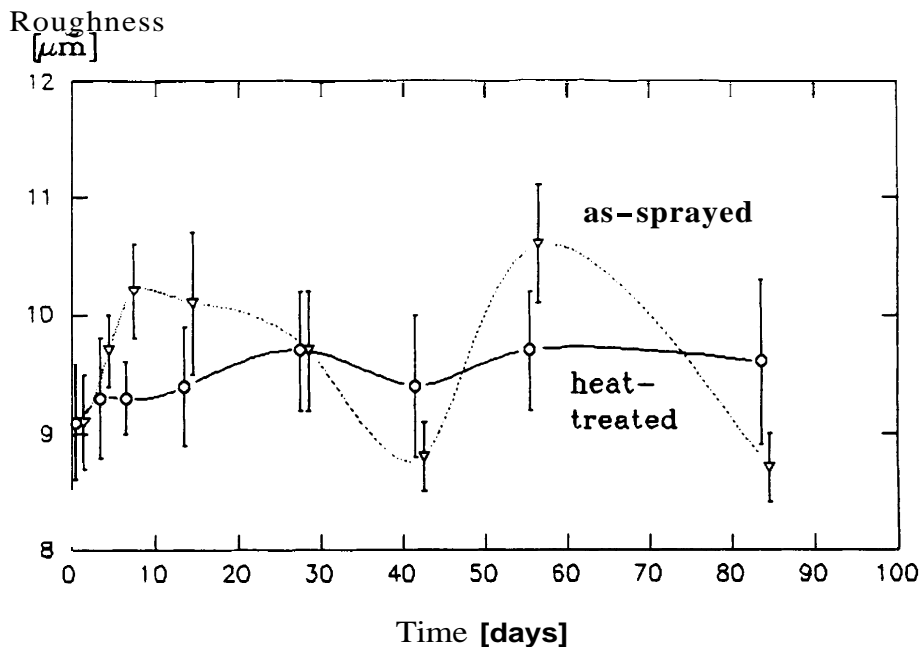


FIG. 2--Roughness of as-sprayed and heat treated samples aged in Ringer's solution.

Structure and Composition Measurements

The as-sprayed coatings were amorphous whereas the heat-treatment gave rise to crystalline coatings (Fig. 3). This increase in crystallinity was determined by the use of X-ray diffraction and is observed by a higher peak height. The heat-treated coating showed no structural change after immersion and this was ascertained from an identical HAP diffraction spectrum. As-sprayed HAP coatings showed an apparent increase in crystallinity over a period of 12 weeks (Table 1). This was measured by the change in peak height.

Table 1: Increase in crystallinity with aging time (indicated by higher peak height).

Time (days)	0	1	2	4	7	14	28	42	56	84
Main HAP peak height (cps)	274	274	285	285	260	331	361	546	625	1056

The amorphous coating contained traces of tricalcium phosphate and tetracalcium phosphate (Fig. 3). Initially the main peaks of HAp and tricalcium phosphate (TCP) were identical in height but with time, peaks other than HAp became smaller in comparison.

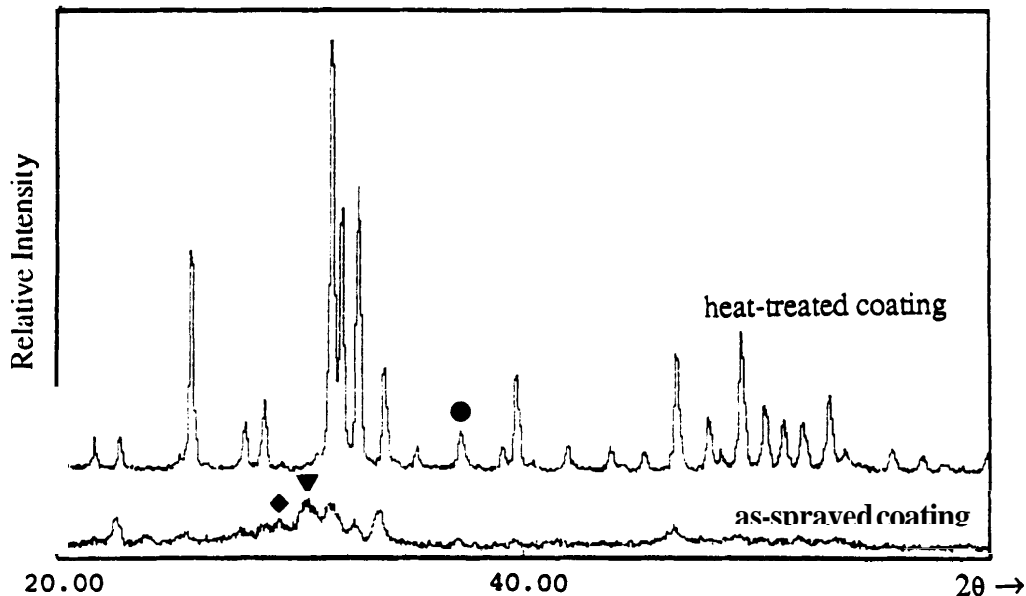


FIG. 3-- Crystallinity and composition for as-sprayed and heat treated coatings. Presence of calcium oxide (●), tricalcium phosphate (▼) and tetracalcium phosphate (◆) are indicated.

Surface Morphology Examination

Amorphous coatings -- The coating morphology changed after immersion in Ringer's solution. Initially, the microstructure exhibited a surface with well molten splats, fine cracks and small 1 μm unmolten particles on the surface (Fig. 4a). Little spheres about 5 μm or less can also be found and they are remnants from the impact event of molten particles on a substrate. The surface morphology underwent a change after 1 day in Ringer's solution. Cracking is more intensive and the size of the cracks are twice as large compared to the as-sprayed coating. Some particles, as a result of this cracking, have rough edges and can be identified as splat fragments which are slightly removed from the splats (Fig. 4b). Small pores were also identified in the splats.

Cracking was observed to take place on a greater scale as the aging time increased. This imparted a rougher appearance to the surface and large regions of material were sometimes removed from many locations within the coating. Fractured splats could be found both on low and high points on the coating. A side elevation can be seen where many splats had deposited on one another and these splats are all fractured (Fig. 4c). The fractured aides of splats that were originally rough in appearance, now exhibited smooth sides and gave a more rounded appearance to the whole splat.

After a period of 4 weeks, small spheres containing calcium and phosphorous are present and located at low points or valleys in the coating (Fig. 4d). The crack frequency is observed to be significantly lower and the fragments seem to be more rounded. The coating surface has completely different characteristics after 6 weeks. The population of small (2-5 μm) calcium and phosphorous containing spheres increases (Fig. 4e). These small spheres cover the coating in the form of a dense blanket after 8 weeks, rendering the typical coating morphology barely recognizable (Fig. 4f).

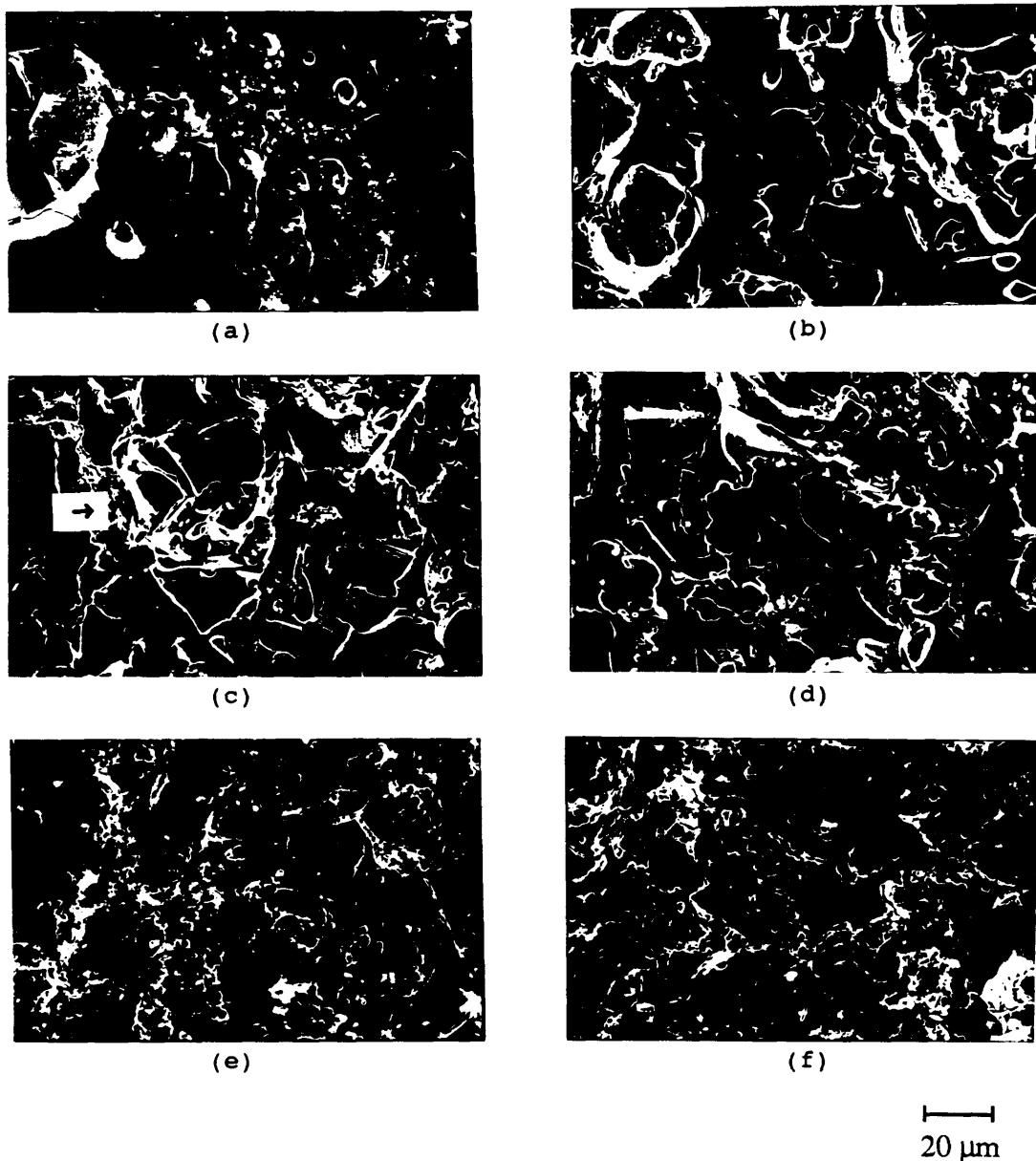


FIG. 4.--Surface morphology of as-sprayed hydroxyapatite coatings after aging for (a) 0 days, (b) 1 day, (c) 1 week, (d) 4 weeks, (e) 8 weeks and (f) 12 weeks in Ringer's solution.

Crystalline coatinus--Heat-treated coatings displayed a relatively clean surface with small residue particles which gives evidence of the splat process in thermal spraying. Compared with the as-sprayed samples, the crack width is twice as large, about $0.8\ \mu\text{m}$ (Fig. 5a). Upon immersion these cracks open up further (Fig. 5b). The cracks eventually lead to delamination of some particles and evidence of this is shown in Figure 5c. Half a lamella is observed which is still intact with the underlying substrate. Sections of lamellae that had de-adhered from the coating were not present on the surface. Not many de-adhered regions

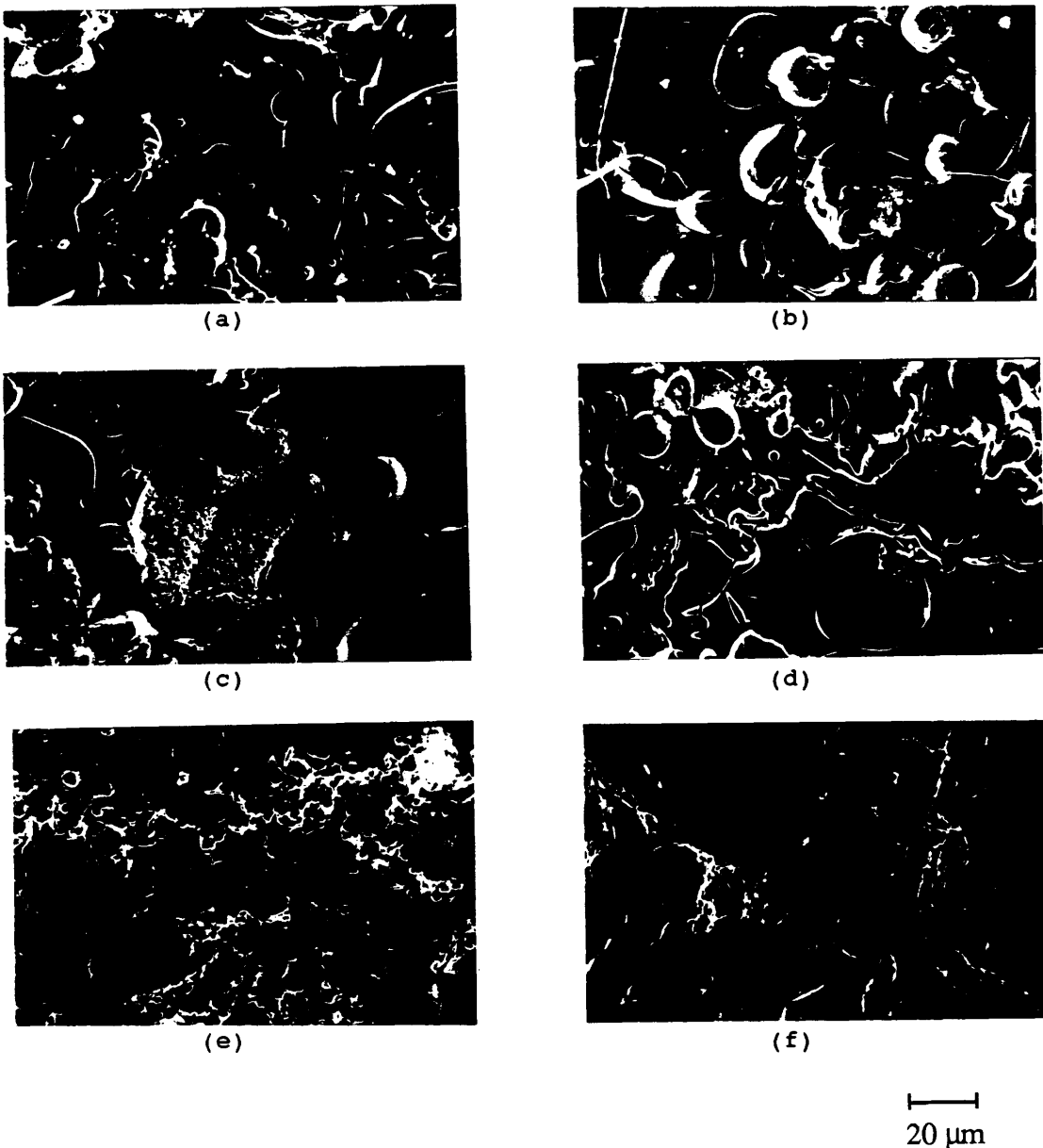


FIG. 5--Surface morphology of heat treated hydroxyapatite coatings after being aged for (a) 0 days, (b) 1 day, (c) 1 week, (d) 4 weeks, (e) 8 weeks and (f) 12 weeks in Ringer's solution.

were found on the coating surface. Small square blocks were revealed in locations where rust spots were observed and these were identified as being comprised of mainly calcium, phosphorus and a trace of iron.

After 4 weeks, no changes were observed with well molten splats (Fig. 5d). Small 1 μm sized spheres were found on some locations of the surface. Both the calcium to phosphorous molar ratio and the size of these spheres increased after 8 weeks. The microspheres increased to 5-10 μm in size and were already a dominating feature after 8 weeks. Just like with the amorphous coatings, the spheres began growing on splats and at cracks but after 12 weeks they were found everywhere. Calcium rich planar crystallites have been detected, about 80 μm in size at several sites on the surface. This phenomena was only observed after 12 weeks.

DISCUSSION

It is known that the thermal history of the powder during plasma spraying and the post heat treatment of the coating influence, among other properties, the composition and the crystallinity of the coating [4]. As-sprayed coatings which were amorphous in structure behaved differently than the heat-treated coatings which were crystalline and this finding has been confirmed by Wolke et al. [5]. Each will be discussed in turn, starting with the crystalline coatings, which displayed a more stable behavior in terms of low degradation.

Crystalline Coatings

A 5.3 wt.% increase in coating weight was observed. This weight increase is the sum of various contributing components, for example, uptake of moisture by absorption into the cracks and cavities of the coating [6, 7] or the probable reaction of CaO with water to form $\text{Ca}(\text{OH})_2$. These mechanisms are assumed to take place fairly rapidly and could explain the initial weight gain immediately after immersion. Using the method of Salsbury [8], the amount of calcium oxide was calculated by dividing the area of the calcium oxide peak by the sum of the amorphous peak, centered at 30 degrees, and the crystalline HAp peaks, between 2θ of 27° and 35° . The composition of the crystalline coating is 5 wt.% calcium oxide and 95 wt.% crystalline HAp. The increase in weight due to the CaO changing to $\text{Ca}(\text{OH})_2$ would be 1.6 mg or $5.8 \mu\text{g}/\text{mm}^2$. This is a significant increase and can be assumed to take place if the CaO is on the surface of the coating where it can be accessed by water to undergo the indicated reaction.. Calcium oxide is active and hydration therefore takes place immediately upon immersion.

Another contributing factor to the weight gain could arise from the small localized areas of corrosion that appeared as small square blocks after a period of 1 week. This can be treated as a negligible effect since there is no weight increase (Fig. 1). The last mechanism of weight gain occurs at a later time (after 4 weeks) and is identified as the precipitation of hydroxyapatite spheres, 5-10 μm in size. If this correlation can be made then the weight of the spheres, determined from Figure 1, is $10 \mu\text{g}/\text{mm}^2$ and the rate of formation is $1.3 \mu\text{g}/(\text{mm}^2 \cdot \text{week})$.

By referring to the micrographs of the surface features after different aging times, one can observe that there is no evidence of coating degradation that would lead to a weight decrease. Some loose lamellae might de-adhere but this is not a general feature to be observed over the entire coating surface. The remaining coating segment remains sharp edged which infers that no dissolution takes place on cracked lamellae. Dissolution measurements on crystalline coatings however have been conducted showing the increase in calcium and phosphorous ion concentration in physiological solution [9]. The magnitude of dissolution is less for crystalline coatings and occurs on the surface of the lamellae. This implies that the interlamellar interface is more resistant to dissolution than the exterior surface of the lamellae directly in contact with the solution. Just like with sintered apatite, the porosity does not appear to play a large role with respect to the degradation kinetics [10]. Surface reactions are thus the rate determining steps.

Cracking is probably due to the release of residual stress that forms during the cooling stage of plasma spraying [11, 12]. This is also detected as the shift of the main hydroxyapatite peak in the X-ray diffraction pattern and the opening of the crack width from 0.5 μm to 1.5 μm after heat treatment. The opening of cracks infers that the cracks pass through numerous splats. The precise depth of these cracks (orthogonal to the substrate surface) is presently unknown but is expected to be in increments of lamellae. This release of residual stress by cracking of the splat leads to the eventual dislodgment of the occasional particle in the Ringer's solution.

A model can be proposed to describe the behavior of the coatings upon in-vitro testing. Heat treatment leads to wider cracks which is indicated by thicker lines (Fig. 6B). The number of cracks increase with immersion time to a lesser extent than with as-sprayed coatings. Some coating fragments are removed and the geometry of the cracked splat remains jagged. Crystalline hydroxyapatite spheres then form on the surface with no preferred growth initiation sites. These HAP spheres can be found in cracks, on interlamellar boundaries and on splats and increase in number with time.

Amorphous Coatings

Hastings et al. [13] and Harris [14] have reported that amorphous coatings are undesirable for implantation since they are believed to be resorbed by bone tissue. The resorbability and effectiveness of amorphous coatings has been investigated by a number of workers. In-vitro tests performed by de Bruijn et al. [15] show that there is no difference in calcium ion release if samples of various degrees of crystallinity are immersed in a solution containing osteoblast-like cells. Maxian et al. [16] shows that the concentration of dissolved calcium is slightly less for amorphous HAP compared to crystalline HAP from an in-vitro experiment, but the surface in contact with cancellous bone does not depend on the crystallinity of the coating. These findings of bone contact to the HAP differ from the work by Gabbi et al. [17] who show that higher bond strengths were attained by using a more crystalline coating. At this stage, more information needs to be

gathered to understand the behavior of coatings with varying degrees of crystallinity for different implantation sites within the body. The importance of each type of coating is now very actively being investigated and the preferred structure is under dispute.

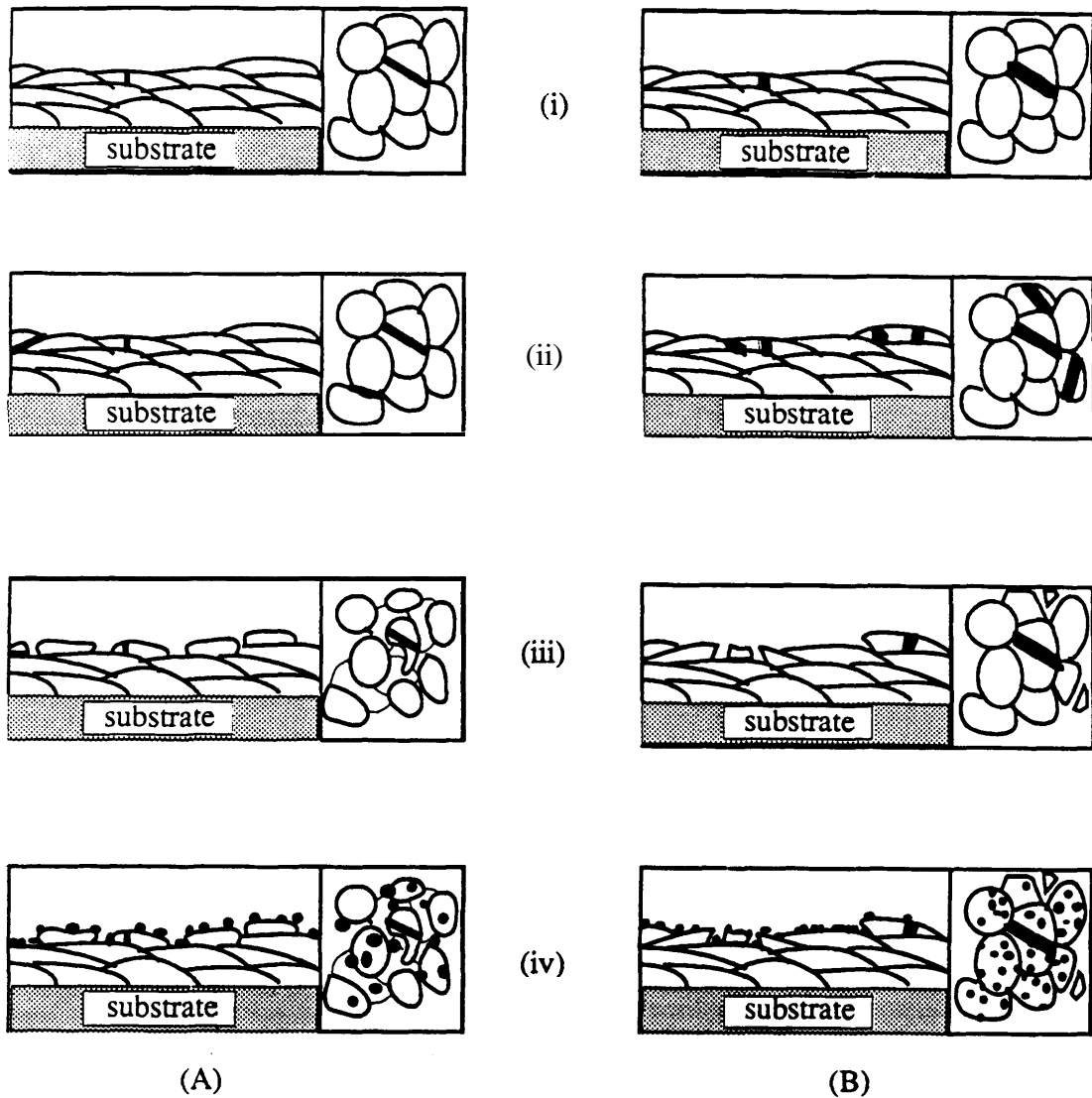


FIG. 6.--Proposed model showing the changes in the microstructure of as-sprayed and heat treated specimens after in-vitro aging. Profile and **planar views** are presented of;
 (A) as-sprayed coatings, and
 (B) heat treated coatings.

Dearadation mechanisms --The model (Fig. 6) will help in explaining the changes in microstructure with time. Reference will be made to the figures showing the variation of weight and roughness with time to better understand the phenomenon taking place. The initial coating has cracks, both interlamellar (*i.e.*, between) and intralamellar (*i.e.*, within), and debris on the surface from the thermal spraying

process. Upon immersion the crack density increases (Fig. 4) and loose debris is removed from the surface. In comparison to heat treated coatings, the amorphous coatings do not show any crack opening. The cracks typically appear to form randomly and do not pass through many lamellae to produce a deeper crack. A weight decrease of $10 \mu\text{g}/\text{mm}^2$ is recorded (Fig. 1) and no change in roughness is reported. A shift in the peak positions of the X-ray traces gives evidence to the release of residual stresses.

The second stage of weight loss occurs when small coating fragments, separated from the coating by cracking, are carried away by solution. This suggests that the interlamellar boundaries are weaker than the lamellae. The remaining splats on the coating possess sharp jagged edges. The presence of other calcium phosphate phases which are more resorbable could aid in the fragmentation of lamellae since the other phases such as tricalcium phosphate, tetracalcium phosphate and calcium oxide are known to have a higher resorbability. Fragmentation of lamellae is observed up to an immersion period of 4 weeks. The solubility of the amorphous coatings is greater than the crystalline coatings and this occurs by dissolution which is observed by the rounding of the fractured splats on the surface. Splats become smaller in size. The roughness begins to decrease after 1.5 weeks, which is interpreted as the onset of dissolution which, in effect, creates a less rough surface. This trend continues for a period of 6 weeks.

Hydroxyapatite precipitation -- Crystalline hydroxyapatite microspheres appear on the surface of the coating. Their crystalline nature is supported by the increase in hydroxyapatite peak height evidenced on X-ray diffraction patterns. This was also found by Klein et al. [18]. It may appear that amorphous hydroxyapatite transforms to a crystalline state but the findings of this work support the view that the crystallinity arises from a thin layer of crystalline spheres which covers the amorphous coating. Nucleation of these spheres is thought to take place between the first and second week which corresponds to the time when changes in peak height are registered for the first time, Table 1. Hyakuna et al. [19] have found that a calcium phosphate layer consisting of $1 \mu\text{m}$ sized balls forms on the surface of sintered hydroxyapatite and Adam et al. [20] who aged tricalcium phosphate in water at 80°C , found that the outer layer also transforms to hydroxyapatite. Ducheyne and coworkers [21] have shown that the formation of a calcium phosphate occurs on tetracalcium phosphate, tricalcium phosphate and various forms of hydroxyapatite. The induction time before deposition of the calcium phosphate is apparently dependent on the presence of certain ionic species in solution [22], material purity, composition, and the dissolution time necessary to create a sufficiently high calcium and phosphorous concentration within the solution. The spheres appear two weeks earlier for the as-sprayed coating which implies that the bonding to bone will occur more rapidly [19].

A quantitative analysis -- The weight change has three main regimes: (i) an initial weight loss on immersion, (ii) splat de-adhesion and dissolution of the fractured and other splats, and (iii) the nucleation and growth of hydroxyapatite spheres. The first two

mechanisms bring about a weight loss but the latter leads to a weight gain. The inflection point is at the 4 week period. Klein et al. [18] confirms the position of this inflection point and the general shape of the curve by measuring an increase of calcium and phosphorous ion concentration in a physiological solution. By measuring the gradient of the second regime one can measure the degradation rate up to 4 weeks. This degradation rate is $9.5 \mu\text{g}/(\text{mm}^2 \cdot \text{week})$. Once spheres start appearing it is assumed that they blanket the underlying coating and prevent further degradation. The duration of the experiment was insufficient to have spheres covering the entire surface, so it can be assumed that degradation of the coating continues, for purposes of the following calculation. The rate of HAP microsphere precipitation can be calculated by subtracting the gradient of the third regime ($2.7 \mu\text{g}/(\text{mm}^2 \cdot \text{week})$) from the degradation rate of the second regime, $9.5 \mu\text{g}/(\text{mm}^2 \cdot \text{week})$. Thus, HAP microspheres form at a rate of less than $6.8 \mu\text{g}/(\text{mm}^2 \cdot \text{week})$. This value is significantly higher than the value calculated for crystalline coatings ($1.3 \mu\text{g}/(\text{mm}^2 \cdot \text{week})$). In fact, the degradation rate should decrease during the formation of HAP spheres due to the blanketing effect and so the calculated rate of HAP formation should be lower than that which has been calculated. If an accurate value of the degradation rate could be determined at any time, then the growth rate of HAP spheres could be calculated and furthermore, the effect of surface structure could also be evaluated. It may be likely that HAP prefers to nucleate on an amorphous structure as opposed to a well defined crystalline material. At the beginning of the HAP precipitation the roughness increases and decreases again. There is insufficient information to determine the precise mechanism taking place during this period. For every decrease of $10 \mu\text{g}/\text{mm}^2$, it can be assumed that one splat is removed which should be about $5 \mu\text{m}$ thick [23]. The weight loss can then be thought of in terms of the number of splats which are removed from the coating and an appropriate curve may be plotted (Fig. 1).

Microstructural considerations -- It has been shown that coating degradation can take place not only by particle removal (coating deterioration) but also by dissolution. The microstructure of the hydroxyapatite coating is believed to play a large role in the rate of dissolution. Features such as cracks, pores, unmolten or respheroidized HAP drops are sites where dissolution is able to dislodge pieces of the coating into the solution. The initial particle size and hence the resulting splat size also plays an important role. It is anticipated that small splats, imparting a larger boundary area (i.e., more interfaces between splats) to the bulk of the coating would be less resistant to degradation than a coating with large splats. This assumes that the boundary dissolves faster than the individual layers due to the higher stress at the boundary. Indeed, there is some correlation for amorphous coatings where there is a decrease in splat diameter with little change in coating thickness.

Design Aspects

By considering the behavior of crystalline and amorphous HAP coatings, the coating can be designed for the required performance or application in the human body. A combination of amorphous and crystalline phases can be chosen for special applications. For example, by grading the coating,

an amorphous phase can be deposited on the surface of a crystalline HAP coating, which might be preferred for mineralization to take place at a shorter induction time. The mechanism by which mineralization takes place however, should be investigated more thoroughly to determine the conditions necessary for earlier bone formation. If it is solely dependent on the concentration of ionic species, then by careful control of the thermal spraying process, the coating can be designed to have a more resorbable external layer.

CONCLUSION

The in-vitro behavior of hydroxyapatite coatings depends upon the phase and lamellae structures. Crystalline coatings are more stable and seem to increase slightly in weight. They do not show signs of degradation except cracking which is attributable to the release of residual stresses. It is believed that surface reactions are responsible for the dissolution also observed by other workers. Amorphous coatings undergo a weight loss and this has been shown to occur by de-adhesion of cracked lamellae and dissolution of the remaining lamellae on the coating surface. Crystalline hydroxyapatite nucleates and grows in the form of spheres on both structural forms of HAP. The formation of HAP spheres appears first for the amorphous structure. The different mechanisms of degradation operating on amorphous and crystalline HAP coatings are important in recognizing which is more favorable for the successful osteointegration with bone.

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