JOURNAL OF MATERIALS SCIENCE: MATERIALS IN MEDICINE 6 (1995) 818–823

# The effect of hydroxyapatite thickness on metal ion release from stainless steel substrates

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The corrosion behaviour of 350 stainless steel coated with hydroxyapatite, HA, by plasma spraying was studied in Hank's Balanced Salt Solution, HBSS, and compared with that of polished passivated surfaces. Two different nominal thicknesses, 50 μm and 200 μm, corresponding to what one might consider a thin and a thick coating, respectively, were used. Only HA coatings with a thickness of 200 μm were capable of reducing the electrical charge consumed at constant potential to values lower than those measured for polished surfaces. However, no HA detachment occurred for both thicknesses, as opposed to what has been found in a previous work [1] with Ti6Al4V alloy coated with 50 μm HA. No iron, chromium or nickel were detected in solution by Atomic Absorption Spectroscopy with electrothermal atomization (ET-AAS). These elements were also absent from the bulk of the HA coating, both after a 6 month immersion period and electrochemical accelerated tests. The data indicate that in spite of the relatively low corrosion resistance of stainless steel as compared to that of titanium alloys, a thin (50 μm) HA coating prevents the release of metal ions, while remaining adherent to the substrate.

## 1. Introduction

In orthopaedic surgery and particularly in total hip replacement, metals are the most widely used materials due to their good mechanical stability. Nevertheless, it is well established that when in contact with aggressive body fluids or tissues they corrode [2–4]. Inflammation is one of the possible responses of the surrounding tissue to the release of metal ions [5, 6]. The role of stainless steel corrosion products in inflammatory processes was studied by Tracana *et al.* [5], after injecting mice with solutions obtained by anodically dissolving stainless steel in HBSS. Their study showed that iron, chromium, nickel and molybdenum induced an acute inflammatory response for a period of one week, as demonstrated by the influx of polymorphonuclear cells and macrophages. In contrast, neither a chronic inflammation nor an immune response was observed indicating that the stainless steel solution caused a minor tissue response. Vrouwenvelder *et al.* [7] investigated the behaviour of fetal rat osteoblasts cultured on four bone replacing materials: bioactive glass, hydroxyapatite, Ti6Al4V and 316L stainless steel. They found that the release of metal ions from stainless steel retards osteoblast expression or even causes cell death in some cultures, which may be induced by the formation of certain toxic organometallic complexes.

With the idea of diminishing adverse biological effects and improving bone bonding, several surface modifications can be used, namely passivation, and deposition of bioactive and bioinductive layers. Hydroxyapatite is perhaps the best example of such coatings, its *in vivo* [8–10] and *in vitro* [11–13] behaviour being extensively documented. However, the effect HA may have on metal ion release has not been sufficiently clarified and very little is known about its use with stainless steel substrates. If corrosion products are released from stainless steel coated with HA they are expected to interact with the coating, probably influencing its adhesion to the substrate. Furthermore, the type and stability of compounds formed may vary from ion to ion, thus influencing the actual amounts of metals released into the body.

The aim of this work was to investigate the effect of HA thickness on metal ion release from 350 L stainless steel. Two thicknesses, 50 μm and 200 μm, were used. The work involved the use of i) accelerated electrochemical tests to study substrate oxidation, ii) chemical analyses of physiological solutions after prolonged (6 month) periods of immersion, to detect

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metal ions released from the stainless steel, and iii) evaluation of the degree of attack at the metal/HA interface and possible incorporation of metal constituents in the HA barrier.

## 2. Materials and methods

The electrochemical dissolution behaviour of 350 stainless steel coated with HA by plasma spraying, was studied in Hank's Balanced Salt Solution, HBSS (Gibco), and compared with that of polished passivated surfaces. Two different nominal thicknesses of HA, 50 and 200 μm, were used. All samples were supplied by Plasma Coatings, UK. The alloy used is a duplex stainless steel, the composition of which is presented in Table I. Specimens had a cylindrical configuration described in a previous paper [1]. In the same work sample preparation is also described in detail. On the specimens used in the electrochemical experiments an electrical contact was made via a threaded rod. Prior to immersion in the HBSS the upper part of the specimens was protected with a lacquer (Lacomit$^R$).

The tests were carried out at 37 ± 1 °C. In the electrochemical experiments a Saturated Calomel Electrode (SCE) was used as reference electrode. The auxiliary electrode was a platinum square sheet with an area of 800 mm$^2$. The electrochemical cell was made of methylpentene polymer and had a capacity of 100 ml. A potentiostat and a ramp generator were used for applying the electrical potentials. A computer and a multiplexer unit were employed for current and potential data acquisition. The data were recorded at 1 s intervals. Cyclic polarization was done according to ASTM G 61-86 test method [14]; the polarization scan was initiated 30 min after immersing the specimens, starting at the corrosion potential and scanning the potential in the more noble direction at a scan rate of $10^{-3}$ V/s. When 1V was reached the direction of scanning was reversed. The potentiostatic polarization test method were performed applying the potential of + 0.8 V during 15 min, after 30 min under open circuit. Electrochemical impedance tests were done by superimposing an a.c. potential of ± 10 mV on the corrosion potential, $E_{\mathrm{corr}}$, measured after 30 min of open-circuit. A frequency response analyser was used for generating the a.c. potential waves. The frequency was varied between 10 KHz and 1 Hz.

Immersion tests (ASTM G 31-72$^{\mathrm{e}1}$) [15] were conducted in polyethylene closed flasks (250 ml) in a thermostatic bath at 37 ± 1 °C with times of immersion up to 6 months. Uncoated (polished and passivated) and coated (50 μm and 200 μm HA) specimens were used. pH and ion concentration in solution were determined after 6 months. Chemical analysis of solutions was done by atomic absorption spectroscopy, AAS, using flame and electrothermal atomization.

The morphology of the HA/stainless steel interface was observed under a scanning electron microscope, SEM, prior to and after testing. The samples were prepared by mounting them in epoxy resin as indicated in a previous work [1]. The surface for SEM observation was wet-ground with 2000 and 4000 grit SiC papers. Energy Dispersive Spectroscopy (EDS) was performed in order to evaluate Ca/P concentration ratios and to detect the presence of metal at the metal/HA interface and within the coating itself.

TABLE I Semi-quantitative composition of 350 stainless steel (EDS analyses)

| Element | Weight (%) |
|---|---|
| Fe | 56.3 |
| Cr | 21.3 |
| Ni | 13.2 |
| Mn | 5.8 |
| Si | 0.4 |
| Mo | 3.0 |

## 3. Results

### 3.1. Corrosion potential measurements

Some $E_{\mathrm{corr}}$ versus time curves obtained in open circuit measurements are shown in Fig. 1. The curves for coated specimens show large variations of potential, $E_{\mathrm{corr}}$ being higher for the 200 μm thick coating. This may be due to the existence of a large diffusion path within the thick coating, capable of causing large potential drops. As the stainless steel surface was not attacked, these potential drops cannot be associated with localized corrosion.

Table II presents the average and standard deviation of at least four $E_{\mathrm{corr}}$ measurements for each surface treatment, after 30 min immersion. The HA coating shifts the corrosion potential of the stainless steel to more noble values, the shift being more significant for the thicker coating.

### 3.2. Electrical charge versus time curves at constant potential

The electrical charge consumed by the stainless steel during potentiostatic tests at 0.8 V was measured. This electrical charge is a measure of the total amount of metal that has undergone oxidation. The two contributions of electrical charge (one due to film formation and the other to metal dissolution) cannot be discriminated with this type of experiment, and therefore the data indicate the total amount of metal that has undergone oxidation. Taking a polished passivated surface as reference, only coatings with a thickness of 200 μm were capable of reducing the electrical charge used in the oxidation of the stainless steel substrate at constant potential, as it can be seen in Fig. 2. The higher charge obtained for the 50 μm thick coating is a result of a higher substrate area being exposed, due to grit blasting prior to HA deposition. Both coatings remained adherent to the substrate as shown in Fig. 3 and 4.

### 3.3. Cyclic polarization experiments

Cyclic polarization curves are presented in Fig. 5. No film breakdown was observed for any sample, up to

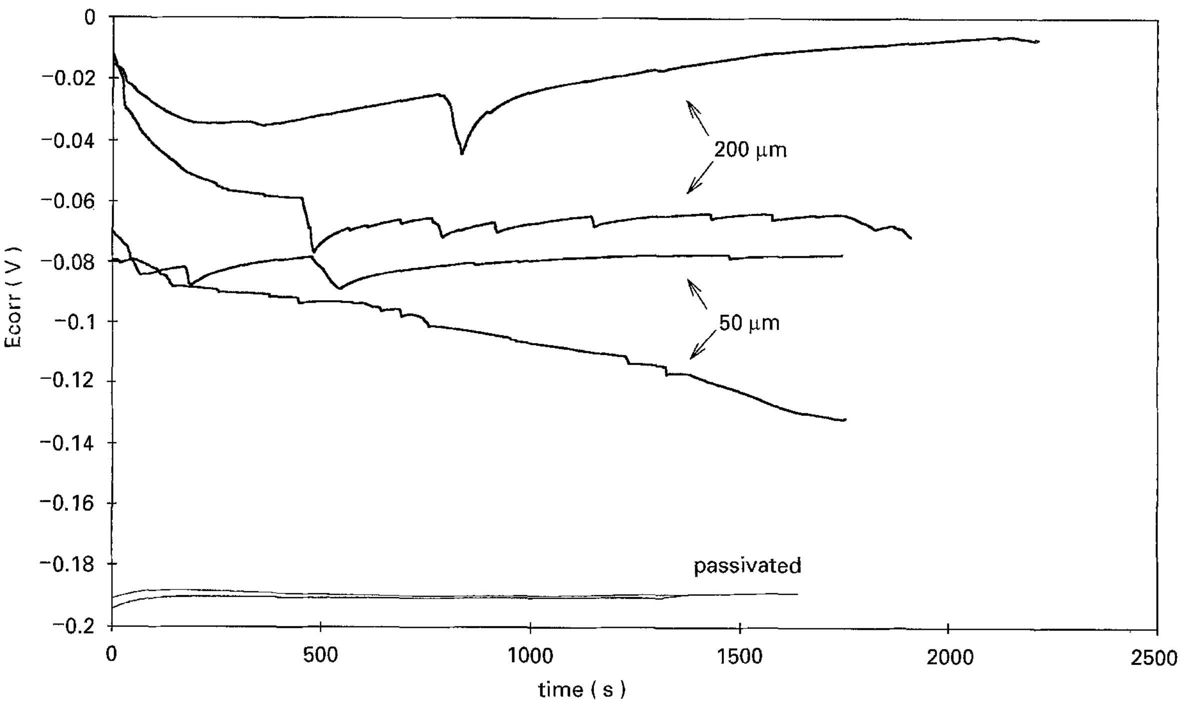

*Figure 1* $E_{corr}$ versus time curves.

TABLE II Corrosion potentials for 350 stainless steel specimens with different surface treatments; time of immersion: 30 min

| Surface treatment | $E_{corr} \pm \sigma$ (mV) |
|---|---|
| Polished and passivated | $-166 \pm 27$ (4) |
| HA - 50 μm thick | $-80.8 \pm 29$ (9) |
| HA - 200 μm thick | $-54.2 \pm 32$ (6) |

In parentheses is indicated the number of tests

1 V. The hysteresis loops observed for coated and uncoated samples are not associated with crevice attack at the metal/HA interface. After cyclic polarization, HA was adherent to the substrate.

Semi-quantitative analyses by EDS were performed on coated samples before and after cyclic polarization tests, in order to detect if any difference in composition within the bulk of the coating were introduced during the testing period. The Ca/P concentration ratio did not alter with the electrochemical experiments. No iron, chromium, nickel or molybdenum were detected.

### 3.4. Electrochemical impedance spectroscopy

Electrochemical impedance experiments were performed at open-circuit potential. Nyquist representations (imaginary versus real components) did not correspond to a simple model and therefore it was

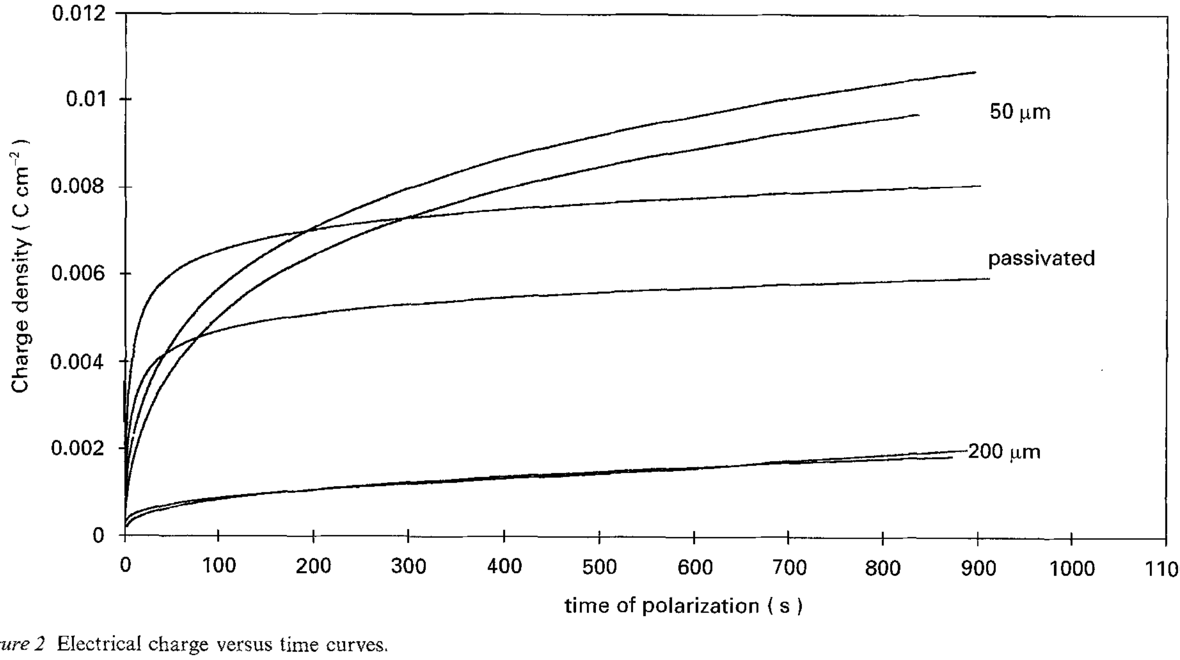

*Figure 2* Electrical charge versus time curves.

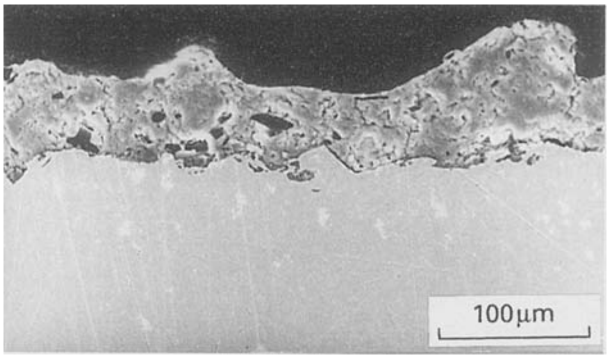

*Figure 3* SEM photomicrograph of a specimen with a 50 μm thick HA coating after potentiostatic polarization, showing no decohesion at the metal/HA interface.

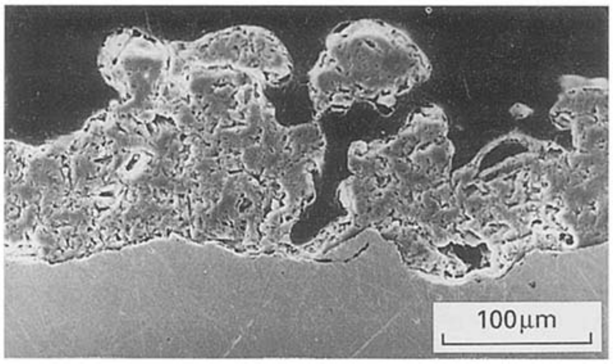

*Figure 4* SEM photomicrograph of a specimen with a 200 μm thick HA coating after potentiostatic polarization, showing no decohesion at the metal/HA interface.

TABLE III Absolute values of impedance $|Z|$, $\Omega \text{cm}^2$, at two frequencies, for HA-coated stainless steel

| f (Hz) | 50 μm | 200 μm |
|---|---|---|
| 1000 | 23.5 | 59.6 |
| 1.00 | 1992 | 2549 |

decided to compare the values of impedance at high (100 Hz) and low frequencies (1 Hz).

Table III gives the results obtained, which are averages of three measurements. At low frequencies charge transfer reactions and mass transfer become the main contributors to impedance. Deposition of a 200 μm thick HA layer increases the impedance values, probably reflecting a significant decrease in real surface area and/or reduction in the oxidation rate of the substrate.

## 3.5. Long term immersion

Fig. 6 shows $E_{\text{corr}}$ versus time curves. After 6 months the corrosion potentials are lower than at the beginning, except for specimens coated with 50 μm thick HA.

pH versus time curves are plotted in Fig. 7. The pH after 6 months of immersion is always lower than at the beginning. The pH of solution has two contributions: one from HA dissolution (increase of pH) and the other from substrate dissolution (decrease of pH). It seems that the predominant effect is hydrolysis of

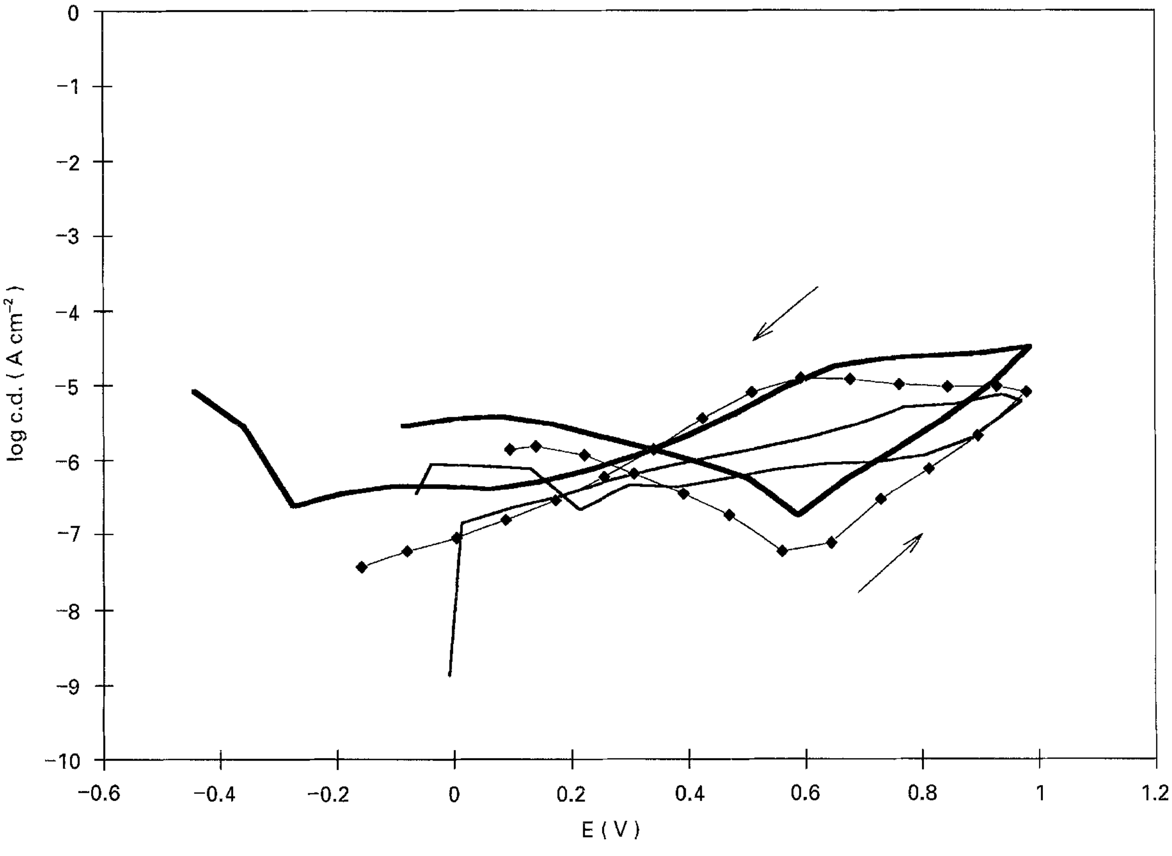

*Figure 5* Cyclic polarization curves. (——), 200 μm; (▬▬), 50 μm; (◆), passivated.

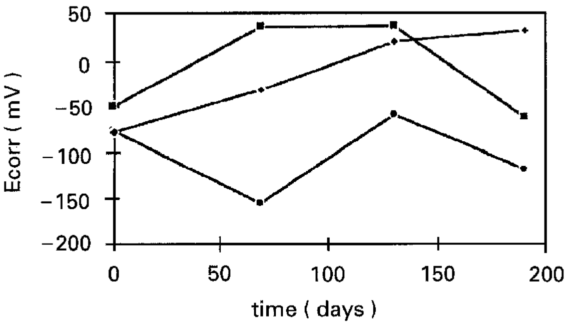

*Figure 6* $E_{corr}$ versus time curves for 6 month immersion tests in HBSS at 37° C. (●), Passivated; (◆), HA–50 μm; (■), HA–200 μm.

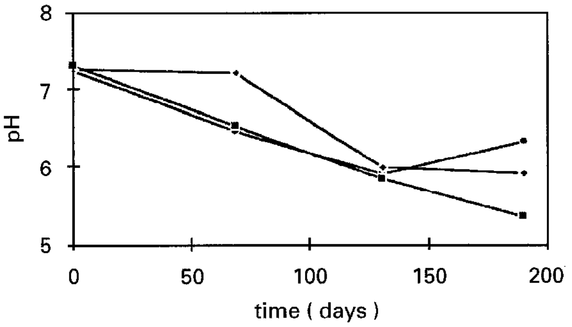

*Figure 7* pH versus time curves for 6 month immersion tests in HBSS at 37° C. (■), Passivated; (◆), HA–50 μm; (●), HA–200 μm.

metal cations, since acidification of the solution is observed.

After 6 months of immersion in HBSS no detachment occurred for both coatings. In another work by the same authors with a titanium alloy, tested under the same conditions, HA decohesion occurred for both thicknesses [1]. The Ca/P concentration ratio within the coating measured after the 6 month immersion period does not significantly change in comparison with the initial values. No iron, chromium or nickel were detected in solution by ET-AAS. These elements were also absent from the bulk of the HA coating after the 6 month immersion period.

## 4. Discussion

When metallic implants are in contact with a biological environment they undergo a serie of modifications, namely protein absorption followed by cell adhesion, and formation of new compounds, e.g. oxide films or metal complexes. In general, stainless steel is known to have a lower corrosion resistance than titanium and titanium alloys [16–18]. Some retrieved implants exhibit fretting corrosion as a cause for metal ion release [2].

Metal ion release can be estimated *in vitro* by electrochemical dissolution followed by measurement of metal levels in solution. In previous studies with 316 L stainless steel [17] we found that proteins improve its pitting resistance. In this study with a duplex 350 stainless steel a good corrosion resistance in protein-free HBSS is observed, as indicated by the lack of localized attack up to 1 V (see Fig. 5). The metal/HA interface remained coherent for both HA thicknesses. Ti6Al4V under identical tests [1] also showed good corrosion resistance up to 3 V but decohesion of the thinner coating was observed.

In both works the smaller thickness could not counterbalance the effect of the increased surface area. This is illustrated by the charge versus time curves in Fig. 2. This figure also shows that a high degree of protection is afforded by the thicker coating. The impedance values shown in Table III confirm that the thinner coating is less protective.

After a 6 month immersion period, HA coatings are still adherent to the metal substrate. Mechanical stresses may alter this behaviour, as shown by Tranquilli Leali *et al.* [18] in an *in vivo* work with the same stainless steel coated with HA. They found that HA coatings were subject to exfoliation/fragmentation, thus exposing the metallic substrate.

## 5. Conclusions

— A 200 μm thick HA coating provides better protection towards metal ion release than passivation of a polished stainless steel surface, which in turn is superior to a 50 μm thick HA coating;

— HA coatings do not induce crevice attack at the metal/HA interface;

— After all the accelerated tests done in this work no detachment of the coating occurred for both HA thicknesses;

— Both HA thicknesses are effective in preventing the release of metallic species into HBSS.

## Acknowledgements

The authors gratefully acknowledge the support received from the Brite/Euram project "Surface Coatings for Biomaterials" (contract No. BREU/0172C) under which the present work was carried out. The authors are also indebted to Dr. Carlos Sá, from CEMUP, for the assistance with the SEM/EDS work and to Plasma Coatings for providing the coated samples.

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*Received 29 June*
*and accepted 4 July 1995*