A STUDY OF ZETA POTENTIAL OF PLASMA SPRAYED HYDROXYAPATITE COATING IN FOUR SIMULATED PHYSIOLOGICAL SOLUTIONS

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Abstract The zeta potential magnitude and the duration of its changes have been thought to be directly related to the surface reactivity, the governing of osteoconductivity and availability of valuable information in determining the anticipated in-vivo performance of implants. In this study the zeta potential of plasma sprayed hydroxyapatite (HA) was evaluated in various simulated physiological solutions. For this purpose the prepared samples were immersed in SBF, Ringers, Tyrodes and Hanks solutions for 3 weeks and kept at 37°C and the time dependent variations of zeta potential results were compared with calcined (2 hr at 850°C) and as-precipitated HA powders. Furthermore, following immersion test, the coating surfaces were examined with SEM and XRD as well as FTIR methods. The results showed that in majority of solutions, the zeta potential increased in the following order: calcined, as precipitated and plasma sprayed. Moreover, the ion concentrations and types in the solutions have significant effects on the zeta potential values. Following the immersion test, the morphology of the samples were determined a function of the thermal history of HA, duration of immersion and the type of the test solution. The results indicated that the surface of the HA coated samples were unstable with significant changes in the observed charge.

Key Words Hydroxyapatite, Zeta potential, Plasma spray coat, Corrosion

چکیده تحقیقات انجام شده نشان میدهند که مقدار و نحوه تغییرات پتانسیل زتا، اثرات تعیین کننده ای بر قابلیت تشکیل پیوند قطعات کاشت شده در داخل بدن دارند. بر این اساس در تحقیق حاضر پتانسیل زتا بیوسرامیک هیدروکسی آپاتیت پوشش داده شده به‌روش پلاسما اسپری در محلولهای فیزیولوژیکی شبیه‌سازی شده بررسی شده است. در این رابطه نمونه‌های آماده‌شده بمدت ۳ هفته در دمای ۳۷°C در داخل محلولهای فیزیولوژیکی شبیه‌سازی شده SBF، رینگرز، تیروید و هانک غوطه‌ور شده و تغییرات پتانسیل زتا آنها بررسی گردید. جهت بررسی اثرات پلاسما اسپری بر روی این بیوسرامیک آزمایشات مشابه بر روی هیدروکسی آپاتیت رسوب داده شده (خام) و بمدت ۲ ساعت در دمای ۸۵۰°C حرارت داده شده (کلسینه) نیز انجام گردید. علاوه بر این با استفاده از روشهای SEM، XRD و FTIR، اثرات خوردگی برروی سطح پوشش هیدروکسی آپاتیت بررسی شد. نتایج بدست آمده نشان میدهند که سطح نمونه‌های پوشش داده شده با هیدروکسی آپاتیت در داخل محلولهای بکار رفته، غیر پایدار بوده و تغییرات زیادی در مقادیر پتانسیل زتا آنها دیده میشود. بعلاوه در بیشتر محلولها، مقدار پتانسیل زتا برای نمونه‌های پلاسما اسپری شده زیادتر از بقیه نمونه‌ها بوده وپس از آن بترتیب نمونه‌های رسوب داده شده و کلسینه شده قرار دارند. در این رابطه اثرات نوع و غلظت یونهای موجود در داخل محلولهای بکار رفته نیز مورد بحث و بررسی قرار گرفته است. همچنین بدنبال غوطه‌وری در داخل محلول، مورفولوژی سطوح نمونه‌های هیدروکسی آپاتیت بر اساس سابقه‌حرارتی، مدت غوطه‌ورشدن و نوع محلول بکار رفته تغییر می‌نماید.

1. INTROCUDTION

Phenomena such as electrode kinetics, electro catalysis, corrosion, adsorption, crystal growth, colloid stability and flow behavior (both of colloidal suspensions and of electrolytes through

TABLE 1. The Molar Ca/P (Measured Analytically) Ratios of Hydroxyapatite Powders, which were used for Experiments. [For more details see References 12 and 13].

Name	Treatment	Molar Ca/P ratio
As-precipitate	-	1.685
Calcined	Heated for 2hr at 850°C	1.666
Plasma sprayed	The calcined powder which was plasma sprayed	1.715

porous media) cannot be properly treated without knowledge of the distribution of charges and dipoles in the interfacial region [1]. Initially, when a single negative colloid is in a solution, attraction from the negative colloid causes some of the positive ions to form a firmly attached layer around the surface of the colloid; this layer of counter ions is known as the Stern layer. A diffuse secondary layer is formed by the other positive ions that are also trying to approach the colloid. The attached counter-ions in the Stern layer and the charged atmosphere in the diffuse layer are what we refer to as the double layer. The boundary where the Stern layer and the diffuse layer meet is called the slip plane. The slip plane [1] or the surface of shear [2] is an imaginary surface, which is considered to lie close to the solid surface, and within which the fluids is stationary [1]. The zeta potential (□) of HA bioceramic, which is the value of the electrical potential of the double layer at the slip plane between HA and surrounding fluids, is related to bone formation and bone tissue attachment. [3,4]. The zeta potential measurement as a function of pH establishes one factor responsible for the observed physicochemical bonding between bone and HA noted by many in the orthopedic community [5]. Suzuki and his co-workers clarified the effects of zeta potential of ceramics on cell adhesiveness [6]. In the presence of an adsorbed layer the sign of the zeta potential depends on the polarity of the adsorbed ions, the surface charge of the solid, and the ion concentration in the fluid. Therefore, the sign of zeta potential can differ from the surface charge of the solid [3]. Some of the results from the important studies on the effects of ions on zeta potential amounts of calcium phosphates are summarized below:

1- K^+ and NO^{3-} ions do not affect the isoelectric point. [1, 3]

2- H^+, OH^-, PO_4^{3-}, Ca^{2+} [2, 3] and F^- [2, 7] and the ions formed by their reactions such as $CaOH^+$ and CaH_2PO^{4+} are the main potential determining ions for the system [1].

3- The negatively charged ions such as OH^-, HPO^{2-} and $H_2PO_4^-$ determine the net negative charge in the beginning of the immersion as well as Ca^{2+}, $CaOH^+$ and $CaH_2PO_4^+$ for the net positive charge on the surface at steady state [3].

4- Fluoride addition causes an increase in negative zeta potential of apatite but the effect is less than that produced by the addition of phosphate [2].

5- The charge at the surface of HA depends on the extent of hydrolysis of each ion. Hydrolysis is greatest for calcium ions at high pH and for phosphate ions at low pH, making the surface more negative in the former case and more positive in the latter [8].

Few electro kinetic studies, including zeta determinates have been performed on calcium phosphate materials in the simulated physiological solutions [6,9]. Investigations on changes of microstructure and the chemical behavior during immersion in simulated physiological solutions give valuable information about the anticipated in-vivo performance [3]. In this study, zeta potential variations with time for plasma sprayed HA coat were monitored. There are various simulated physiological solutions, which are commonly used as test media. In order to study the effect of the solution type on the results, some of the most common solutions (SBF, Ringers, Tyrodes and Hanks) were used, simultaneously [10,11,14,15,16]. It is also the purpose of this work to characterize the solution-mediated changes in the plasma sprayed HA as well as the starting HA powders. The results can reveal further structural information for dissolution mechanism

TABLE 2. Ion Concentrations (mM) of the Physiological Solutions used for Experiments [According to References 11, 15 and 16].

Solutions	Na^+	K^+	Ca^{2+}	Mg^{2+}	Cl^-	HCO_3^-	HPO_4^{2-}	SO_4^{2-}
Ringers	158.70	5.64	2.16	-	168.65	2.40	-	-
Tyrodes	141.85	2.68	1.80	0.49	147.84	12.00	0.26	-
Hanks	142.00	5.81	1.26	0.90	146.00	4.17	0.78	0.41
SBF	142.00	5.00	2.50	1.50	148.80	4.20	1.00	0.50
Human Plasma	142.00	5.00	2.50	1.50	103.00	27.00	1.00	0.50

TABLE 3. Composition (g/L) of the Physiological Solutions used for the Experiments [10,11,14].

Solutions	SBF	Ringers	Tyrodes	Hanks
NaCl	7.996	9.000	8.000	8.000
KCl	0.224	0.420	0.200	0.400
$CaCl_2$	0.278	0.240	0.200	0.140
$NaHCO_3$	0.350	0.200	1.000	0.350
$MgCl_2,6H_2O$	0.305	-	0.100	0.100
$Na_2HPO_4,2H_2O$	-	-	-	0.048
NaH_2PO_4	-	-	0.050	0.100
$MgSO_4,7H_2O$	-	-	-	0.027
$K_2HPO_4,3H_2O$	0.228	-	-	-
Na_2SO_4	0.071	-	-	-
$NH_2C(CH_2OH)_3$	6.057	-	-	-
Glucose	-	-	1.00	1.00
pH	7.40	7.40	7.40	7.40

of the coated samples by combination of the characterization techniques with the zeta potential dissolution test itself.

2. MATERIALS AND METALS

The materials used, their designation and the Ca/P molar ratios are summarized in table 1. The stoichiometric hydroxyapatite was synthesized by the wet chemical method in our laboratory with good heat stability [12]. The calcined samples were prepared by heating the synthesized HA for 2 hr at 850 °C in air and 100 - 200 micrometers size particles were sorted for plasma spraying. The feedstock HA powders and spray conditions used have been reported elsewhere [13]. Ti-6Al-4V

plates (30×30×1.6 mm) were plasma sprayed to have an HA coat with 100 micrometers thickness. For zeta potential measurements, the plasma coatings were scraped and milled with a vibration rotary silicon carbide mill for 20 minutes. The milling was continued to obtain a grain size less than 10 micrometers for all the samples. The same Ringers, Tyrodes and Hanks solutions were prepared by dissolving reagent grade of chemical components in distilled water (Table2). The ion procedure was used in order to prepare for as- precipitated and calcined samples. The SBF, concentrations of the solutions were selected to be close to those in human blood plasma (Table 3).

2.1 Surface Characterization of Coated Samples The HA coated samples was immersed 37°C for 3 weeks. The changes in characteristics of HA-coated plates were in SBF, Ringers, Tyrodes and Hanks solutions at investigated. After 2, 10 and 21 days, the specimens were taken out from the solutions washed in distilled water and subsequently in acetone. The washed specimens were dried in room temperature. After drying, the specimens were examined for coating characteristics including microstructure, phase purity and crystallinity. The surface morphology of HA coated samples was examined by scanning electron microscope (SEM, Hitachi-4500, USA), at an accelerating voltage of 2 KV. The morphology and size distribution of synthesized HA powder were investigated using SEM. A monochromatic copper Ka (Wave length = 1.5418 Å) was selected for XRD studies. The operational tube voltage and current were 30 kV and 30 mA respectively (Siemens diffractometer D5000, Germany). The chemical nature and molecular bond structure of the synthesized HA were determined using FTIR. (ATI MATTSON, Genesis series FTIR spectrometer, USA). Routinely, 50 interferograms collected at 4 cm^{-1} resolutions were co-added and the resultant the resultant interferograms Fourier transformed.

2.2 Zeta Potential Measurements The immersion tests were performed on powder samples in SBF, Ringers, Tyrodes and Hanks solutions (Table 2) at pH 7.4 with a 1-mg/ml weight to volume ratio without stirring. The electrophoretic mobility measurements were performed at 37°C using a zeta Potential Analyzer (Zeta Plus, Brookhaven Instrument corporation, USA) with 15 mV solid-state lasers operated at a laser wavelength of 635 nm. It uses electrophoretic light scattering and the Laser Doppler Velocimetry (LDV) method to determine particle velocity and, from this calculates the zeta potential of the particles using the Smoluchowski equation [1].

The factors that can influence the value of the zeta potential have been described before [3]. In this study, the tendency for different equilibrium condition due exclusively to particle concentration, shape and size were minimized. The effect of particle size and shape was also kept in check, for only the intense peak in the histogram was used to determine the electrophoretic mobility, and hence the zeta potential. For each test, at least three samples were used. For each time point examined, the zeta potential data (n = 5) presented here were calculated from average electrophoretic mobility values based on five separate histograms and standard error of the mean were calculated.

3. RESULTS AND DISCUSSION

Figures 1a to 1d show surface morphology of the plasma sprayed Hydroxyapatite samples before and after soaking in SBF. This surface reveals significant changes after to 3 weeks of immersion. In the first few days, the surface was cracked and appeared to have new material deposited on the surface dissolution, and nucleation of globular precipitation occurred on the surface as well as more microcracks on its surface (Figure1b). This disssolution seems to have occurred with detachment of unmelted cores of starting powder in amorphous matrix [13]. As Duchyne and his co-worker mentioned [17], there are usually two possible methods of degradation: one is dissolution of the soluble phases like alpha tri-calcium phosphate, tetra calcium phosphate and amorphous phases which are present in coatings; another is the loosening or detachment of unmelted cores of starting powder in amorphous matrix [13]. After 3 weeks of immersion, the layer became smoother and many circulated splats of around 10

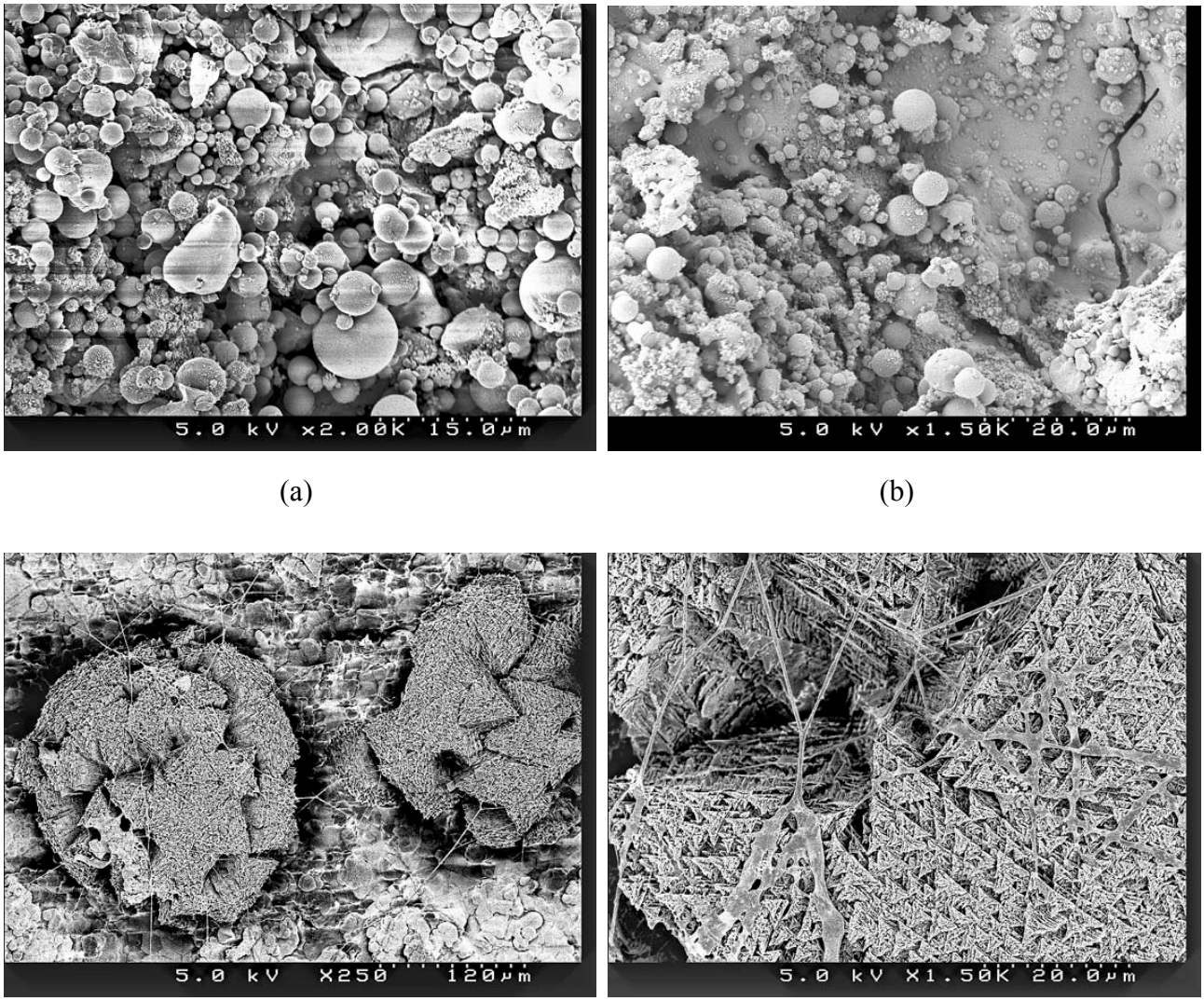


Figure 1. The SEM micrographs of the surface of the plasma sprayed HA sample before (a) and after 2 days (b) and 21 days (c,d) soaking in SBF at 37°C .SEM micrographs obtained at various magnitudes. Notice more microcracks in morphology of the 2 days immersion sample (b) compared with the as-sprayed micrograph (a). Also notice significant changes in morphology of 21 days immersion sample (c) compared with the 2 days immersion sample (b).

micrometers were isolated from the surface matrix (Figure1c). The samples which, were soaked in other solutions, were not significantly different from these mentioned sample. But after 21 days, it seemed that the SBF-soaked samples appeared sparser to be composed of globular material with smaller coating patches compared to the samples, which had been soaked in the other solutions. The net like structure of these surfaces actually is the individual HA crystals (Figure 1c), which can be seen in higher magnification monograph (Figure 1d). The amount of the above-mentioned petal like crystal was less for the sample soaked in Ringers solution compared to other ones.

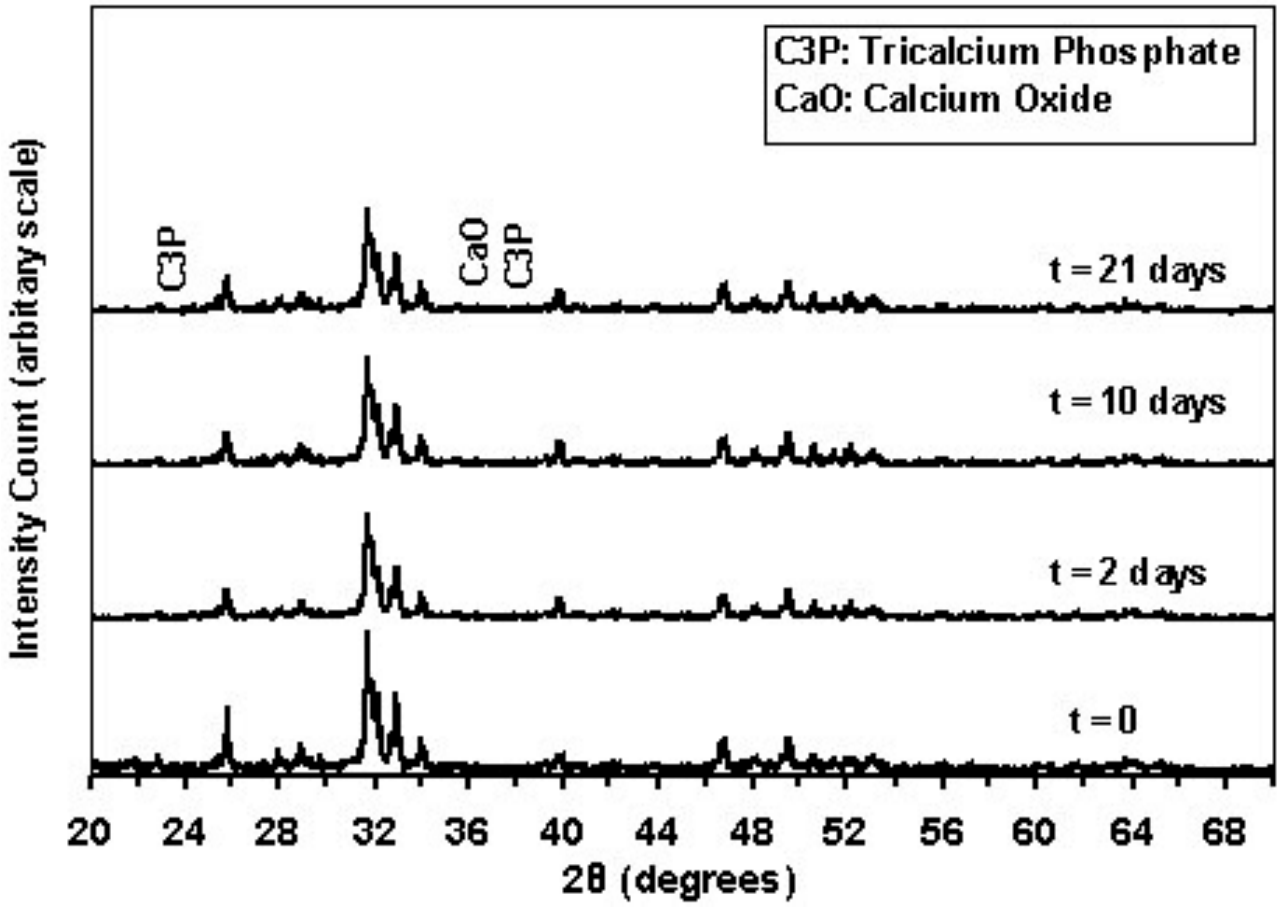


Figure 2. Typical XRD patterns for the plasma sprayed HA coatings before (t = 0) and after 2, 10 and 21 days soaking in SBF at 37°C .

Figure 2 shows typical X-ray diffractions of an HA coated sample before and after 2, 10 and 21 days of immersion in SBF solution respectivly. The crystalline phases of as-plasma coated are primarily a mixture of HA with very small amount of alpha-TCP and calcium oxide [13] which are labeled in this figure. The relative amount of this impurity decreased with the increase of the time of immersion for all solutions.

Figure 3 illustrates the typical infrared analysis of this sample before and after 2, 10 and 21 days immersion in SBF solution. The characteristic features like a very weak OH- band at 3570 cm^{-1} and the absence of hydroxyl band at 633 cm^{-1} in as-plasma sprayed sample are indications of dehydration of HA [13,18]. After 21 days immersion the intensity of the above mentioned peeks [13] increased. This result shows that the dehydroxylation degree [11,19] of the coat decreases with an increase in the time of immersion. The 961 cm^{-1} bands, characteristic of symmetric stretching of PO_4 group also shows some changes that can be related to crystallinity changes [20,21] or dissolving of secondary phases [22]. Some little peaks also appear at 1450-1550 cm^{-1} wave numbers that are characteristics of carbonated apatite [22], as deposited on the surface of samples. These findings are compatible with those Li and his co-worker [23]. The result taken from the samples which were soaked in other

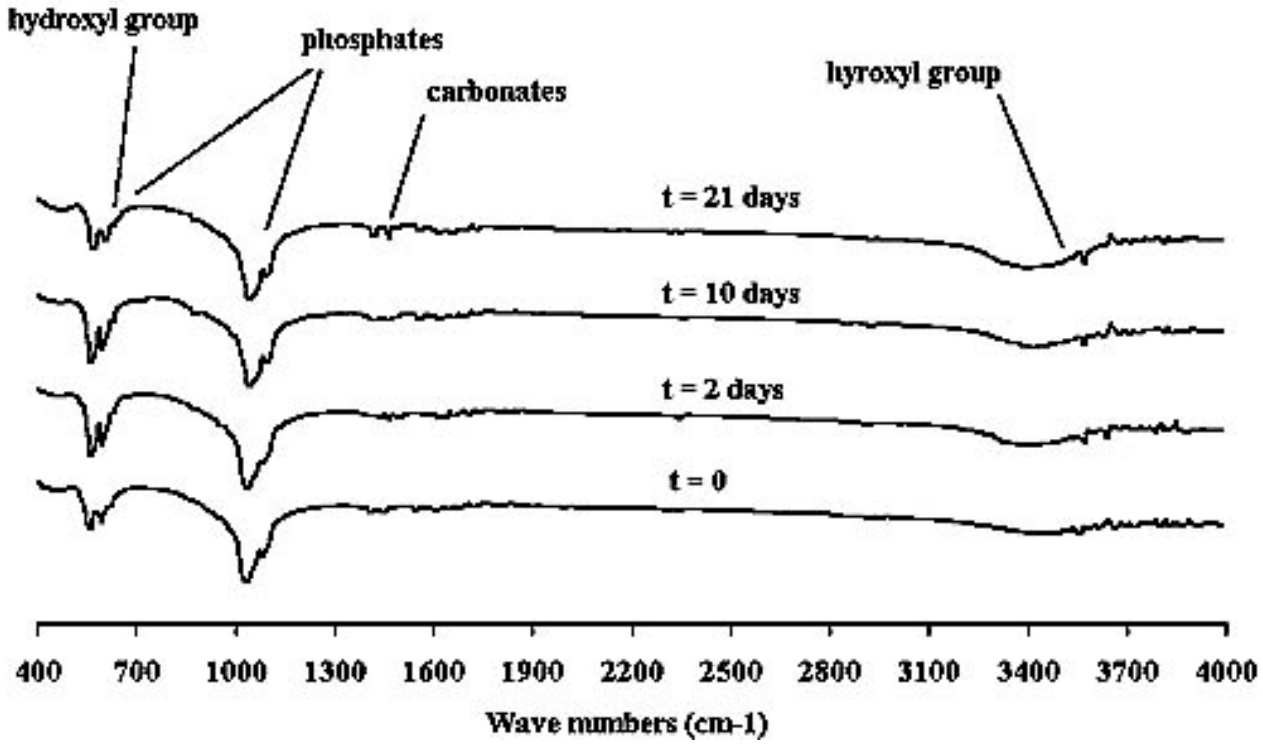


Figure 3. Typical FTIR spectra for the plasma sprayed HA coatings before (t = 0) and after 2, 10 and 21 days soaking in SBF at 37°C .

solutions are also the same. Joint XRD and FTIR analysis indicated that after 10 days of immersion, a more crystalline structure is formed due to loss of the more soluble secondary and amorphous phases [11]. Also, after 21 days immersion, a bone-like apatite layer precipitates on the coat which is found to be poorly crystallized and contained carbonate. These results are similar to those of the samples which had been soaked in other solution.

Time-dependent changes in as-precipitate, calcined and plasma sprayed HA's zeta potentials are shown in Figures 4a to 4c, respectively. Figures 5 and 6 show the variation of zeta potential of several HA powders as a function of immersion time in Ringers, Tyrodes, Hanks and SBF solutions. Each data point represents the mean of five zeta potential values calculated based on average EPM of five histograms (n = 5).

The error bar represents the standard deviation of the mean values. The data showed that the surface was unstable with significant changes in the charge being observed. As shown in Figures 5 and 6, for most solutions, after 21 days immersion, the zeta potential values increase in the following order: calcined, as-precipitated and as-plasma. A comparison between the zeta potential values and the Ca/P ratio values of the samples (Table 1) reveals that with decrease of Ca/P ratio, the zeta potential values decrease toward more negative amounts. This result is compatible with that of Suzuki and his co-workers who have worked on the effects of the Ca/P ratios on zeta potential of calcium phosphates [6]. Also, as the results of XRD showed, the amount of secondary phases have increased in the same order [13]. This reveals that dissolution of secondary phases have significant effects on the zeta potential value of HA.

The zeta potential variations with time are similar for most of the samples since the differences

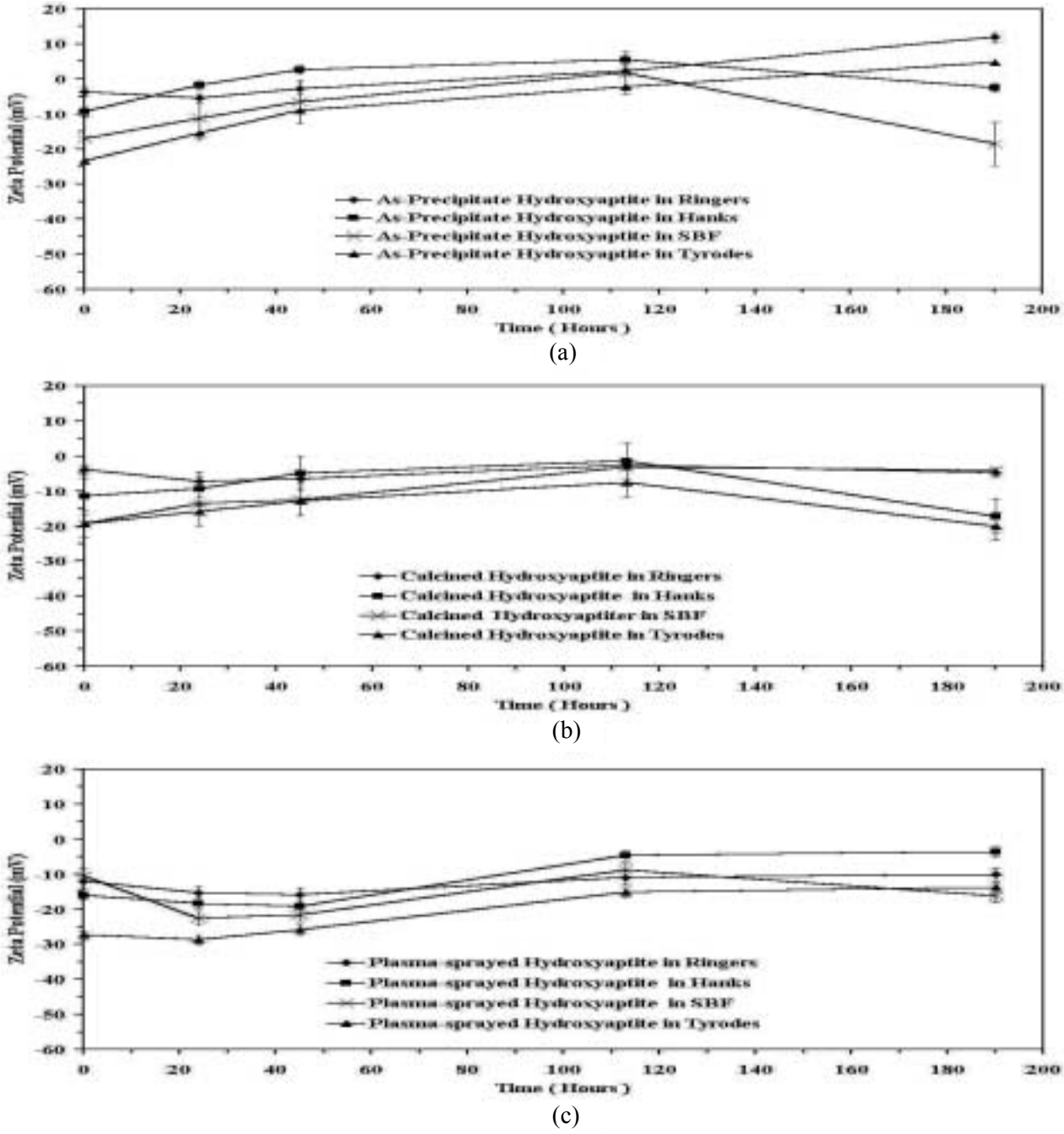
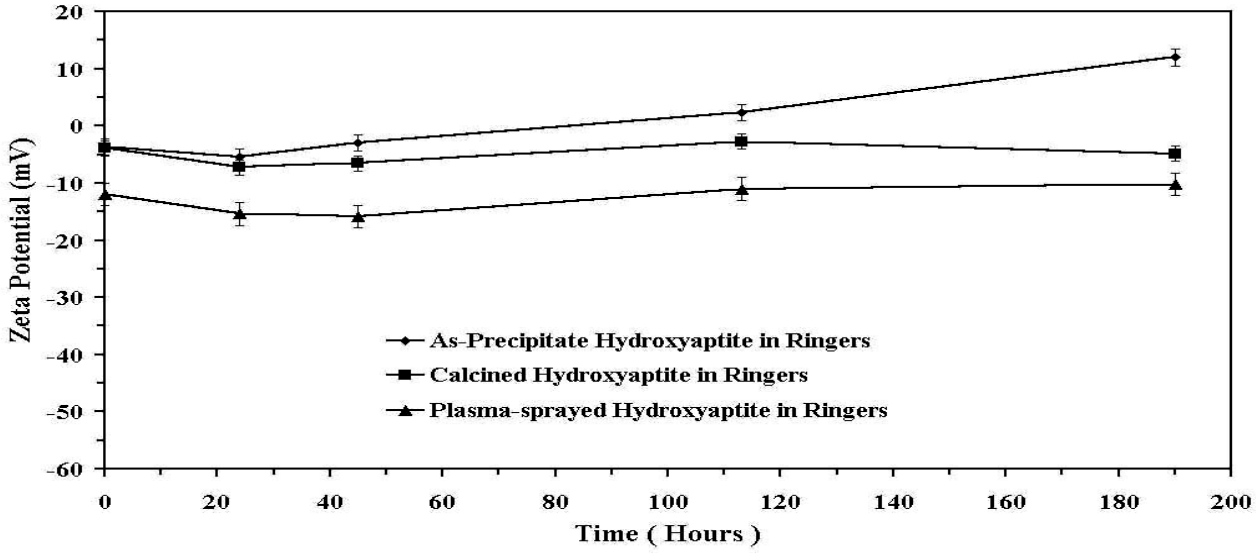
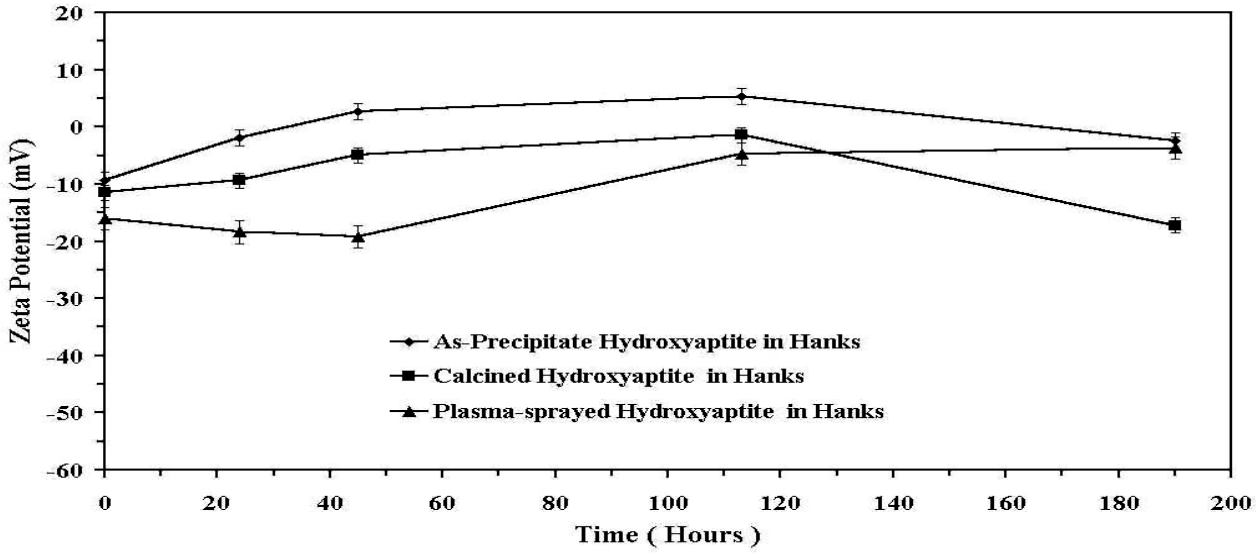


Figure 4. Variation of the zeta Potential as a function of time of immersion of as precipitate (a), Calcined (b) and Plasma sprayed (c) Hydroxyapatite in Ringers, Tyrodes, Hanks and SBF solutions at 37°C .

between them slightly decreased after a long immersion time. The kinetic zeta potential variations corresponded to, and might directly influence, a Ca-P layer formation. Furthermore, it



(a)

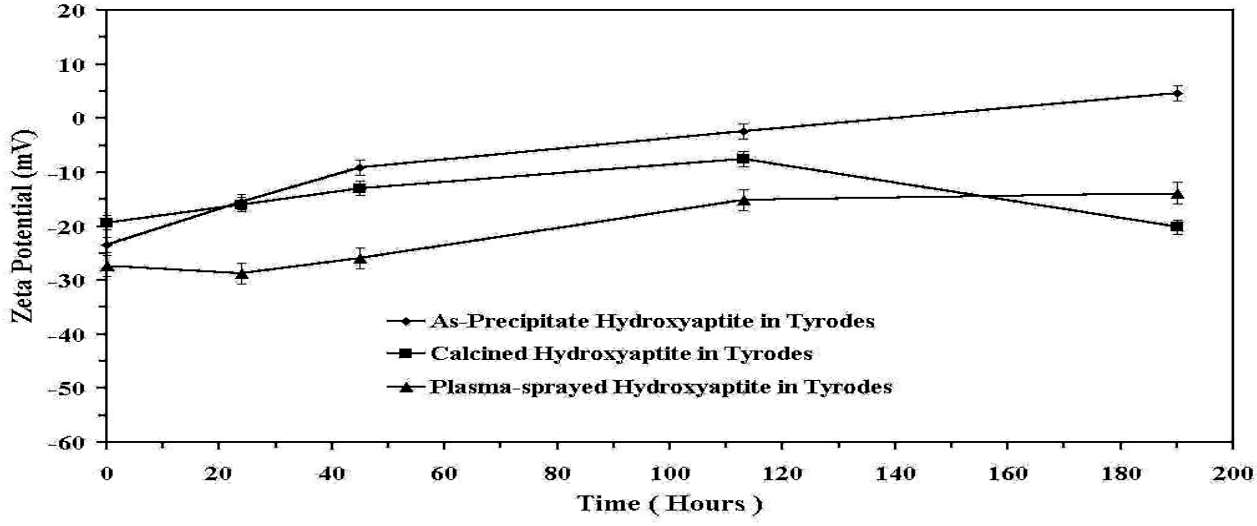


(b)

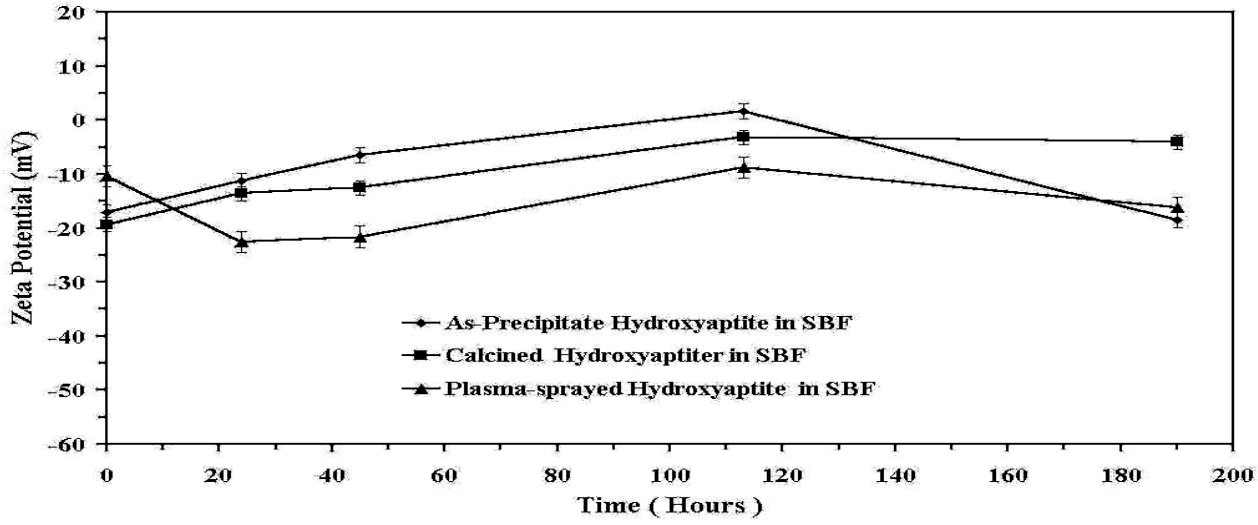
Figure 5. Variation of the zeta Potential as a function of time of immersion of as precipitate, Calcined and Plasma sprayed Hydroxyapatite in (a) Ringers and (b) Tyrodes solution at 37°C.

may be presumed that the precipitated layers which have been deposited on the surface of sample particles, is a determining factor for the Stern layer`s potential.

For the Ringers solution, the zeta potential amount is more positive in comparision with the other solutions, especially at the beginning of immersion. According to Table 3, Ringers solution does not have any phosphate ions by itself. Thus at the beginning of immersion, in the case of Ringers



(a)



(b)

Figure 6. Variation of the zeta Potential as a function of time of immersion of as precipitate, Calcined and Plasma sprayed Hydroxyapatite in (a) Hanks and (b) SBF solution at 37°C .

solution, the effective ions integrated on the surface of the Stern layer and then dissolution of HA followed this reaction [11]:

$$Ca_{10}(PO_4)(OH)_2 \rightarrow Ca(OH)_2 + H_3PO_4 + H_2O$$

OH^- and PO_4^{3-} ions integrated on the surface so calcium ions could be absorbed to this layer and zeta potential lightly decreased. Consequently these calcium ions could produce $Ca(OH)^-$ complexes here and make zeta potential more negative.

The effects of solution compound on the zeta potential of HA are shown in Figure 4a to 4c. Tyrodes and Ringers solutions show respectively

the lowest and the highest zeta potential values, especially at the beginning of immersion. As shown in Table 3, Tyrodes solution has the most HCO_3^- ions concentration compared to other solutions. On the other hand Ringers solution has more Ca^{2+} ions and less HCO_3^- ions and as a result the zeta potential would be more positive.

4. CONCLUSION

The results of XRD and FTIR were shown, the amount of amorphous and secondary phases (TCP, Tet-CP and CaO) in the coat decreased after soaking in the solutions. On the other hand, the increased degree of hydroxylation of the coat after immersion shows that the coat surface absorbs hydroxyl ions. As a result, a relatively large amount of dissolution products as well as hydroxyl ions were found available near the surface of the coat at the beginning of soaking. The negative zeta potential values at the beginning of soaking show the negative charge at the Stern layer. This is caused by the negative ions that were adsorbed on surface of the samples such as hydroxyl ions. These adsorbed hydroxyl ions can also perform hydrogen bonding with phosphate groups [23]. This bonding can form a local saturation [15] of HA at the surface that leads to precipitation of amorphous HA. Furthermore, as the FTIR results show, in the presence of carbon dioxide in air, the deposed HA is carbonated (type A-B) [11]. SEM studies showed that, this precipitate layer became smoother as time progressed. With formation of this layer, the concentration of the adsorbed negative ions ($Ca(OH)^-$ HCO_3^- OH^- and PO_4^{3-}) decreased and so the zeta potential values started to increase. The decrease enhancement of zeta potential amount, as time progressed, is due to diffusion of the ions from a longer distance. On the other hand, this observation is reasonable considering that the solution had considerable amounts of sodium bicarbonate as a buffer. Thus, the possibility exists that some type of dissolution-precipitation reaction would have occurred. Another possibility for the increase in the carbonate vibrational band in the FTIR spectra for the immersed HA, is CO_3^{2-} ions substitution into the apatite lattice. Such a substitution will decrease the solubility of the HA coating and has been proposed for plasma sprayed HA by Whitehead et al. [24]. This may be the reason for the smoother changes of zeta potential and it seems that this reaches a steady state condition. The amount and speed of each of change of zeta potential is dependant upon the properties of HA as well as the ion concentration in the test solutions.

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