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Synthesis and Characterization on Atomospheric Plasma Sprayed Amorphous Silica Doped Hydrxoyapatite Coatings

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Abstract

Plasma sprayed hydroxyapatite (HA) coatings have been widely investigated for application on surface of biomedical metals to improve bonding and interaction between body tissues and implant. In this study, a small amount of amorphous silicon dioxide (silica, ~ 1, 2, 5wt%) was introduced into HA slurry which was subsequently spray-dried into powder form. The powder feedstock was then sprayed onto Ti-6Al-4V alloy substrates by an atmospheric plasma spraying technology. To improve the bonding strength of as-sprayed coatings, the substrates were pre-heat treated to 250 °C by plasma. The characteristics of the as sprayed coatings were investigated by various technologies inclusive of scanning electron microscopy (SEM), X-ray diffraction and X-ray photoelectron spectrometry, and Raman spectrometry. The adhesive bonding strength of the as-sprayed coatings was specified using a universal testing system. Modulus and hardness of the coatings were investigated using nano-hardness tester. The results showed that, with pre- substrate heat treatment, the presence silica have enhanced the hardness, modulus and adhesive strength of coatings. Incorporation of Si has affected the structure of HA crystal involving the exchange of oxygen and silicon. In addition, in vitro osteoblasts cellular responses were assessed and discussed to investigate the cell proliferation and differentiation on the as sprayed coatings.

Keywords: hydroxyapatite; silica; atmospheric plasma spraying; coatings; osteoblasts;
1. Introduction

The majority of bone consists of extracellular matrix proteins and the mineral calcium phosphate which contains various ionic substitutions. Due to its chemical composition similar to the bone mineral, hydroxyapatite (HA, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) based materials are widely used for bone replacement in biomedical applications. The osseointegration of plasma sprayed HA is faster than uncoated implant [1]. However, its inferior mechanical properties and relatively long remodeling time limit its medical applications. To improve the performance of HA, effort has been invested on improving mechanical properties, biocompatibility and bioactivity of HA, as these properties are critical for long term applications of implant [1-3]. Currently, experimental syntheses of chemically doped or ion-substituted HA have drawn great interest due to the importance of the ions in developing artificial bone [4-6].

Silicon (Si) is concentrated at the mineralisation front of growing bone [7]. It has been stated that silicon plays a role during the bone remodelling process and it is essential for maintaining healthy bone and connective tissue. The presence of Si element could increase the osteoblast cell proliferation therefore resulting in an increased bioactivity. There is 1-2 grams of silicon present in the body. In its pure form, silicon cannot stand alone in a natural state due to its vulnerability to atmospheric water and oxygen. Instead, one such mineral, silica ($\text{SiO}_2$) is found in polymerized combinations with metals as silicates. Silica is also an important food additives but it may be not safe when the $\text{SiO}_2$ quantity is more than 2 wt% of the food weight. Silica surfaces are hydrophilic [7]. Amorphous silicas are generally considered to be non-toxic. The local adsorption of biologically active peptide growth factors onto the surface of an implant may contribute to the unique osteogenic nature of silica-containing bioactive ceramics. Biological silica production has drawn intense attention and several molecules involved in biosilicification have been identified [8-9]. Main advantage utilise was the ability to form lipid bilayers which act as an inert platform for further immobilisation of molecules. Additional research has demonstrated that silica played a vital role in the formation of apatite crystal, the main constituent in bone [10]. Some studies reported that the importance of silicon in bioactive materials for bonding of bone and muscles as well as cross linking agent in connective tissues. Doping HA with Si element would be a potential method to get a Si substituted HA with enhanced bioactivity without compromising its mechanical properties. Morks [11]mixed fused silica powder with HA powder following by plasma spraying and the results showed that the presence of silica significantly improved the adhesive strength of HA/$\text{SiO}_2$ coating due to the increase in bonding strength of the coating at the interface. Plasma spraying was the preferable method commercially available for coating metallic implants with HA [11-13]. HA coating has been well characterized chemically and mechanically as its benefits are demonstrated in both short- and long-term clinical results [14-16]. These coatings are able to support bone ingrowth and osseointegration when used in orthopedic, dental and maxillofacial applications. Moreover, it has the capacity to shorten the healing process of metal based implants. Atmospheric plasma spraying (APS) is a well known deposition technique for bioactive HA coatings. An ideal HA coating must have strong cohesive strength, low porosity, high degree of crystallinity, high purity, phase stability and suitable crystal structure for the osteoconductive process and for the bone to bond directly and preventing its contact with the bare metal.

There is only one interesting study on the plasma sprayed 10 wt% and 20wt% of silica mixed HA powder which investigated the microstructure and mechanical properties [11]. The focus of this paper is to extend an understanding of the chemical and physical characterization of plasma sprayed HA with trace amounts of silica. In this study, a small amount of amorphous silica (~1, 2 and 5 wt%) was introduced into a chemically synthesized HA slurry which was subsequently spray-dried into powder. A silica doped HA coating (2SiHA coating) was then deposited onto Ti-6Al-4V alloy substrates by APS technology. The surface chemistry that would directly influence bone forming cell proliferation was characterized through advanced analysis methods inclusive of SEM, XRD, XPS,
and Raman spectrometer. Moreover, the bonding strength of the as-sprayed coatings were measured using a universal testing system. The microstructures of the detached surfaces were carefully examined using SEM. The cell growth of osteoblasts on the plasma sprayed coatings were also examined.

2. Experimental details

An HA slurry was prepared using a wet chemical approach by reacting 0.6 mol of orthophosphoric acid (H$_3$PO$_4$) with 1 mol of calcium hydroxide (Ca(OH)$_2$). The precipitation reactants were carried out at 40 ± 5 °C and the reaction was terminated when the pH reached 9 through the addition of H$_3$PO$_4$. After the complete mixing of the reactants, the precipitate was stirred for two hours and left overnight to settle. Amorphous precipitated silica at about 1, 2 and 5 wt% was added into the HA slurry and the slurry was stirred for 4 more hrs before transportation into a spray dryer (Ohkawara LT-8, Japan), where the atomization and drying processes took place. The results from the laser particle size analyzer (Fritsch 22, Germany) show that all the powders have median diameters of 14-17 μm. The processing steps used for all materials result in similar particle size distributions, regardless of the doped silica. This implies that the low amount of silica doesn’t influence the separation of HA during the spray drying steps. A typical picture of the particle size distribution is shown in Figure 1(a). It is found that the median particle size of spray-dried HA powder is about 15.9 μm. From the two main peaks of the size distribution graphs in Figure 1(a), it can be concluded that around 10% of the powders are less than 5μm and 90% of the powders falls in the range of 5-40 μm. Figure 1(b) showed typical SEM images of spray dried HA powder doped with 5wt% of silica. It could be observed that the silica has distributed homogeneously in the spray dried powder feedstock.

The above synthesized spray dried nanostructured HA powder was utilized for the coating deposition on commercial Ti-6Al-4V substrates via plasma spraying process using a Sulzer-Metco F4-MB (Switzerland) plasma gun mounted on an ABB IRB2400 robot(Sweden). Before coating deposition, degreasing and grit blasting were carried out to make the substrate surface clean and coarse (roughness Ra=5.1±0.3μm). The plasma spraying distance was about 90 mm. Purified argon was used as a main arc forming gas and carrier gas. Helium was used as an auxiliary gas. The main gas, auxiliary gas and carrier gas flow rates, were 40, 20 and 3 liter per minute, respectively. The current is 600 A. Consider the high wetting ability of silica at high temperatures, some of the substrates were pre heat treated at about 250 °C by the plasma. Coating sample codes, staring powder and plasma spray process parameters were listed in Table 1. The coating thickness was calculated by measuring the substrate thickness before and after plasma spraying using a micrometer.

Microstructures of the coating samples were analyzed using a scanning electron microscopy (SEM, JEOL JSM 5600LV). Phases and lattice parameters were identified using X-ray diffraction (XRD) (Philips MPD 1880, the Netherland) using Cu K$_α$ radiation at 30 kV and 20 mA. The XRD analysis was carried through the 2θ from 20° to 60° at a scan rate of 0.04 degree per second. Lattice constants (a, b and c) of hydroxyapatite in the as sprayed coatings were refined by software Maud using XRD data. The space group is P6$_3$/m. To get complementary detailed information about the molecular environment and crystallization state of calcium phosphate, Raman spectra of the samples were recorded at room temperature and atmospheric pressure on Renishaw Raman Imaging Microscope (RM1000, United Kingdom). The excitation source was formed by a 633 nm line of a HeNe laser at a maximum power of 50mW attached to the microscope to provide a confocal illumination of the sample via a holographic beam splitter. The exposure time is 10 s. The optical objective is 50X with one time of accumulation.

The chemical composition of the samples was determined by X-ray photoelectron spectroscopy (XPS). XPS spectra were recorded on a Kratos spectrometer (Japan) operated using Al Kα (1486.6
eV) monochromatic X-ray source. The XPS pressure was about $10^{-9}$ Pa. The calibration was done by referencing to C 1s (284.5 eV).

The adhesive strength of the coatings was measured using a peel-off method. A universal testing system, Instron 4204, was used for the measurements according to the ASTM C633-79 standard. The coated samples of 2 cm$^2$ were affixed from both sides of coat and substrate between two stainless steel rods using an FM® 1000 adhesive film (Cytec, USA). The cross head speed was 1mm/min. Each adhesive strength value is the average of three tested samples.

The hardness (H) and Young’s modulus (E) of the coatings surfaces were studied using a Nano II$^{TM}$ nanoindenter with a Berkovich indenter tip (USA). Pure fused silica with a Young’s modulus of ~72GPa was used as a calibration material for the nano indentation. The maximum indentation depth was 100 nm with a dwell time of 10 s. During the tests, every two points had a distance of ~200 µm to avoid the stress-strain influence caused by previous loading the preceding indentation. The average values were calculated from at least eight readings for each compacted sample.

Human osteoblast like cells (hFOB 1.19 (ATCC, CRL-11372)) were used to assess the cellular responses to the sprayed coatings. The culture medium was a 1:1 mixture of Ham’s F12 medium and Dulbecco’s Doped Eagle Medium containing 2.5 mM L-glutamine and 0.3 mg/mL G418 and supplement with 10 vol% of fetal bovine serum and 5vol% of antibiotics. After sub-culturing in a humidified atmosphere of 5% CO$_2$ 95% air at 37°C, the cells were washed with phosphate-buffered saline (PBS), detached with trypsin-EDTA solution (0.3% trypsin) at 37°C for 5min, and centrifuged and resuspended for further cell tests. The coatings samples were sterilized via autoclaving, the resuspended cells were seeded with an initial density of $5 \times 10^4$/cm$^2$ on the sterilized samples in a 6-well Petri dish. Based on the capacity of viable cell to metabolize tertrazolium to formazan crystals, a methyl thiazo lone tertrazodium (MTT) method was used to determine the cell proliferation after culturing for various periods [17]. The cells cultured on the sterile plastic Petri dish were used as a control group. The procedures followed the steps described in a previous report [17]. The number of the cells was determined according to a plot of absorbance against the standard numbers of cells. For the MTT tests, three of each kind of samples were employed and three separate experiments have been carried. The functional activity of the proliferated cells was examined by measuring the alkaline phosphatase (ALP) activity. The cells were cultured for 1 and 2 weeks. An ALP gene assay kit (APF, Sigma) was used to determine the ALP activity. A microplate fluorescence reader(FL600, BioTek) which was set to 360nm excitation and 440nm emission was used to measure the absorbance intensities.

3. Results and Discussion

Figure 2 shows the typical surface microstructures of the as plasma sprayed coatings which are generally porous. The porous structure might be beneficial to the biomedical application involving the mechanical fixation through bone ingrowth. The plasma sprayed HA coatings contained fully melted, partially melted and few unmelted particles as well as few micro cracks. The coatings thicknesses are about 150 µm. It was noted that H1 coating and the other coating with substrates exposed pre plasma heat treatment predominantly consisted of well flattened lamellae. The regular formation of neatly stacked disc like splats produced a flat, less porous and smooth surface profile. Quite porous surface microstructures were observed in the S1 coatings using silica doped HA powders (Figure 2c). The presence of silica appeared significantly change the morphological feature of the surface without exposing pre plasma heat treatment. As observed, a significant percentage of HA grains did not melt during the spraying. This relatively poor coating microstructures consisted of micro and macro pores, irregular splats and unmelted particles. Because of limited interparticle cohesion, these silica doped HA coatings have poor structural integrity. Due to the high wettability of silica, the presence of silica couldn’t form a splat upon bouncing on the substrates, which resulted in the formation of ball like particles. This ball like particles lead to the poor structures of as-sprayed coatings. From an in vitro
point of view, this rough microstructure in the silica doped coatings may give rise to the decrease in cell proliferation activity.

XRD analysis of the plasma sprayed coatings (Figure 3) revealed the presence of alpha tricalcium phosphate (TCP, Ca$_3$(PO$_4$)$_2$), tetra calcium phosphate (TTCP, Ca$_4$P$_2$O$_9$), amorphous phase and minor of calcium oxide in addition to crystalline HA. These phases came from the decomposition of the apatite induced by the high temperature plasma spraying process. Plasma sprayed HA coatings are known to contain other bio-resorble phases such as alpha TCP, beta TCP, TTCP, CaO and amorphous calcium phosphate. In this study, no obvious calcium silicates or calcium phosphate silicates were detected. This was possibly due to the relatively low amount of silica added. Moreover, the addition of silica has affected the relative crystallinity of the as sprayed coating by comparing the highest peak (211) with that of the HA coatings. The crystalline HA peaks became less sharp and broaden, which indicated the decrease in degree of crystallinity. This might be due to the presence of amorphous silica adjacent to or in contact with HA particles affected the melting degree of HA particles [11].

Fig. 4 shows the Raman patterns of coatings deposited on the substrates. The results are supportive and complementary to the XRD results described in the previous section. The spectrum of HA contains the phosphate bands and the bands due to hydroxyl ion stretching and vibrational modes. The vibrational spectra of calcium phosphates exhibit a strong molecular character associated with the internal modes of the PO$_4^{3-}$ tetrahedra. The P-O stretching bands at 1090, 1029, and 962 cm$^{-1}$ were clearly observed. The strongest line situated at around 962 cm$^{-1}$ was attributed to the symmetric stretching $v_1$ mode of PO$_4^{3-}$. The phosphate $v_1$ modes are notably broaden showing shoulder peaks, which indicates the presence of amorphous phase [18]. The peaks situated at about 1040-1090 cm$^{-1}$, 571-603 cm$^{-1}$ and 430-450 cm$^{-1}$ were assigned, respectively, to the antisymmetric stretching $v_3$, the antisymmetric bending $v_4$ modes and the bending $v_2$ of the PO$_4^{3-}$ ions [19-21]. Compared to the band of P-O in the powder, broad bands corresponding to the P-O stretching mode could be seen and no distinctive difference was noticed among the coatings, which was attributed to the poor crystallinity of the coatings. No obvious peak belong to Si-O was detected. The bands originating from the stretching vibrations of the Si-O bond overlapped with the bands of the PO$_4^{3-}$ [22]. This was due to the similar types of vibrations between the connected SiO$_4$ and PO$_4$ tetrahedron. Additional analysis method should be employed to characterize the chemical presence of silicate in the coatings. As shown in Fig. 4, it was found that the intensities of hydroxyl absorption band at 3571 cm$^{-1}$ were similar to the intensities in the coatings without exposure pre plasma heat treatment. The intensities of hydroxyl absorption band in Si-4 coatings were slightly lower than that of phase pure H1 coatings. It indicated that the presence of silica might accelerate the breakdown process of the hydroxyl group in HA, and some vacancies or oxygen ion may substitute the hydroxyl sites and lead to a distortion of the hexagonal symmetry. The distortion of the hexagonal symmetry would lead the decomposition of apatite into alpha TCP [22]. Moreover, the presence of silica also influence the decompositon of HA to form coarse microstructure as shown in Fig. 1 due to its low melting points in the amorphous state.

The surface chemical compositions of the as sprayed coating surfaces were analysed by XPS. Fig. 5 shows the representative survey spectra. Ca$_2p$, Ca$_{2s}$, P$_{2s}$, P$_{2p}$, O$_{1s}$, and C$_{1s}$ were identified for all test conditions.

Figure 6 shows the core values of O$_{1s}$, P$_{2p}$ and Si$_{2p}$ in the as sprayed coatings. Compared to the peak tested on the H1 coating, the O$_{1s}$ peak in the plasma sprayed silica doped HA coating (Figure 6a) showed an asymmetry and was fitted using the peak deconvolution approach. The O$_{1s}$ peak is doublet containing two sub-peaks. The exact binding energy of an electron depends not only upon the level from which photoemission is occurring, but also upon:

1. The formal oxidation state of the atom;
2. The local chemical and physical environment.
Changes in either (1) or (2) give rise to small shifts in the peak positions in the spectrum. Atoms of a higher positive oxidation state exhibit a higher binding energy due to the extra coulombic interactions between the photo-emitted electron and the ion core. This ability to discriminate between different oxidation states and the chemical environments is one of the major strengths of the XPS technique. Accordingly, the variations in binding energies in Fig. 6a reflected the differences in the electronic environment of the O element. The main component at 532.1 eV corresponded to the O linked only to a phosphorous atom as in PO$_4^{3-}$ ions. The second at higher energy was attributed to polymeric Si-O peak. As shown in Fig. 6b, P$_{2p}$ peaks were quite stable at 133.6 eV because the binding energy of this element did not change much inapatite due to the screening of the P atom by oxygen atoms, and to a close crystalline potential [23]. Though no obvious calcium silicate peaks were observed in XRD pattern, it was still assumed that HA decomposed into TCP and CaO and these chemicals could react with silicate to form calcium silicate. Incorporation of Si might have affected the structure of HA crystal. Possible formation mechanism involves the exchange of oxygen and silicon between Si$^{1+}$: Si$_3$O, Si$^{2+}$: SiO, Si$^{3+}$: Si$_2$O$_3$ and Si$^{4+}$: SiO$_4$. Compared to that of S1 coating, higher oxidationstates of Si was obsevered in S2, S3 and S4 samples. The effect might be significantly influenced by the processing technique. Moreover, the increased lattice parameters (Table 2) of the as sprayed coatings with the introduction of silicon indicated the possibility of substitution of phosphate by silicate [22]. In our study, we believe the rapid quenching (~10$^5$ K/s) of the melt particles upon impingement on substrate/pre-coating should be responsible for the structure without presence of any calcium silicate base materials such as, Ca$_{10}$[(OH)$_2$ (SiO$_4$)$_3$(PO$_4$)$_3$] or (Ca$_{40}$[(OH)$_2$ (SiO$_4$).PO$_4$)$_6$].

The mechanical properties of the as-sprayed coatings were tabulated in Table 3. In our experiments, the tensile strength from H1 coating was about 14.65 MPa. When the substrate was pre-heated, the decrease of adhesion strength of HA coating might be due to micro-cracks formation in the coating which was caused by the influence of increased substrate temperature [24]. Further studies on the effects of substrate temperatures on the mechanical properties of the as received coatings are under proceeding. The silica doped HA coating without pre plasma heat treatment has a low bond tensile strength of 3.00±0.25 MPa which was due to the porosity. With pre substrate heat treatment up to 250°C, the highest adhesive strength of 14.73MPa was achieved in S2 coatings. From the implantable medical device industry point of view, a value of 7 MPa in adhesive strength was acceptable for HA coating on metallic implants. The porous characteristics of thermal sprayed ceramic coatings were initially thought to benefit biomedical application because of the possibility of mechanical fixation through bone in growth. However, too porous structures sacrificed the bonding strength limiting the possibility of achieving the desired coating characteristics for biomedical application. The successful use of plasma sprayed HA coatings require strong adhesion between the ceramic coating and the underlying metal substrates. Bond strength was one of the important mechanical properties that can affect the ultimate success of the coatings on metallic implant. Generally, the coating failure mode affecting coating deterioration was classified into the following failure types: adhesion (between the coating and substrate), cohesive (within the coating), and adhesive (between the epoxy and the coating) failures [25]. Failure of HA coated titanium alloy implants during in vivo testing could happen: (a) at HA/Bone interface; (b) inside the lamellar splat layer of coatings; or (c) at the HA/substrate interface. Usually, the failure was neither completely cohesive nor completely adhesive. Measurement of the success of a particular joint was based on the relative percentage of cohesive to adhesive failure. Fig. 7 shows that the broken surface of the coatings after tensile test. In this experiment, it was found that the failure started at some sites of coating/substrate interface and spread within the coatings. This in turn indicates that the coating/substrate interface plays a key role in determining the adhesions [26]. The enhancement of the adhesion between HA coating and Ti substrate was more important than the strengthening of the HA coating [27]. Results from Young's modulus and hardness (Table 3) showed the highest in modulus and hardness values were obtained in S2 coatings compared with other coatings because of
the decrease of porosity and increase in the bonding strength of HA by adding 2 wt% silica. These results confirmed that the pre substrate heat treatments were important for HA with the presence of silica in the plasma processing and our plasma processing parameters were adequate for obtaining strong 2 wt% silica doped HA coatings with adequate mechanical properties on Ti-6Al-4V substrate.

We selected three kinds of coatings (H1, S2 and S4) with higher adhesion strength to examine the influence of as sprayed coatings on osteoblast cell number. Fig. 8 shows that on the early culture time points, the coating surfaces had fewer osteoblasts compared with those on Petri dish. The secondary calcium phosphate phases are highly dissoluble to culture media which would contribute to an high mineral content in the cultures. Adams et al. [28] explored the possibility that an increase in calcium level could elevate the intracellular calcium concentration and thereby promote the death cascade. They reported that a combined increased calcium and phosphate ions was a powerful inducer of the death response. The results from Oreffo et al. [29] showed that the amorphous calcium phosphate phase was cytotoxic to cultured osteoblasts. However, Iwakami et al.[30] reported that it was almost impossible for the inhibition of osteoblast cell proliferation to be caused by soluble factors generated by HA but by direct contact between the cells and hydroxyapatite. The reason for inhibition might be the direct contact contact between HA and the cells [31]. The results from Sun et al. [32] suggested that the effect of sintered HA particles with different sizes on cell proliferation varies according to the physical and chemical characteristics of the HA. According to these results, it seems that inhibition in the silica doped coatings, was not due to chemical components released into the medium by the coating. We speculated that the presence of silica resulting in the rougher microstructure shown in Figure 1 might play a more important role than the decomposition of HA in the cell proliferation.

Alkaline phosphatases (ALP) exists with high concentration in vivo within teeth and bone, and plays a very important role in cell proliferation and calcification. Alkaline phosphatases act by splitting phosphorus (an acidic mineral) creating an alkaline pH [33]. As shown in Figure 9, ALP activity increased with increasing in culturing period. At week 1, the cells on both coatings samples showed similar ALP levels as compared between various coating samples. After 2 weeks, the differentiation of the cells was significantly higher on the Petri Dish than those on the coating samples. Furthermore, pure HA coatings showed slightly higher ALP levels than those on those coatings with addition of silica. According to Amutha et al. [33], an increased serum ALP may be due to rapid bone growth. A decreased serum alkaline phosphatase may be due to low phosphorus levels. Our data indicated that the cell grew faster on H1 coatings than those cultured on silica doped coating samples and there was few phosphate ions released from the S2 and S4 coatings. The minor amount of SiO\textsubscript{2} in our case did not significantly affect the HA structure, consequently, the coating showed competitive ALP activity as compared to pure HA coating. In fact, cell behavior is presumably decided by protein adsorption behaviors on the material surface. In the meanwhile, the mechanism of the adsorption of the proteins from serum is not very well understood yet. We postulate that the experiment phenomenon that Si did not change apatite structure should account for the lowest ALP activity of S4 coatings. The results obtained from our in vitro tests may not be completely compatible with the results from in vivo studies [34-36]. However, our study giving good understanding of such a silica added HA coating would contribute to rational design of next generation biomaterials for medical devices.

4. Conclusions

This study investigated the influence of minor amount of silica on the properties of plasma sprayed HA coatings. Relative differences in the microstructure, phase composition, adhesive strength were investigated. From the results, it was found that, without any pre-substrate heat treatment, the presence of silica did not improve the mechanical properties of the HA coatings. This was due to the highly porous microstructure with low crystallinity in the silica doped HA coatings.
However, with pre heat treatment of substrate by the plasma, the mechanical properties of the as sprayed coating with doped silica improved significantly, which were competitive to those of plasma sprayed pure HA coating. Our studies also demonstrated that the various plasma sprayed coatings showed competitive cell proliferation and differentiation among the coatings with or without silica dopants. Our research nevertheless gives good understanding of such a silica added composite coating for biomedical applications, and hence contributes to selection of next generation biomaterials for medical surgeries.
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Figure 2  SEM microstructures of surface morphologies of:(a) H1; (b) H2; (c) S1; (d) S2; (e) S3 and (f) S4 coatings.

Figure 3  XRD patterns of the various plasma sprayed coatings. The major peaks of HA, alpha TCP, CaO and TTCP are marked by ●, ○, * and ♦, respectively.

Figure 4  Raman spectra of the various plasma sprayed coatings.

Figure 5  Representative XPS survey spectra of the plasma sprayed coatings.

Figure 6  Binding energies (eV) of O_{1s} (a), P_{2p} (b) and Si_{2p} (c) in plasma sprayed coatings as referred C_{1s} to 284.5 eV.

Figure 7  Typical fracture surface of plasma sprayed H1 coatings. Black arrow pointed the fracture starting at some sites of coating/substrate interface and spreading within the coatings(white arrows)

Figure 8  Proliferation of hFOB 1.19 celss examined with an MTT assay.

Figure 9  Effect of HA coating on AKP extracted from hFOB 1.19 cells.
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<th>He flow rate (l/min)</th>
<th>Carrier gas flow rate (l/min)</th>
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Table 1
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Table 2
<table>
<thead>
<tr>
<th>Samples</th>
<th>Adhesion strength (MPa)</th>
<th>Young’s modulus (GPa)</th>
<th>Hardness (MPa)</th>
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</thead>
<tbody>
<tr>
<td>H1</td>
<td>14.65±1.90</td>
<td>17.7±7.5</td>
<td>156±43</td>
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<td>H2</td>
<td>7.55±1.03</td>
<td>25.3±3</td>
<td>228.8±47</td>
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<tr>
<td>S1</td>
<td>3.00±0.25</td>
<td>10.3±1.8</td>
<td>360±86</td>
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<tr>
<td>S2</td>
<td>14.73±1.82</td>
<td>37.1±5.1</td>
<td>362.7±81</td>
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<tr>
<td>S3</td>
<td>3.36±1.05</td>
<td>18.4±1.4</td>
<td>281.06±53</td>
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<tr>
<td>S4</td>
<td>6.71±0.78</td>
<td>18.6±3.3</td>
<td>255.1±44</td>
</tr>
</tbody>
</table>

Table 3
Fig. 1
Fig. 2
Fig. 3
Fig. 4
Fig. 5
Fig. 6

(c)
Fig. 9